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APPLICATION NUMBER:

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MEDICAL REVIEW(S)

CLINICAL REVIEW

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Reviewer Name	Yuliya Yasinskaya, M.D.
Medical Team Leader	John Alexander, M.D., MPH
Review Completion Date	November 14, 2012
Established Name	Raxibacumab
Trade Name	None
Therapeutic Class	Human monoclonal antibody against protective antigen of *Bacillus anthracis*
Applicant	Human Genome Sciences
Formulation	Intravenous
Dosing Regimen	40 mg/kg single dose
Indication	Inhalational anthrax
Intended Populations	Adults and children

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Table of Contents

1 RECOMMENDATIONS/RISK BENEFIT ASSESSMENT 8

1.1 Recommendation on Regulatory Action 8
1.2 Risk Benefit Assessment 9
1.3 Recommendations for Postmarketing Risk Evaluation and Mitigation Strategies 10
1.4 Recommendations for Postmarketing Requirements and Commitments 10

2 INTRODUCTION AND REGULATORY BACKGROUND 10

2.1 Product Information 12
2.2 Tables of Currently Available Treatments for Proposed Indications 12
2.3 Availability of Proposed Active Ingredient in the United States 13
2.4 Important Safety Issues With Consideration to Related Drugs 13
2.5 Summary of Presubmission Regulatory Activity Related to Submission 13
2.6 Other Relevant Background Information 19

3 ETHICS AND GOOD CLINICAL PRACTICES 20

3.1 Submission Quality and Integrity 20
3.2 Compliance with Good Clinical Practices 20
3.3 Financial Disclosures 21

4 SIGNIFICANT EFFICACY/SAFETY ISSUES RELATED TO OTHER REVIEW DISCIPLINES 21

4.1 Chemistry Manufacturing and Controls 21
4.2 Clinical Microbiology 21
4.3 Preclinical Pharmacology/Toxicology 21
4.3.1 Natural History Studies in NZW rabbits and Cynomolgus Macaque Models of Inhalational Anthrax 21
4.3.3 Nonclinical Study 1103-CG923704 Evaluation of Raxibacumab as Therapeutic Treatment Against Inhalation Anthrax in the New Zealand White Rabbit Model.. 24
4.3.4 Nonclinical Study 1141-CG920871 Added Benefit of Raxibacumab with Levofloxacin vs. Levofloxacin as Post-exposure Treatment in the New Zealand White Rabbit Inhalational Anthrax Model 50
4.4 Clinical Pharmacology 71
4.4.1 Mechanism of Action 71
4.4.2 Pharmacokinetics and Pharmacodynamics 72

5 SOURCES OF CLINICAL DATA 73

5.1 Tables of Studies/Clinical Trials 74
5.2 Review Strategy 76
5.3 Discussion of Individual Studies/Clinical Trials 76

6 REVIEW OF EFFICACY 76

First Cycle Review Efficacy Summary 76

Reference ID: 3220416

Resubmission Review Efficacy Summary 78
6.1 Indication 78
6.1.1 Methods 79
6.1.2 Subject Demographics and Disposition 79
6.1.3 Analysis of Primary Endpoint(s) 79
6.1.4 Analysis of Secondary Endpoints(s) 81
6.1.5 Analysis of Secondary Endpoints(s) 81
6.1.6 Other Endpoints 82
6.1.7 Subpopulations 82
6.1.8 Analysis of Clinical Information Relevant to Dosing Recommendations 82
6.1.9 Discussion of Persistence of Efficacy and/or Tolerance Effects 82
6.1.10 Additional Efficacy Issues/Analyses 83

7 REVIEW OF SAFETY 83

Safety Summary 83
7.1 Methods 83
7.1.1 Studies/Clinical Trials Used to Evaluate Safety 83
7.1.2 Categorization of Adverse Events 86
7.1.3 Pooling of Data Across Studies/Clinical Trials to Estimate and Compare Incidence 86
7.2 Adequacy of Safety Assessments 87
7.2.1 Overall Exposure at Appropriate Doses/Durations and Demographics of Target Populations 87
7.2.2 Explorations for Dose Response 87
7.2.3 Special Animal and/or In Vitro Testing 88
7.2.4 Routine Clinical Testing 89
7.2.5 Metabolic, Clearance, and Interaction Workup 89
7.2.6 Evaluation for Potential Adverse Events for Similar Drugs in Drug Class 89
7.3 Major Safety Results 89
7.3.1 Deaths 89
7.3.2 Nonfatal Serious Adverse Events 89
7.3.3 Dropouts and/or Discontinuations 90
7.3.4 Severe Adverse Events 91
7.3.5 Submission Specific Primary Safety Concerns 92
7.4 Supportive Safety Results 93
7.4.1 Common Adverse Events 93
7.4.2 Laboratory Findings 93
7.4.3 Vital Signs 93
.4.4 Electrocardiograms (ECGs) 94
7.4.5 Special Safety Studies/Clinical Trials 94
7.4.6 Immunogenicity 94
7.5 Other Safety Explorations 95
7.5.1 Dose Dependency for Adverse Events 95
7.5.2 Time Dependency for Adverse Events 95

Reference ID: 3220416

7.5.3 Drug-Demographic Interactions 95
7.5.4 Drug-Disease Interactions 95
7.5.5 Drug-Drug Interactions 97
7.6 Additional Safety Evaluations 97
7.6.1 Human Carcinogenicity 97
7.6.2 Human Reproduction and Pregnancy Data 97
7.6.3 Pediatrics and Assessment of Effects on Growth 97
7.6.4 Overdose, Drug Abuse Potential, Withdrawal and Rebound 98
7.7 Additional Submissions / Safety Issues 98

8 POSTMARKET EXPERIENCE 98

9 APPENDICES 99

9.1 Literature Review/References 99
9.2 Labeling Recommendations 99
9.3 Advisory Committee Meeting 99
9.4 Field Study Protocol Synopsis/Review 101

Reference ID: 3220416

Table of Tables

Table 1: Approved Drugs and Doses for *B. anthracis* Exposure in Adults – Treatment and Post-Exposure Prophylaxis 12
Table 2: Presubmission Regulatory History 13
Table 3: Efficacy Results in Raxibacumab Monotherapy Studies (Bacteremic Animals, FDA analysis) 15
Table 4: Efficacy Results in Raxibacumab Combination Studies with Antimicrobials 16
Table 5: Comparison of Histological Findings in Non-survivors by Treatment Group in NZW Rabbits and Cynomolgus Macaques (Monotherapy Studies) 17
Table 6: Interspecies Comparison, Inhalational Anthrax (FDA) 22
Table 7: The Appearance, Formulation, and Strength of the Study Drug and Placebo 25
Table 8: Histopathology Findings from Efficacy Studies in Animals 26
Table 9: Animal Allocation 27
Table 10: Blood Collection and Assay Schedule 28
Table 11: Summary of Demographics and Baseline Characteristics (ITT Population) 32
Table 12: Mean Spore Challenge Dose (LD_{50}) by Challenge Day and Treatment Group 32
Table 13: Time to Fever (Temperature Elevation of >2F above Baseline) 34
Table 14: Time to Bacteremia 35
Table 15: Positive Blood Cultures and Survival Timeline on the Study 35
Table 16: Time to PA Toxemia 36
Table 17: PA Toxemia Levels at Treatment and Death for Non Survivors 37
Table 18: Positive PA (Quantitative ECL) and Survival Timeline on the Study 37
Table 19: Positive PA (Quantitative ECL) Terminal Samples in Non-Survivors 38
Table 20: Time to Treatment Initiation 38
Table 21: Time from Positive PA to Treatment 38
Table 22: Time from Positive Blood Culture to Treatment 39
Table 23: CSF PA in Non-Survivors 40
Table 24: Immunogenicity (Anti-Raxibacumab Antibodies) 41
Table 25: TNA Titers in Surviving Rabbits 42
Table 26: Incidence of Pathology Findings on Necropsy 43
Table 27: Incidence of High Grade Lesions in Treated Animals 44
Table 28: CNS Histopathology Results by Treatment Group and Survival Status 44
Table 29: Primary Efficacy Analyses 47
Table 30: The Appearance, Formulation, and Dosage Strength of the Study Drug, Study 1141 . 51
Table 31: Rabbit Survival in HGS Studies – Placebo-Treated Animals 52
Table 32: NIH/Battelle Studies with Levofloxacin Treatment of Anthrax-Infected Rabbits 52
Table 33: Correlation between Survival Time and Other Event Parameters 53
Table 34: Animal Allocation 53
Table 35: Blood Collection and Assay Schedule 54
Table 36: Treatment Assignments by Challenge Set and Day, Study 1141 57
Table 37: Animals with Fever (>2F above Baseline for ≥2 Consecutive Measurements), Study 1141 60
Table 38: Time to Bacteremia, Study 1141 60

Reference ID: 3220416

Table 39: Positive Bacteremia and Survival Timeline, Study 1141 61
Table 40: Time to PA Toxemia 62
Table 41: Positive PA Toxemia and Survival Timeline on the Study 62
Table 42: PA Toxemia Levels at Treatment for Treated Animals and at Death for Untreated Animals, Study 1141 64
Table 43: Time to Randomization / Treatment Initiation, Study 1141 65
Table 44: Time from Positive PA to Treatment, Study 1141 65
Table 45: Time from Positive Blood Culture to Treatment, Study 1141 65
Table 46: TNA Titers in Surviving Rabbits, Study 1141 66
Table 47: Incidence of Pathology Findings on Necropsy, Study 1141 68
Table 48: Incidence of High Grade Lesions in Treated Animals, Study 1141 68
Table 49: Primary Efficacy Analyses, Study 1141 69
Table 50: Studies Submitted and Reviewed during the BLA First Cycle 74
Table 51: Studies Submitted and Reviewed for the Current BLA Cycle 75
Table 52: Efficacy Results in Raxibacumab Monotherapy Studies 76
Table 53: Efficacy Results in Raxibacumab Combination Studies with Antimicrobials 77
Table 54: Primary Efficacy Analysis, Study 1103 80
Table 55: Studies in Healthy Volunteers Included in the Safety Analysis 83
Table 56: Demographics of Subjects Enrolled Across Studies 85
Table 57: Number of Raxibacumab-Treated Subjects with Treatment Emergent AEs 87
Table 58: Serious Adverse Events Reported Across All Studies 89
Table 59: Subjects with AR Resulting in Discontinuation of Treatment 91
Table 60: Severe Adverse Events across Studies (FDA Analysis) 91
Table 61: Nervous System Disorders across All Studies (FDA Analysis) 92
Table 62: Study Calendar 103

Reference ID: 3220416

Table of Figures

Figure 1: Animal's Disposition 31
Figure 2: Survival Time During 28-day Study Period in Rabbits Toxemic and/or Bacteremic at Treatment Initiation 48
Figure 3: Time from Challenge to Death in Non-Survivors 49
Figure 4: Animal's Disposition, Study 1141 ..... **57**
Figure 5: Plasma PA Concentration-Time Profile in Treated Rabbits that Died (A) and Survived (B) 64
Figure 6: Survival Time, Study 1141 70
Figure 7: Survival Time During 28-day Study Period in Rabbits Toxemic and/or Bacteremic at Treatment Initiation, Study 1103 81
Figure 8: Survival Time During 35-day Study Period in Rabbits Survived to Treatment, Study 1141 82

Reference ID: 3220416

1 Recommendations/Risk Benefit Assessment

1.1 Recommendation on Regulatory Action

The clinical reviewer recommends approval of raxibacumab for the treatment of inhalational anthrax. The BLA submission under review has fulfilled all four criteria for approval under 21 **CFR §601.90-95** "*New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible*,"[1] for **Biologics, also referred to as** "*The Animal Rule*":

- There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;
  The pathophysiology of inhalational anthrax is reasonably well understood and is similar between humans and animal species selected by the applicant for efficacy studies: New Zealand White (NZW) rabbits and cynomolgus macaques, manifesting as a life-threatening infection with multiorgan failure and a cardinal feature of hemorrhagic mediastinitis. *B. anthracis* toxins [Lethal toxin (LT) and Edema toxin (ET)] are thought to be responsible for the morbidity and high mortality characteristic of systemic anthrax infection. Raxibacumab is a recombinant, human IgG1λ monoclonal antibody directed at neutralizing the protective antigen (PA) of *B. anthracis*, binding component of the anthrax toxins, thereby preventing downstream toxic effects of LT and ET: cell death, loss of endothelial barrier function, immune suppression/dysregulation, and edema.

- The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;
  Two animal models of inhalational anthrax were evaluated in adequate and well controlled studies: NZW rabbits and cynomolgus macaques. Inhalational anthrax in these models closely resembles inhalational anthrax in humans with respect to susceptibility, disease pathophysiology, clinical presentation, outcome, gross and microscopic pathology findings. Treatment effect of raxibacumab on reducing the risk of mortality was demonstrated in these animal models.

- The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and mortality
  Untreated inhalational anthrax is a highly fatal disease (>95%), therefore, a relevant endpoint for the animal efficacy study, survival, was selected.

[1] Final Rule published in the Federal Register, Vol. 67, No. 105, May 31, 2002, pages 37988-37998; Regulations: 21 CFR § 314.600-650 (New Drugs), 21 CFR § 601.90-95 (Biologics)

Reference ID: 3220416

- The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.
  The human dose of 40 mg/kg IV over 2 hours resulted in exposures that are similar or greater than those achieved in animals given 40 mg/kg in a single dose (the dose found to be efficacious in NZW rabbits and cynomolgus macaque models of inhalational anthrax).

Raxibacumab, when administered as a single intravenous dose of 40 mg/kg dose is found to be safe for the treatment of inhalational anthrax. This safety conclusion is based on the pooled safety analyses of 326 adult subjects treated with the recommended dose of raxibacumab of 40 mg/kg. The database included 303 subjects who received a single dose, 23 subjects who received a 2nd dose 2 weeks after their first dose, and 20 subjects who received a 2nd dose more than 4 months after their initial dose (study HGS1021-C1069). No serious, fatal or life-threatening events attributed to raxibacumab were observed in clinical studies. The most common adverse reaction reported in raxibacumab treated subjects were headache, upper respiratory infections and rash. In study HGS1021-C1064, 6/27 (22%) of volunteers without prior administration of diphenhydramine developed rash and urticaria after infusion of raxibacumab. As a result of this finding, patients are to receive diphenhydramine pretreatment before raxibacumab infusion.
Nonclinical findings of exaggerated CNS pathology (bacteria, inflammation, and hemorrhage) in the raxibacumab-treated non-survivors as compared to placebo-treated non-survivors were observed in monotherapy animal studies in both species. However, when taken together with a survival benefit offered by raxibacumab, negative brain tissue cross-reactivity study results, absence of exposure/response for the CNS findings as well as greater pathologic changes in the organs other than CNS in placebo-treated animals, the CNS findings in raxibacumab-treated non-survivors do not appear to be a direct toxic effect of raxibacumab.

1.2 Risk Benefit Assessment

Untreated inhalational anthrax is a highly fatal (>95%) disease in humans. The reviewer finds that the potential survival benefit of raxibacumab treatment in human inhalational anthrax as evidenced by its reproducible findings of efficacy of 40 mg/kg single dose in three monotherapy studies in two representative animal models of inhalational anthrax in NZW rabbits (35-44% survival) and cynomolgus macaques (69% survival) when treatment was initiated postexposure at the time when all animals were toxemic/febrile, as well as a trend of improved survival of 17% (not statistically significant, p=0.089) over antimicrobial alone when the treatment is delayed to 84 hours postexposure, outweighs the risk of any known or potential adverse reactions associated with raxibacumab treatment.
Safety of raxibacumab 40 mg/kg single intravenous infusion was established in a database of 326 healthy human volunteers, allowing for exclusion with 95% confidence of serious adverse reactions related to raxibacumab that occur at frequency of 1% or greater.

Reference ID: 3220416

1.3 Recommendations for Postmarketing Risk Evaluation and Mitigation Strategies

There are no recommendations for postmarketing risk evaluation and mitigation strategies.

1.4 Recommendations for Postmarketing Requirements and Commitments

Approval under the Animal Rule is subject to a requirement for a postmarketing field study(s) to be conducted with due diligence, to verify and describe the drug's clinical benefit and to assess its safety when used as indicated when such study (ies) is feasible and ethical. The applicant was asked to design a protocol to collect and evaluate efficacy, pharmacokinetics, and safety of raxibacumab for the treatment inhalational anthrax. The protocol was included in the current BLA re-submission and was found adequate. The proposed field study should be ready for implementation in the event of an intentional release of *B. anthracis* in the US population. Coordination of the field study with the government agencies (BARDA, CDC) is recommended.

2 Introduction and Regulatory Background

The "Animal Rule"

Products for the treatment or prevention of human disease are generally studied in adequate and well-controlled clinical trials that enroll patients with the disease, and these clinical trials, along with other investigations, serve as the basis for approving or licensing the product. However, inhalational anthrax is extremely rare and human trials are not feasible. The efficacy studies in this BLA submission for raxibacumab for the treatment of inhalational anthrax will be assessed for approval under the "Animal Rule". The "Animal Rule" is a regulatory mechanism to review efficacy studies in animal models for diseases that cannot be studied in humans. FDA published a Final Rule in 2002 entitled "*New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible*,"[2] that is also referred to as "*The Animal Rule*." Under the *Animal Rule*, products intended to reduce or prevent serious or life-threatening conditions in humans may be approved by the FDA based on evidence of effectiveness derived from controlled studies in animal models of the disease with additional supporting safety and pharmacokinetic data. This rule is summarized in the Code of Federal Regulations (CFR), in Title 21 CFR 314.600 (Subpart I) for New Drugs and in Title 21 CFR 601.90 for Biologic Products. Raxibacumab is a monoclonal antibody, a biologic product; and therefore, the provisions of 21 CFR 601.90 (Subpart H) apply.

Criteria for Submission

All animal studies subject to the Animal Rule regulations must be conducted in accordance with good laboratory practices. Products evaluated for effectiveness under Subpart I are expected to be evaluated for safety under preexisting requirements for establishing the safety of new drugs.

[2] Final Rule published in the Federal Register, Vol. 67, No. 105, May 31, 2002, pages 37988-37998; Regulations: 21 CFR § 601.90-601.95 (Biologic Products)

Reference ID: 3220416

Criteria for Approval of a Drug under the Animal Rule
The Animal Rule states that a drug can be approved on the basis of adequate and well-controlled animal studies when the results of those animal studies establish that the biological product is reasonably likely to produce clinical benefit in humans.
To ensure applicability of animal data to human disease the following would need to be established:

- **Pathophysiology of the disease and product's** mechanism of action are reasonably well understood;
- The product efficacy is demonstrated in more than one animal species, unless the model in a single animal species is sufficiently well characterized for predicting the response in humans;
- The animal study endpoint is clearly related to the desired benefit in humans; and
- Data on product pharmacokinetics and pharmacodynamics in animals and humans allow selection of an effective human dose.

In assessing the sufficiency of animal data, the FDA may take into account other data, including human data in other indications. For example, product efficacy in diseases of similar pathophysiology might provide supportive information for the indication being sought. Therefore, data from appropriate studies to address each of the above bullet points would need to be provided to support the conclusion that the product is effective.

In addition, approval under this regulation will be subject to three requirements:

1. **Postmarketing studies – The applicant must** conduct postmarket studies, such as field **studies, to verify and describe the drug's clin**ical benefit and to assess its safety when used as indicated when such studies are feasible and ethical (for example during or following a bioterrorism event).
2. Approval with restrictions to ensure safe **use – If FDA concludes that a drug product** shown to be effective under this regulation can be safely used only if distribution or use is restricted, FDA will require such postmarket restrictions as are needed to ensure safe use of the drug product.
3. Information to be provided to patients – **For drug products or specific indications** approved under this subpart, applicants must prepare, as part of their proposed labeling, an information sheet to be provided to patients that includes the following: a disclaimer that the drug's approval was based on solely animal efficacy studies, the indication, directions for use, foreseeable risks and benefits, and any other relevant information required by FDA at the time of approval.

FDA may withdraw approval if:

- A postmarket clinical study fails to verify clinical benefit;
- The applicant fails to perform the postmarket study with due diligence;
- Use after marketing demonstrates that postmarket restrictions are inadequate to ensure safe use of the drug product;

Reference ID: 3220416

- The applicant fails to adhere to the postmarket restrictions applied at the time of approval under this subpart;
- The promotional materials are false or misleading; or
- Other evidence demonstrates that the product is not shown to be safe or effective under its conditions of use.

2.1 Product Information

Inhalational anthrax is caused by the exotoxin-producing Gram-positive bacterium, *Bacillus anthracis*. In the absence of adequate treatment, this is a highly fatal disease. While *B. anthracis* replication can be affected by antibiotics, it is the secreted anthrax toxin that is primarily responsible for the symptoms, signs, and high mortality rates in infected individuals.

Anthrax toxin is a protein complex consisting of lethal factor (LF), edema factor (EF) with protective antigen (PA) constituting the binding moiety. The PA moiety of anthrax toxin binds the anthrax toxin receptors (ATR or CMG2) on host cells to initiate a cascade of molecular events that manifests as clinical disease.

Raxibacumab is a recombinant human immunoglobulin (IgG1λ) monoclonal antibody to *B. anthracis* protective antigen (PA). Raxibacumab blocks the binding of PA to cell surfaces and prevents the anthrax toxins from entering host cells and exerting their downstream toxic effects.

2.2 Tables of Currently Available Treatments for Proposed Indications

Raxibacumab is a first-in-class agent, with no other approved antibody therapies available for the proposed indication of treatment of inhalational anthrax. However, antibiotics approved for the treatment of anthrax disease include penicillin G procaine and antimicrobials of the tetracycline class (doxycycline; tetracycline and minocycline are recommended in situations where penicillin is contraindicated).

In addition, several antimicrobials were approved for the inhalational anthrax (post exposure) in the wake of the US anthrax bioterrorism event in 2001. The evidence for efficacy of these antimicrobials came from the studies in rhesus macaques. These approvals antedated the Animal Rule and were based on subpart H regulations (3).

Table 1: Approved Drugs and Doses for *B. anthracis* Exposure in Adults – Treatment and Post-Exposure Prophylaxis

Drug	Dose	Duration	Notes
Treatment of anthrax and post-exposure prophylaxis			
Doxycycline (doxycycline	100 mg po q12h 100 mg IV q12h	60 days 60 days	Members of the tetracycline class are generally not indicated for the treatment of

3 21 CFR 314.510 (**Subpart H** – Accelerated Approval of New Drugs for Life-Threatening Illnesses)

Reference ID: 3220416

Drug	Dose	Duration	Notes
calcium, doxycycline hyclate)			patients under 8 years of age (tooth discoloration, enamel hypoplasia, delays in bone development with prolonged use)
Penicillin G procaine	1.2 million units IV q12h	60 days	
Post-exposure prophylaxis of anthrax			
Ciprofloxacin	10 mg/kg IV (maximum 400 mg/dose) q12h 15 mg/kg po (maximum 500 mg/dose) q12 hours	60 days	Approved for post-exposure inhalational anthrax based on pharmacokinetic data (human exposures matched exposures that proved efficacious as post-exposure prophylaxis in rhesus monkeys exposed to inhalational anthrax)
Levofloxacin	500 mg po QD	60 days	Approved for post-exposure inhalational anthrax based on pharmacokinetic data (human exposures matched exposures that proved efficacious as post-exposure prophylaxis in rhesus monkeys exposed to inhalational anthrax)

2.3 Availability of Proposed Active Ingredient in the United States

Raxibacumab is manufactured in the United States by Human Genome Sciences (HGS) located in Rockville, MD, USA.

2.4 Important Safety Issues With Consideration to Related Drugs

Not applicable. Raxibacumab is a first in class monoclonal antibody against PA of *B. anthracis*.

2.5 Summary of Presubmission Regulatory Activity Related to Submission

The following table summarizes the key presubmission regulatory activities and actions.

Table 2: Presubmission Regulatory History

Date	Type of correspondence	Action
28 August 2002	Meeting request and package	Pre-IND submitted for raxibacumab
22 May 2003	Applicant submission	IND filing
15 August 2003	Fast track designation letter	Fast track development program for the investigation of PA mAb for post exposure prophylaxis and adjunctive therapy of inhalational anthrax disease designated
12 November 2003	Organ drug designation letter	Request for orphan drug designation granted

Reference ID: 3220416

Date	Type of correspondence	Action
12 July 2006	Type B (pre-Phase 3) meeting minutes	Agreed on development plans for raxibacumab; agreed on therapeutic treatment indication
27 September 2006	Follow-up Type B meeting minutes	Confirmed immunogenicity study with wash-out period was required in humans
17 November 2006	FDA written correspondence	Defined scope of animal efficacy studies needed to support licensure (2 animal models required; demonstration that the monoclonal is superior to placebo; compare the efficacy of the monoclonal with antimicrobial to the antimicrobial alone
11 December 2006	Type A meeting minutes	Discussion of design for rabbit efficacy studies
6 February 2007	FDA written correspondence	Confirmed embryo-fetal toxicity study was required
3 December 2007	FDA written correspondence	Confirmed scope of studies required **for licensure (Efficacy – require 2 models of monoclonal with antimicrobial; Safety – established that** PAM-NH-01 study cannot be counted towards the 300 subjects safety database because its safety profile (M10) is different from the safety profile of the M11 product.
21 October 2008	Type B (pre-BLA) meeting minutes	Reviewed data to support submission of a BLA (FDA agreed that therapeutic efficacy studies reviewed to date were sufficient to file the BLA; discussed presentation of safety data, namely separate presentation of the M10 and M11 data, separate presentation of single dose (n=326) and double dose with and without washout also separately presented)
30 January 2009	FDA written correspondence	Confirmed raxibacumab efficacy, safety and product quality data provided by HGS were sufficient to support the use of a single 40 mg/kg IV dose for the treatment of inhalational anthrax under IND **11,069 ("safe to proceed" issued by FDA on Jan 23, 2009)**
13 July 2009	FDA written correspondence	Raxibacumab BLA filed with FDA
10 October 2009	HGS and FDA presentation	Anti-Infective Drug Advisory Committee meeting
14 November 2009	FDA written correspondence	Complete response letter for the BLA
29 January 2010	Type A meeting correspondence	Discussion of how to resolve the deficiencies identified in the complete response letter (CRL) for raxibacumab
10 July 2010	FDA written correspondence	The revised bioanalytical assay for determination of raxibacumab concentrations in human serum found to be acceptable
14 October 2010	FDA written correspondence	Special protocol assessment (SPA) agreement letter for study 1141-CG920871 (added benefit study)
17 November 2010	FDA written correspondence	FDA letter granting 2-year extension for the resubmission of the raxibacumab BLA
28 October 2011	FDA written correspondence	Advice/information request for PK modeling/simulation to determine raxibacumab doses for pediatrics
19 March 2012	Type B meeting correspondence	Review of data to support resubmission of the raxibacumab BLA
23 March 2012	FDA written correspondence	**Advice/information request regarding FDA's modeling and simulation** and recommendations for a pediatric dosing regimen for raxibacumab
15 June 2012	BLA re-submission	Resubmission to complete response letter

Reference ID: 3220416

The original BLA for raxibacumab was submitted to the FDA on May 14, 2009. The application contained a number of proof-of-concept studies in various animal models, safety pharmacology and clinical pharmacology studies in animals and humans, safety studies in healthy human volunteers, and four GLP animal efficacy studies of raxibacumab for the treatment of inhalational anthrax in New Zealand white (NZW) rabbits and cynomolgus macaques in lieu of adequate and well controlled studies in humans.

Efficacy Findings

NZW rabbits and cynomolgus macaques were selected for the efficacy studies of raxibacumab for treatment of inhalational anthrax. In the original BLA, four treatment studies with raxibacumab were submitted. The two efficacy studies tested raxibacumab versus placebo and two others evaluated the efficacy of raxibacumab plus antimicrobial (two studies each in rabbits and in monkeys). The animals were challenged with aerosolized *B. anthracis* spores at 200x LD_{50}. In rabbit studies, sustained elevation of body temperature above baseline for 2 hours or a positive result on PA toxemia screen assay served as trigger for intervention (average 28 hours postexposure). In monkey studies, intervention commenced at the time of positive PA result (average 42 hours postexposure).

The review revealed that in the population of all treated bacteremic animals raxibacumab at 40 mg/kg IV single dose was superior to placebo in the rabbit and monkey raxibacumab monotherapy studies. In the monkey study, raxibacumab 20 mg/kg was also superior to placebo, but in the rabbit superior efficacy was limited to the 40 mg/kg arm. In the population of all treated animals regardless of bacteremia status prior to treatment both doses of raxibacumab were superior to placebo in monkey and rabbit animal studies.

Table 3: Efficacy Results in Raxibacumab Monotherapy Studies (Bacteremic Animals, FDA analysis)

Treatment group	Cynomolgus macaques at 28 days			NZW rabbits at 14 days		
	Number (%) of survivors	P value vs. placebo[1]	95% CI of raxibacumab-placebo[2]	Number (%) of survivors	P value vs. placebo[1]	95% CI of raxibacumab-placebo[2]
Placebo	0/10 (0%)			0/13 (0%)		
20 mg/kg raxibacumab	5/12 (42%)	0.0396	(7.2, 68.7)	4/16 (25%)	0.1067	(-2.2, 50.9)
40 mg/kg raxibacumab	9/13 (69%)	0.0016	(31.1, 88.9)	6/17 (35%)	0.0237	(7.3, 59.6)

[1] P value based on 2-sided Fisher's exact test for the comparison vs the placebo control group.

[2] 95% CIs are exact confidence intervals

When given in combination with antimicrobials, the efficacy of the combination was high and superior to placebo, but the efficacy of antimicrobials alone was also high, approaching 100% in both animal models, raising the question whether the animal models adequately represented advanced anthrax disease in humans, where high mortality (45%) was observed despite antimicrobial treatment.

Reference ID: 3220416

Table 4: Efficacy Results in Raxibacumab Combination Studies with Antimicrobials

Treatment group	Cynomolgus macaques at 28 days		NZW rabbits at 28 days	
	Number (%) of survivors	95% CI of Cipro/raxibacumab-ciprofloxacin[2]	Number (%) of survivors	95% CI of Levo/raxibacumab-levofloxacin[2]
Placebo	0/10 (0%)	-	0/10 (0%)	-
Antimicrobial alone[1]	13/13 (100%)	-	19/20 (95%)	-
Antimicrobial[1] + 40 mg/kg raxibacumab	11/13 (85%)	(-45.5, 11.4)	16/17 (94%)	(-23.9, 19.6)

[1] NHP study antimicrobial was 75 mg ciprofloxacin twice daily x 3 days
NZW rabbit study antimicrobial was 50 mg/kg levofloxacin daily x 3 days
[2] 95% CIs are exact confidence intervals

These animal studies were discussed in an AIDAC meeting in October of 2009. Based on the information from the natural history of inhalational anthrax disease in man, in rabbits, and monkeys; the mechanism of action of raxibacumab; and the efficacy of raxibacumab 40 mg/kg IV in the animal studies, FDA and the majority (16/24) of AIDAC committee members concluded that NZW rabbit and cynomolgus macaque models of inhalational anthrax disease are reflective of human disease and the efficacy findings from the therapeutic studies in these models could be extrapolated to humans.

However, several limitations of the efficacy findings in pivotal and combination animal studies and in animal models themselves were discussed.

First, the timing of intervention is likely to differ between animals and humans. In the experimental settings, animals were closely monitored for either an elevation in temperature (rabbits) or a positive plasma PA (rabbits and monkeys), and once detected, a therapeutic intervention was delivered. Humans may have delayed presentation to the healthcare setting or be given alternative diagnoses prior to the definitive diagnosis of anthrax being made. As the effects of delayed administration of raxibacumab after the onset of clinical symptoms has not been studied in the animal models, it is unclear how long raxibacumab administration can be delayed and still provide survival benefit.

Second, the combination efficacy studies demonstrated very high efficacies (nearly 100%) of levofloxacin in NZW rabbits and ciprofloxacin in monkeys. In contrast, during the 2001 anthrax attacks 5 of the 11 people who developed inhalational anthrax died despite best available antimicrobial and supportive treatment. In addition, a question regarding efficacy interaction between raxibacumab and antimicrobial when given together for the treatment of inhalational anthrax remained unanswered. Twelve of twenty four AIDAC committee members pointed out a number of deficiencies in the combination study design that precluded definite conclusions

Reference ID: 3220416

regarding efficacy interactions, e.g. timing of intervention, antibiotic regimen, and the absence of a raxibacumab alone arm. A majority of the committee members (17/24) recommended additional evaluation of the contribution offered by raxibacumab to the efficacy of antimicrobial alone.

Third, the conditions of an animal experiment do not include any supportive care that human patients with inhalational anthrax will receive in a healthcare or clinical trial setting, including but not limited to mechanical ventilation, fluid resuscitation, pleural fluid drainage, dialysis, etc.

Fourth, due to concerns about the reliability of raxibacumab and antimicrobial PK data generated for the original BLA, FDA was unable to conclude that the proposed human dosing regimen would result in the exposures similar to those seen at the efficacious dose in animal studies. Because of these concerns, it was also unclear that the antimicrobial exposures in the combination animal studies did not exceed human exposures achieved with the approved human dose regimen.

Safety Findings

During the BLA review in 2009, an exaggerated inflammatory response in the CNS of the raxibacumab treated non-survivors compared to the placebo non-survivors was found on histopathological examination in the raxibacumab monotherapy animal studies. The duration of bacteremia prior to treatment / prior to death and time to death were similar between the placebo and raxibacumab non-survivors for both the monkey and the rabbit studies. However, within the raxibacumab groups, it appears the animals with CNS pathology lived longer than those without. Non-survivors in the raxibacumab 20 mg/kg dose group had a higher incidence and severity of CNS pathology as compared to the non-survivors the 40 mg/kg group, suggesting an absence of dose/exposure-response relationship for brain histopathology.

Table 5: Comparison of Histological Findings in Non-survivors by Treatment Group in NZW Rabbits and Cynomolgus Macaques (Monotherapy Studies)

Organ/animal	Placebo (%)	Raxibacumab 20 mg/kg (%)	Raxibacumab 40 mg/kg (%)	P-value (20 vs. p)*	P-value (40 vs. p)*
Cynomolgus Macaque (As treated)					
Mortality	**12/12 (100)**	**7/14 (50)**	**5/14 (35.7)**	**0.006**	**0.001**
CNS	**1/12 (8)**	**6/7 (86)**	**3/5 (60)**	**0.002**	**0.053**
Liver	3/12 (25)	1/7 (14)	0/5 (0)	1.0	0.515
Spleen	11/12 (92)	5/7 (71)	4/5 (80)	0.523	0.515
Mediastinitis	10/11 (91)	4/7 (57)	4/5 (80)	0.245	1.0
Pneumonia	5/12 (42)	0/7 (0)	1/5 (20)	0.106	0.6
Nephritis	2/12 (17)	0/7 (0)	0/5 (0)	0.509	1.0
NZW Rabbit (As treated)					
Mortality	**16/16 (100)**	**12/17 (70.5)**	**11/19 (57.9)**	**0.044**	**0.004**
CNS	**2/16 (12)**	**9/12 (75)**	**6/11 (55)**	**0.001**	**0.033**
Liver	6/16 (38)	3/12 (25)	3/11 (27)	0.687	0.692
Spleen	11/16 (69)	2/12 (17)	2/11 (18)	0.009	0.018

Reference ID: 3220416

Organ/animal	Placebo (%)	Raxibacumab 20 mg/kg (%)	Raxibacumab 40 mg/kg (%)	P-value (20 vs. p)*	P-value (40 vs. p)*
Mediastinitis	15/16 (94)	7/12 (58)	8/11 (73)	0.057	0.273
Pneumonia	10/16 (63)	0/12 (0)	0/11 (0)	0.001	0.001
Nephritis	4/16 (25)	1/12 (8)	2/11 (18)	0.355	1.0
Cardiac	0/16 (0)	0/12 (0)	2/11 (18)	Ns	0.157

*** two-sided Fisher's exact test**

These findings were presented at the AIDAC and their implications for humans were discussed. Additional studies to elucidate raxibacumab effect on CNS in survivors and non-survivors were recommended.

Animal Studies

The main limitation of the pivotal efficacy studies with respect to the evaluation of the CNS findings was a lack of pathological examination of the survivors to allow comparison to the animals that died. In the monotherapy efficacy studies, surviving rabbits were euthanized and disposed of, while surviving monkeys were observed for the duration of the study and confined to the Biosafety Level 3 (BL3) husbandry for 30 days post exposure, followed by a downgrade to a BL2 husbandry for an additional 60 days and then a release into the colony if in good health to be used in other experiments.

Although survivors were sacrificed in the combination levofloxacin/raxibacumab rabbit study, the high efficacy of the fluoroquinolone in this study limited the number of animals that died from anthrax in the active treatment arms. Only one animal in an active treatment group (levofloxacin alone) died of anthrax. Although it was reassuring that none of the surviving animals in the two treatment arms exhibited CNS pathology attributable to anthrax, all treated animals received a fluoroquinolone in this study.

Similar findings were noted in the combination ciprofloxacin and raxibacumab study in monkeys. No animals in the ciprofloxacin group died during 28 days following exposure. Only one animal in the ciprofloxacin/raxibacumab treatment group died of anthrax disease. None of the surviving animals were sacrificed for microscopic examination.

Human volunteers

No serious, fatal or life-threatening reactions attributed to raxibacumab were observed in clinical studies. The most common adverse reactions reported in raxibacumab-treated subjects were headache, upper respiratory infections and rash. In study HGS1021-C1064, 6/27 (22%) volunteers without prior administration of diphenhydramine developed rash and urticaria after infusion of raxibacumab. As a result of this finding, patients are to receive diphenhydramine pretreatment before raxibacumab infusion.

In a safety database of 326 subjects only events that occur with frequency of 1% or greater could be excluded with 95% confidence. As the safety database consisted only of healthy volunteers, any possible study agent/disease interaction could not be assessed. While CNS AE were

Reference ID: 3220416

reviewed in healthy volunteers, it appears from the animal models that systemic anthrax disease must be present for CNS pathology to be observed. CNS safety review in humans was therefore limited.

Absence of data in the pediatric age range was noted as a limitation of the raxibacumab safety evaluation.

To address the deficiencies identified during review and AIDAC discussion of the original submission the sponsor conducted additional studies that are subject of the BLA resubmission dated June 15, 2012.

2.6 Other Relevant Background Information

Following the anthrax attacks of 2001, the CDC[4] recommended the use of two or three antimicrobials in combination in persons with inhalational anthrax based on susceptibility testing with epidemic strains. Limited early information suggested that patients treated intravenously with two or more antimicrobials active against *B. anthracis* had a greater chance of survival. It was also recommended to consider antimicrobial penetration into the central nervous system. CDC recommendations advocate use of ciprofloxacin over doxycycline, plus augmentation with chloramphenicol, rifampin, or penicillin when meningitis is established or suspected.[4]

Raxibacumab for Strategic National Stockpile

In October 2005, an End of Phase 1 meeting was held between the FDA and HGS to discuss the issues related to the licensure and use of raxibacumab under Emergency Use Authorization (EUA) for the post-exposure treatment of anthrax. During this meeting, the Agency indicated that unmet medical need exists for the treatment of anthrax rather than postexposure prophylaxis and expressed the need for studies in healthy volunteers and animal efficacy studies.

In December 2007, agreement was reached on the data necessary to support the Centers for Disease Control and Prevention (CDC) IND submission to use raxibacumab in the Strategic National Stockpile (SNS), and subsequent additional studies needed in the BLA submission to support licensure.

In January 2009, DSPTP determined that a single dose of raxibacumab 40 mg/kg IV was **acceptable for use under HGS' and the CDC's IND** for the treatment of inhalational anthrax. This decision was based on the review of various preclinical studies (including pivotal efficacy studies in animals as required under the Animal Rule), human safety and PK studies, acceptable product quality assessments, and the Division of Scientific Investigation (DSI) and **Biotechnology Manufacturing Team's inspections.**

4 Update: Investigation of Bioterrorism-Related Anthrax and Interim Guidelines for Exposure Management and Antimicrobial Therapy, October 2001 *MMWR. 2001;50(42):909-19*

Reference ID: 3220416

3 Ethics and Good Clinical Practices

3.1 Submission Quality and Integrity

The Division of Scientific Investigations (DSI) inspected Human Genome Sciences, as well as two clinical sites that enrolled the majority of subjects in safety studies HGS1021-C1063 and HGS1021-C1064. The studies appeared to have been conducted adequately, and therefore, the data generated were deemed acceptable to support the proposed indication. Please refer to the DSI consults in DARRTS for complete details.

In September 2009, Division of Scientific Investigations (DSI) inspections for the analytical portions of human studies HGS1021-C1063 and HGS1021-C1064 were conducted. During these inspections, multiple issues with the analysis of raxibacumab and ciprofloxacin concentrations were identified. Inspection findings raised concerns regarding the reliability of the pharmacokinetic data submitted in the application and utilized for selection of an effective dose in humans for therapeutic treatment of anthrax. On 14 November 2009, the FDA issued a Complete Response letter to the applicant requesting revisions of the analytical procedures for raxibacumab to address the DSI inspection findings, re-analysis of the PK samples from human studies HGS1021-C1063, HGS 1021-C1064, and HGS1021-C1069, and re-calculation of the PK parameters for raxibacumab from these re-analyses. Upon revalidation of the assay for analysis of serum raxibacumab samples, the analytical sites underwent re-inspection by DSI focusing on the methods and data supporting Clinical Study HGS1021-C1064. Following inspection, the modified raxibacumab assay as used for the reanalysis of Study HGS1021-C1064 was deemed acceptable by DSI inspectors.

On April 23, 2010, the applicant submitted information supporting the revalidated assay for concentrations of raxibacumab in human serum along with PK comparison reports for the original raxibacumab assay (CLI-2879) and the results obtained with the modified assay (CLI-3192) and relevant bioanalytical validation and quantitation reports. Upon review of the submissions of data obtained by both the original and modified bioanalytical methods, the pharmacokinetic data for raxibacumab were considered reliable and sufficient for extrapolation of animal efficacy findings to humans for determination of the efficacious dose in humans for therapeutic treatment of anthrax. Therefore, pharmacokinetic data for raxibacumab obtained by the original bioanalytical method could be used for labeling.

3.2 Compliance with Good Clinical Practices

All animal monotherapy and the raxibacumab/antibiotic combination efficacy studies were conducted in accordance with GLP and the Animal Welfare Act. Human clinical trials for safety data were performed in compliance with GCP and ICH guidelines.

Reference ID: 3220416

3.3 Financial Disclosures

The Applicant obtained certification from each investigator and sub-investigator who enrolled subjects in the clinical (human) trials, as well as the pivotal nonclinical efficacy studies in rabbits and monkeys. No investigator or sub-investigator had any disclosed information to reveal.

4 Significant Efficacy/Safety Issues Related to Other Review Disciplines

4.1 Chemistry Manufacturing and Controls

Please refer to the final chemistry manufacturing and control (CMC) review by Dr. Chen Sun. for complete details. Please also refer to the CMC review by Dr. David Frucht for the original BLA submission.

4.2 Clinical Microbiology

Please refer to the final microbiology reviews by Dr. Lynette Berkeley for complete details. Based on the review of the animal studies in this resubmission, Dr. Berkeley concluded that raxibacumab offers protection against infection with *B. anthracis* and does not exacerbate CNS damage from anthrax. Please also refer to reviews of the original BLA submission by Drs. Maureen Davidson and Lynnette Berkeley.

4.3 Preclinical Pharmacology/Toxicology

Please refer to the final review by Dr. Terry Miller of the animal studies, the subject of this resubmission. Please also refer to the reviews of Drs. Terry Miller, Susan McCune, Tracy McGill, Ying Mu, and Yuliya Yasinskaya for the reviews of the animal studies submitted in the original BLA.

4.3.1 Natural History Studies in NZW rabbits and Cynomolgus Macaque Models of Inhalational Anthrax

In order to be able to develop a therapeutic product for the treatment of inhalational anthrax, it was important to identify and characterize animal models of inhalational anthrax to be used to evaluate the product's efficacy. Specifically, as provided in the Animal Rule, it was important to study the natural history of the anthrax infection in the animals, with the goal to understand the pathophysiological mechanism of the toxicity of the substance (*B. anthracis*) and prevention or substantial reduction of disease and/or mortality by the product. After consideration of rodent and larger animal species, the natural history of inhalational anthrax in NZW rabbit and cynomolgus macaque was evaluated with a goal to establish models in each of these species to

Reference ID: 3220416

be used for the efficacy evaluation of therapeutic products in randomized comparative treatment studies of inhalational anthrax.
NZW rabbit and cynomolgus macaque models of inhalational anthrax have been evaluated in two natural history studies conducted by the Battelle Biomedical Research Center (BBRC) laboratories. These two animal models are proposed to be used for evaluating efficacy of products for treatment of inhalational anthrax under the Animal Rule.
The design was fairly similar between the studies. Animals were exposed to 200xLD_{50} of *B. anthracis* spores via head only aerosol. Animals were monitored for clinical signs of the disease, and subjected to laboratory investigations that included hematology and serum chemistry evaluations and daily blood cultures. Following natural death for the disease or euthanasia due to moribundity animals underwent necropsy examination. As for both studies non naïve animals were used, survival up to 25% was observed. Experimental conditions were refined subsequently and only naïve animals were procured for the therapeutic experiments resulting in uniform mortality in the exposed untreated animals in both models.
The table below summarizes the data from the natural history of inhalational anthrax in NZW rabbits and cynomolgus macaques in the experiments conducted by BBRC and those published in the literature and compares the findings with historical data on inhalational anthrax in humans.

Table 6: Interspecies Comparison, Inhalational Anthrax (FDA)

DATA ELEMENTS	Animal Model		Human
	New Zealand White rabbit (NZW)	Nonhuman primate (NHP, cynomolgus macaque)	
A. Characteristics of the CBRN Agent that Influence the Disease or Condition			
1. The challenge agent	Ames strain *B. anthracis* spores	Ames strain *B. anthracis* spores	Many strains, but Ames strain used in 2001 bioterrorism attack
2. Pathogenic determinants	Antiphagocytic capsule, tripartite exotoxin	Antiphagocytic capsule, tripartite exotoxin	Antiphagocytic capsule, tripartite exotoxin
3. Route of exposure	Aerosol challenge with 200x LD_{50} of *B. anthracis*	Aerosol challenge with 200x the LD_{50} of *B. anthracis*	Inhalation
4. Quantification of exposure	200x LD_{50} LD_{50} = 1.1x10^5 cfu	200x LD_{50} LD_{50} = 0.6-1.1 x10^5 cfu	Infectious dose 2-55x10^4 cfu estimated from NHP studies and contaminated mills studies
B. Host Susceptibility and Response to Etiologic Agent	Susceptible[1]	Susceptible	Moderately susceptible
C. Natural History of Disease: Pathophysiologic Comparability			
1. Time to onset of disease/condition	16-53 hours postexposure to positive PA/fever. More rapid than humans[2]	28-58 hours postexposure to positive PA	Incubation period mostly < 7 days; longest up to 2 months
2. Time course of progression of disease/condition	More rapid than humans. Succumb to disease 2-9 (median 3) days postexposure	Similar to that seen in humans but dependent on the dose used for the challenge.[4,5] At 200x LD_{50} death 3-9 (median 4) days.	Prodromal phase 1-6 days; fulminant phase less than 24 hours[7] Overall mortality of 85% with a mean time from

Reference ID: 3220416

	Animal Model		
			symptom onset to death of 4.8 days[7] In 2001 attacks, symptoms began 4-6 days.[8] In Sverdlovsk incident, death in 1-4 days.[9]
3. Manifestations (signs and symptoms)	Lethargy, not eating, rapid respirations, respiratory distress, seizures and/or moribund shortly prior to death	Few clinical signs until 1-2 hours prior to death, then progressively less responsive to external stimuli. Lethargy, tachycardia, and fever, although abnormal signs varied and presented shortly before death.[6]	Flu-like symptoms for several days followed by severe respiratory collapse
4. Pathology	Mediastinitis, pneumonia, hemorrhagic meningitis 30%	Mediastinitis, pneumonia, hemorrhagic meningitis 30%	Hemorrhagic mediastinitis, meningitis 50%
D. Trigger for Intervention	Positive PA level or temperature elevation	Positive PA level	Variable, circumstance **specific – medical** history, epidemiologic history, signs and symptoms
E. Characterization of the Medical Intervention			
1. Product class	Raxibacumab, human monoclonal antibody		
2. Mechanism of action	Human monoclonal antibody which binds the protective antigen (PA) of *B. anthracis* and prevents the binding of PA with its receptors		
4. Activity in disease/condition of similar pathophysiology	N/A		
5. PK in unaffected animals/humans	Raxibacumab PK in infected and healthy NZW rabbits was similar		
6. PK/PD in affected animals/humans	Healthy adult humans achieve similar to or greater exposure to raxibacumab at 40 mg/kg single IV dose compared to animals receiving the same dose: • Mean raxibacumab Cmax in humans was similar to or greater than mean Cmax values in monkeys and rabbits. • Mean raxibacumab AUC in humans following a single 40 mg/kg IV dose was 2.4 and 4.6-fold that of the mean AUC values in monkeys and rabbits, respectively. • $T_{1/2}$ raxibacumab was longer in humans compared to mean half-lives observed in monkeys and rabbits (20.6 ± 6.5 days versus 10.1 ± 2.4 days and 4.1 ± 0.85 days, respectively).		
7. PK interactions with medical products likely to be used concomitantly	No PK interaction with levofloxacin	No PK interaction with ciprofloxacin	No PK interaction with ciprofloxacin
8. Synergy or antagonism of medical products likely to be used in combination	No interaction with levofloxacin	No interaction with ciprofloxacin	
F. Design Considerations for Animal Efficacy Studies	GLP, randomized, double-blind, placebo controlled study with mortality endpoint		
1. Endpoints	Survival	Survival	Survival

Reference ID: 3220416

	Animal Model		
2. Timing of intervention	Based on detection of positive PA or 2 consecutive temperature elevations, confirmed by culture	Based on detection of positive PA level, confirmed by culture	Variable upon suspicion, time of diagnosis, or **general condition** – empiric
3. Route of administration	IV	IV	IV
4. Dosing regimen	Single dose raxibacumab 40 mg/kg IV	Single dose raxibacumab 40 mg/kg IV	A 40 mg/kg single IV dose resulted in an exposure similar or exceeding those found efficacious in animals
HUMAN SAFETY INFORMATION			326 human subjects exposed to single and repeat dose raxibacumab

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4. Vasconcelos D, Barnwell R, Babin M, Hunt R, Estep J, Nielsen C, Carnes, Carney J. Pathology of inhalation anthrax in Cynomolgus monkeys (Macaca fascicularis). Lab. Invest. 2003;83:1201-1209.
5. Twenhafel NA, Leffel E, Pitt MLM. Pathology of inhalational anthrax infection in the African Green monkey. Vet. Pathol. 2007; 44:716-721.
6. Rossi C, Ulrich M, Norris S, Reed DS, Pitt LM, and Leffel EK. Infect Immun. 2008; 76:5790-5801.
7. Holty JEC, Bravata DM, Liu H, Olshen RA, McDonald KM, Owens DK. Systematic review: a century of inhalational anthrax cases from 1900 to 2005. Ann. Intern. Med. 2006;144:270-280.
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9. Abramova, FA, Lev M. Grinberg LM, Yampolskaya, OV, And. Walker, DH. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979 Proc. Natl. Acad. Sci. USA 1993; 90:2291-4.

4.3.3 Nonclinical Study 1103-CG923704 Evaluation of Raxibacumab as Therapeutic Treatment Against Inhalation Anthrax in the New Zealand White Rabbit Model

INVESTIGATOR: Daniel Sanford, PhD,

STUDY CENTER: Battelle Biomedical Research Center (BBRC)

STUDY PERIOD: 7 July 2010 to 19 December 2011

OBJECTIVES:
Primary:

- To assess terminal pathology in both non-surviving and surviving rabbits after anthrax exposure and therapeutic treatment with placebo or raxibacumab.

Reference ID: 3220416

Secondary:

- To evaluate the efficacy of raxibacumab when administered as a therapeutic treatment against lethality due to inhalation exposure to *B. anthracis* in NZW rabbits

METHODOLOGY: Parallel-group, randomized, blinded, single center, GLP-compliant study to evaluate the histopathology in surviving and non-surviving animals after anthrax exposure and therapeutic treatment with placebo or raxibacumab.

NUMBER OF ANIMALS: Forty-eight out of 54 screened and quarantined New Zealand White rabbits free of disease and malformations were randomized by gender and body weight into 2 treatment groups (24 animals, ~ 50% male and 50% female each): raxibacumab or placebo. The rest of the animals served as replacements.

STUDY DRUG: IV raxibacumab given as a single bolus infusion at 40 mg/kg dose.

Raxibacumab, also known as HGS1021, PA mAb, or ABthrax, is a recombinant, fully human IgG1λ mAb that binds *B. anthracis* PA with high affinity and inhibits its biological activity. Raxibacumab is expressed in the NS0 mouse myeloma cell line and secreted into culture media, from which it s purified by a series of chromatography and filtration steps.

Table 7: The Appearance, Formulation, and Strength of the Study Drug and Placebo

Study Drugs	Appearance	Formulation	Dose Unit	Batch numbers
Raxibacumab	Clear liquid	Raxibacumab sterile solution for injection	50mg/ml	71128
Placebo (raxibacumab formulation buffer)	Clear liquid	n/a	n/a	71043

MO comment: *The test article was raxibacumab (Lot #71128) the same as added benefit study.*

DURATION OF TREATMENT: Blinded raxibacumab treatment as a single IV bolus dose of 40 mg/kg or matching placebo were given to NZW rabbits at the time of positive plasma PA.

TREATMENT COMPLIANCE: Study drug infusion information, including dosage and volume prescribed, and volume administered, was recorded.

STUDY DESIGN: Parallel-group, randomized, blinded, single center, GLP-compliant study to evaluate the histopathology in surviving and non-surviving animals after anthrax exposure and therapeutic treatment with placebo or raxibacumab

Background: To date the Sponsor has evaluated raxibacumab in several animal species and under various conditions. Efficacy of raxibacumab was evaluated in a proof-of concept rat model of Lethal Toxin infusion in pre-exposure and post-exposure setting. In addition, when used for

Reference ID: 3220416

pre-exposure prophylaxis raxibacumab showed dose-dependent survival benefit in rabbits and monkeys. Time-related effect on survival was also observed in a post-exposure prophylaxis setting in a rabbit model. Monotherapy studies of raxibacumab administered to rabbits and cynomolgus macaques at the time of diagnosis of inhalational anthrax disease showed a statistically significant survival benefit relative to placebo treatment.
The complete response letter for the original BLA identified that the non-survivors that received raxibacumab had a greater rate and severity of central nervous system (CNS) disease than did placebo non-survivors. The reasons for this greater rate and severity of CNS disease as well as the potential relevance to humans were unclear.

Table 8: Histopathology Findings from Efficacy Studies in Animals

Organ/animal	Placebo	Raxi 20	Raxi 40	P-value (20 vs. p)*	p-value (40 vs. p)*
Cynomolgus macaques (as treated)					
Mortality	**12/12 (100%)**	**7/14 (50%)**	**5/14 (35.7%)**	**0.0064**	**0.0007**
CNS	**1/12 (8%)**	**6/7 (86%)**	**3/5 (60%)**	**0.002**	**0.05**
Liver	3/12 (25%)	1/7 (14%)	0/5 (0%)	0.397	0.324
Spleen	11/12 (92%)	5/7 (71%)	4/5 (80%)	0.26	0.441
Mediastinitis	10/11 (91%)	4/7 (57%)	4/5 (80%)	0.199	0.485
Pneumonia	5/12 (42%)	0/7 (0%)	1/5 (20%)	0.068	0.32
Nephritis	2/12 (17%)	0/7 (0%)	0/5 (0%)	0.386	0.485
NZW Rabbit (as treated)					
Mortality	**16/16 (100%)**	**12/17 (70.5%)**	**11/19 (57.9%)**	**0.0445**	**0.0038**
CNS	**2/16 (12%)**	**9/12 (75%)**	**6/11 (55%)**	**0.001**	**0.025**
Liver	6/16 (38%)	3/12 (25%)	3/11 (27%)	0.255	0.282
Spleen	11/16 (69%)	2/12 (17%)	2/11 (18%)	0.008	0.012
Mediastinitis	15/16 (94%)	7/12 (58%)	8/11 (73%)	0.034	0.150
Pneumonia	10/16 (63%)	0/12 (0)	0/11 (0)	0.001	0.001
Nephritis	4/16 (25%)	1/12 (8%)	2/11 (18%)	0.222	0.338
Cardiac	0/16 (0%)	0/12 (0)	2/11 (18%)	Ns	0.157

*** Fisher's exact test**

The Agency recommended HGS to conduct a study evaluating the effect of raxibacumab on the CNS in an animal model of inhalational anthrax and characterize the clinical course and histological appearance of the CNS lesions in survivors and non-survivors. The protocol for this study, submitted on May 4, 2010, was reviewed by FDA and comments regarding study design were communicated to the sponsor.

Inclusion criteria:

- Disease and malformation free New Zealand White (NZW) rabbits 50% male and 50% female weighing 2.7-4.3 kg supplied by (b) (4)
- Implanted vascular access ports and telemetry (temperature) transponder
- Quarantine period of 7 days

Exclusion criteria:

- Malformations

Reference ID: 3220416

- Clinical signs and symptoms of disease
- Outside approved weight range
- Veterinarian discretion

Treatments and Study Groups:

Table 9: Animal Allocation

	Number of Rabbits Planned	Dose	Route/Frequency	Treatment Point
Group 1 (placebo)	24	Placebo[1]	IV/single dose	Individual treatment times based on serum PA levels[2]
Group 2 (raxibacumab)	24	40 mg/kg	IV/single dose	

1 Raxibacumab buffer, administered at a dose equivalent in mL/kg volume to Group 2.
2 Each individual rabbit was to receive raxibacumab or placebo immediately upon its 1st positive PA result via qualitative ECL assay. If an animal was not treated by the 48 hour post-challenge time point (negative for PA by ECL), the animal was to be treated after the results of the 48 hour ECL assay were known regardless of the result

Study procedures: The study was conducted under GLP.

- Animals were housed in the individual cages.
- Study animals were randomized by treatment group and gender prior to challenge
- Study animals were randomize to one of two aerosol challenge days and a challenge order
- Aerosol challenge at targeted 200 x LD_{50} of *B. anthracis* Ames strain spores
- Temperature monitoring was done twice daily for 3 days prior to challenge until 28 days post challenge with the exception of every 6 hours between 18-168 hours post challenge
- Single dose IV study treatments were to be administered immediately upon positive PA result
- Clinical observations were done every 6 hours from 18 hours until 168 hours postexposure and twice daily thereafter
- Animals were euthanized if found moribund on the study or survived until the end of the study on Day 28 postexposure
- Necropsy and histopathology were conducted on all euthanized and all found dead animals in a blinded fashion
- Brains and spleens of all animals were subjected to immunohistochemistry for raxibacumab, glial fibrillary acidic protein (GFAP) to detect healed damage in survivors and for endogenous rabbit IgG
- Four brain sections that included cortex, cerebellum, hippocampus/thalamus, basal ganglia and corpus callosum were also subjected to Bielschowsky and Fluoro-Jade C stains to detect neuronal and neurofibrillary degeneration

Reference ID: 3220416

Table 10: Blood Collection and Assay Schedule

Time Point	Blood Tube Type/ Approximate Blood Volume	Bacteremia (Culture)	Plasma PA (screening assay)	Plasma PA (quantitative assay)	Raxibacumab PK	Immunogenicity and TNA
Day -6	EDTA ~2.0 ml	X	X	X	X	X
16hr PC	EDTA ~2.0 ml	X	X	X		
20hr PC	EDTA ~2.0 ml	X	X	X		
24hr PC	EDTA ~2.0 ml	X	X	X		
28hr PC	EDTA ~2.0 ml	X	X	X		
32hr PC	EDTA ~2.0 ml	X	X	X		
36hr PC	EDTA ~2.0 ml	X	X	X		
40hr PC	EDTA ~2.0 ml	X	X	X		
44hr PC	EDTA ~2.0 ml	X	X	X		
48hr PC	EDTA ~2.0 ml	X	X	X		
PTT	EDTA ~2.0 ml	X	X	X	X	
5min PTx	EDTA ~1.5 ml			X	X	
4hr PTx	EDTA ~1.5ml			X	X	
10hr PTx	EDTA ~1.5 ml			X	X	
24hr PTx	EDTA ~1.5 ml	X		X	X	
48hr PTx	EDTA ~1.5 ml	X		X	X	
72hr PTx	EDTA ~1.5 ml	X		X	X	
7 days PC	EDTA ~2.0 ml	X		X	X	
14 days PC	EDTA ~2.0 ml	X		X	X	
21 days PC	EDTA ~2.0 ml	X		X	X	
28 days PC	EDTA ~2.0 ml EDTA ~ 2.0 ml	X		X	X	X
Terminal	EDTA ~2.0 ml	X		X	X	

PC Post-Challenge; PTx Post-Treatment; PTT Prior to Treatment
Adapted from Sponsor's study report p.21

Raxibacumab PK and PA concentrations and TNA

The PK analyses of raxibacumab concentration-time profiles for all raxibacumab-dosed rabbits were conducted using population analysis techniques (mixed effect modeling), with the NONMEM software. Plasma raxibacumab concentration-time data, serum PA concentration-time data, individual raxibacumab PK parameters, and individual PA kinetic parameters were summarized using descriptive statistics. The TNA titers in plasma samples from surviving rabbits were determined using a cell killing assay.

Immunogenicity

Reference ID: 3220416

Plasma samples were assessed for anti-raxibacumab activity using a 2-part ECL-based bridging assay.

MIC
Minimal inhibitory concentrations (MICs) of levofloxacin were performed on *B. anthracis* challenge material and on positive cultures from terminal blood samples.

CSF
Cerebrospinal fluid (CSF) was collected from all rabbits prior to euthanasia (including survivors euthanized on study day 28) or on animals found dead, whenever possible. CSF was collected for culture, PA and raxibacumab concentration assessments.

Endpoints:
Primary efficacy endpoint was survival in the ITT study population on Day 28

Analysis Populations:
- ITT population is defined as all randomized rabbits that received the study agent

MO comment: *additional sensitivity analyses will be performed by the FDA in bacteremic population (animals that were bacteremic at the treatment onset).*

Statistical Plan:
The primary efficacy endpoint was 28-day survival, defined as the proportion of animals that survive to Day 28 after challenge. Based on previous experience (natural history of inhalational anthrax in NZW rabbits and results from the placebo arms in the previous efficacy studies), the sample size of 48 animals allows evaluation of a sufficient number of survivors (approximately 10 survivors). This design also provides 88% power to detect a survival benefit of 42% (10/24) in the raxibacumab-treated group compared to a 4% (1/24) survival rate in the placebo group. Histopathology data will be summarized using descriptive statistics.
The primary analysis of efficacy is to compare the percent of animals alive at study Day 28 post challenge between the placebo control group and the raxibacumab treatment group. The comparison will be performed using a 2-sided likelihood ratio test (or the Fisher's exact test, if any expected cell count in the contingency table is < 5). The secondary efficacy endpoint is animal's survival time from the initiation of spore challenge. Survival time will be compared between the placebo group and the raxibacumab treatment group using a log-rank test. Mortality will be recorded as it occurs to the nearest observation interval.

PROTOCOL CHANGES:
The original protocol was submitted and reviewed by the Division on October 10, 2010. Subsequently, the protocol was amended 4 times:

Amendment 1 (01 July 2010)
- Prioritizing testing of CSF samples in the following order: culture, PA and raxibacumab concentrations

Reference ID: 3220416

- Increasing frequency of clinical observations from 12 to every 6 hours between 18-168 hours post challenge
- Clarifications regarding brain tissue collection and staining

Amendment 2 (08 September 2010)

- Provision was added to euthanize the replacement animals to serve as negative controls for histological evaluation

Amendment 3 (17 January 2011)

- Unblinding of the treatment assignments will occur at the time of the completion of the in-life portion of the study to the study director, but not to the pathologist to allow analyses of raxibacumab PK and immunogenicity as well as efficacy to proceed while ensuring that the pathology evaluation will proceed in a blinded fashion.

Amendment 4 (27 July 2011)

- Clarifications were made to the location of the lab for immunohistochemistry, GFAP, raxibacumab and rabbit IgG at BBRC

RESULTS

Animal Disposition

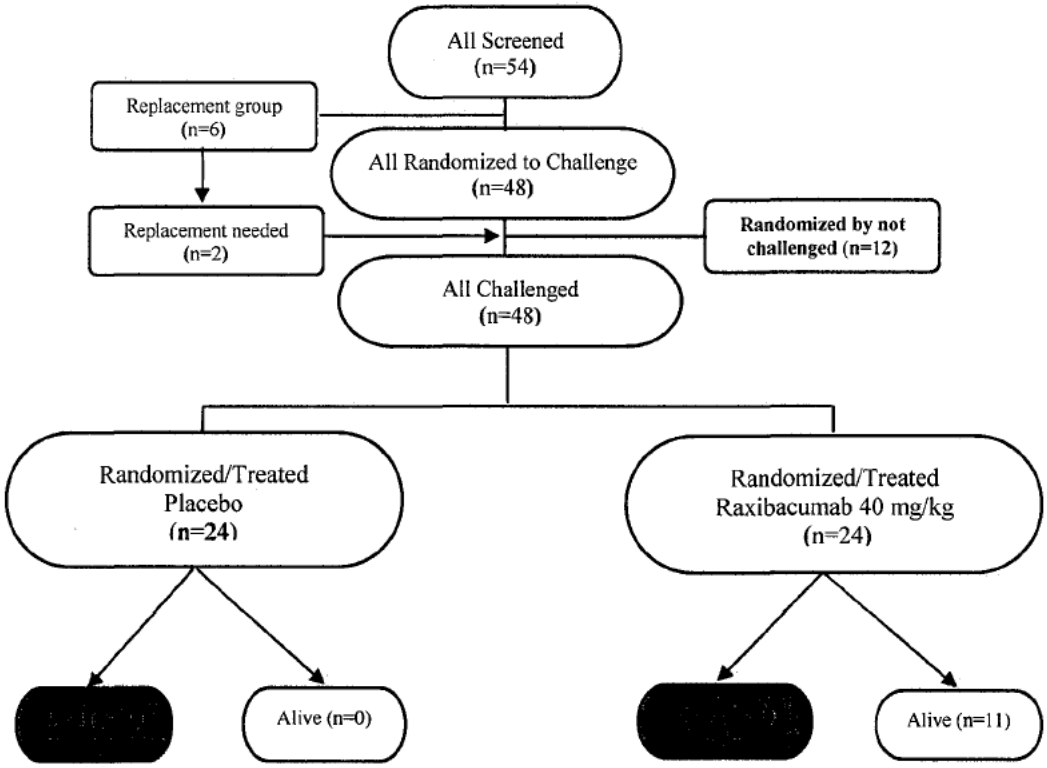
Study centers: 1 study center (b) (4)

A total of 54 animals were available for the study. Forty eight were initially selected and randomized, 2 of them were replaced due to health conditions unacceptable for the study. Forty eight animals received spore challenge. All rabbits received the treatment to which they were assigned.

The following flowchart and the tables summarize the composition of the populations for analysis, the primary reasons for exclusion and the demographics of the study population.

Reference ID: 3220416

Figure 1: Animal's Disposition



Two rabbits L35567 and L35572 were randomized, but not challenged, and were replaced by rabbits L35557 and L35569, respectively. Animal L35541 on raxibacumab arm, a survivor, did not have a positive bacteremia result prior to treatment or for the duration of the study. The animal, however, had developed a febrile response. It also had a positive PA screen assay result at 30 hours post challenge, although quantitative PA assay was negative for the duration of the study. In addition, there were no findings on gross necropsy of this animal suggestive of anthrax, while microscopic examination showed minimal inflammatory changes in the lymph nodes and spleen. This animal did not develop a TNA titer at the end of the study.

MO comment: *It appears that animal L35541 did not develop anthrax disease and did not respond to infection immunologically. The reviewer will conduct efficacy analyses with and without the inclusion on this animal.*

Reference ID: 3220416

Demographic and Baseline Characteristics

Table 11: Summary of Demographics and Baseline Characteristics (ITT Population)

	Placebo N = 24	Raxibacumab 40 mg/kg N = 24	P value[1]
Sex			1.0
Male	13 (54.2%)	13 (54.2%)	
Female	11 (45.8%)	11 (45.8%)	
Weight(kg)			0.37
Mean ± SD	3.4 ± 0.3	3.5 ± 0.3	
Median	3.3	3.4	
Range	(2.9, 4.0)	(2.9, 4.2)	
Age at randomization (months)			
Mean ± SD	7.7 ± 0.0	7.7 ± 0.0	
Median	7.7	7.7	
Range	(7.7, 7.7)	(7.7, 7.7)	
Toxemic at baseline	1 (4.2%)	1 (4.2%)	1.0
Bacteremic at baseline	0 (0.0%)	0 (0.0%)	

Adapted from Sponsor's study report p.29
2 P-value for comparison across 2 treatment groups obtained from likelihood ratio chi-square or Fisher's exact test for categorical data, and 1-way ANOVA for continuous data.
3 Toxemic is defined as detectable PA.

MO comment: *All study animals were the same age. Treatment groups had comparable demographic composition. One animal on each treatment arm found to have reached a low limit of quantitation on the quantitative ECL PA assay (0.56 ng/mL): L35553 in the raxibacumab treatment group registered PA levels at 0.542 ng/mL, while L35556 in the placebo treatment group had it recorded at 0.587 ng/mL). Neither animal was positive for PA by the qualitative screening assay. Both animals succumbed to anthrax.*

Extent of Anthrax Exposure

Table 12: Mean Spore Challenge Dose (LD_{50}) by Challenge Day and Treatment Group

	Mean Challenge Dose ± SD	Median	Range
Challenge Day			
A (n=24)	142 ± 45	144.0	70.0-240.0
B (n=24)	149 ± 36	151.0	77.0-222.0
Group			
Placebo (n=24)	138 ± 39	143.5	70.0-196.0
Raxibacumab (n=24)	154 ± 39	151.0	83.0-240.0
All animals (n=48)	146 ± 41	150.0	70.0-240.0

MO comment: *The challenge spore dose was close to 150x LD50 for the majority of animals in the study regardless of treatment assignment. Animals on the raxibacumab arm received an average challenge dose that was 16 LD_{50} higher than animals on placebo arm. (two-sided p value of 0.3472)*

Reference ID: 3220416

Clinical Observations

Abnormal clinical signs were first documented in the majority of animals at approximately 48-72 hours post-challenge. The most common abnormal clinical signs reported post-challenge were lethargy, loss of appetite, stool and respiratory abnormalities.
Twenty three of 24 (96%) placebo-treated animals and 8/13 (61.5%) raxibacumab-treated animals were found dead. A single placebo-treated animal L35556 was euthanized due to severe facial and neck swelling with impaired breathing. This animal was toxemic and bacteremic at the time of death. One raxibacumab treated animal L35562 had impaired hind limb use on Day 2 that became severe enough to warrant euthanasia on Day 3 per the staff veterinarian. This animal in addition to hind limb impairment exhibited loss of appetite, lethargy and respiratory abnormalities at 30-48 hours post exposure. The spore challenge dose of 93xLD_{50} was achieved in this animal; PA screening assay and blood culture became positive at 21 hours, while the treatment was initiated at 25 hours postexposure. At the time of treatment PA level was recorded at 33.3 ng/ml; it continued to rise to a maximum of 922 ng/ml at 10 hours post treatment then declined to 362 ng/ml at the time of euthanasia.
Three additional raxibacumab-treated animals: L35554, L35548 and L35561 were euthanized after ataxia or seizures were noted on Days 4-5 post challenge. They have received spore challenge doses 111-233 x LD_{50}. Neurologic symptoms were preceded by lethargy, inappetence, and respiratory abnormalities for 48-72 hours. These animals were toxemic and bacteremic prior to treatment. PA toxemia in these animals reached peak levels around 10 hours post treatment and declined steadily until prior to death. Animals were persistently bacteremic until prior to death.

MO comment: *Neurological signs were observed in these animals on Day 4-5 postexposure and approximately 3 days post treatment with raxibacumab. Histopathological examination revealed extensive hemorrhagic, inflammatory, and necrotic changes in the various parts of the brain. CSF PA and raxibacumab concentrations were not determined in these animals as no CSF was collected.*

Animal L35568 was euthanized 11 days post-challenge because of inappetence, no stool, and lethargy. This animal was treated with raxibacumab at 28 hours post-challenge after receiving 179 LD_{50}, had a positive PA assay at 22 hours and was found to be bacteremic at 26.5 hours. It was lethargic and inappetent with increased lacrimation from 36 to 72 hours post challenge. For the next 42 hours his symptoms seemed to have subsided. However, at 114 hours lethargy, lacrimation, and inappetence returned; respiratory abnormalities became evident, and animal met criteria for euthanasia on Day 11 postchallege. Gross necropsy was negative, and, in contrast to all other animals that died on study, in this animal microscopic findings were not consistent with anthrax infection: minimal hemorrhages in brain and minimal heterophilic inflammation in the mediastinal lymph nodes, but moderate chronic active nephritis, suggestive of other potential pathogens, e.g., *Encephalitozoon cuniculi* (as discussed in the report by the pathologist). Brain cultures and CSF PA assay in this animal were also negative. L35568 had the lowest CSF raxibacumab concentration, negative raxibacumab IHC staining results, and the longest time to

Reference ID: 3220416

death (263.7 hours) compared with other raxibacumab-treated rabbits that succumbed to anthrax. Upon review of all data collected for this animal, the study pathologist considers it more likely that this rabbit did not die of anthrax.

MO comment: *The reviewer will perform efficacy analyses with and without inclusion of animal L35568.*

Two raxibacumab-treated animals L35581 and L35566 were documented to have bilateral facial edema at a single time point during the study; both survived.
In raxibacumab-treated animals that survived to Day 28, most animals returned to normal by Day 7 with the exception of a few animals that demonstrated some hind limb issues classified as minor. Three animals L35530, L35531 and L35550 were noted to favor a limb following resolution of acute symptoms postexposure (Day 11, 12, and 17 respectively). Abnormal limb use was sporadic and resolved within a few days of onset. For 2 of the animals (L35530 and L35531) these limb issues resolved by Day 13 post challenge while abnormal limb observations for animal L35550 resolved on Day 18 post-challenge. L35550 was examined by veterinarian and noted to have a limb injury.

MO comment: *Pathology reports for the above animals did not detect any gross or microscopic abnormalities in the tissues examined, including brain.*

Temperature monitoring
All challenged animals demonstrated increases in body temperature on Days 2 though 5 following exposure to *B. anthracis*. In the majority of animals increased temperature lagged the appearance of PA or bacteremia. Two of 24 raxibacumab treated animals had febrile response prior to treatment initiation. On placebo arm, 3/24 animals had fever at the time of blinded intervention. In total, 34/48 animals developed febrile responses to infection at some point post-challenge: 18/24 on raxibacumab arm and 16/24 on the placebo.

MO comment: *Only 34/48 (71%) animals on the study developed febrile responses to infection and only 5/48 (10%) were febrile prior to treatment, in contrast to the previous rabbit monotherapy study 682, where all animals were febrile at some point post challenge with 65% of them febrile prior to treatment. In addition, average time to fever is almost 24 hours longer in this study compared to study 682.*

Table 13: Time to Fever (Temperature Elevation of >2F above Baseline)

Time to Fever	Placebo N = 24	Raxibacumab N = 24
Febrile animals	**16**	**18**
Mean ± SD (h)	35.3 ± 8.6	44.0 ± 15.7
Median	32.3	44.7
Range	**25.1 – 61.4**	**23.6 – 82.9**

Reference ID: 3220416

MO comment: Although in the TXTIMES dataset TMPstudy column specified that 14/48 animals did not meet criteria for fever during the study, the time to onset of fever (>2F above baseline on 2 consecutive measurements) was provided on TM2_TEMP column for all animals.

Bacteremia Results

All animals except for 1 on raxibacumab arm (L35541) and 2 on the placebo arm (L35543 and L35563) were found to be bacteremic prior to treatment administration. Animal L35541 remained negative for bacteremia following treatment. Two animals L35543 and L35563 on placebo arm were not bacteremic prior to blinded treatment administration; they became bacteremic at 24 hours post treatment time point.

MO comment: Animal L35531 on raxibacumab arm had positive blood culture at 36 hours postexposure, was missing a blood sample at the time of treatment administration (38h), but had positive blood culture at 24 and 48 hours after treatment administration. This reviewer considers Animal L35531 as being bacteremic prior to treatment.

The treatment intervention in this study occurred at the time of positive PA by screening ECL assay. Blood culture was collected at the predetermined time points similar to those for PA assay, but the results became available >24 hours after incubation. The following table examines time to first positive blood culture and its relationship to survival.

Table 14: Time to Bacteremia

Time to Bacteremia	Placebo N = 24	Raxibacumab N = 24		
		Non-survivors N=13	Survivors N=10[1]	Total N=23[1]
Mean ± SD (h)	31.4 ± 9.5	24.6 ± 3.4	25.3 ± 6.4	24.9 ± 4.8
Median	28.6	23.7	22.7	23.0
Range	**21.3 – 63.4**	**19.5 – 30.3**	**16.3– 36.7**	**16.3– 36.7**

1 Animal L35541 is excluded from the analyses as it had negative bacteremia results for the duration of the study

MO comment: Animals on raxibacumab arm had developed bacteremia on average 5 hours earlier than did the animals on placebo arm. There was no difference in the timing of bacteremia onset in survivors as compared to non-survivors.

The following table examines the timeline of survival in study arms and the pattern of bacteremia clearance post treatment.

Table 15: Positive Blood Cultures and Survival Timeline on the Study

	Placebo N = 24		Raxibacumab N = 24	
	Survivors (%)	Bacteremic (%)	Survivors (%)	Bacteremic (%)
Prior to treatment	24 (100.0%)	22 (91.6%)	24 (100.0%)	23 (95.8%)
24 h post tx	23 (95.8%)	19 (82.6%)	22 (91.6%)	19 (86.3%)
2 days post tx	10 (41.6%)	10 (100.0%)	19 (79.2%)	17 (89.5%)

Reference ID: 3220416

3 days post tx	3 (12.5%)	3 (100.0%)	15 (62.5%)	12 (80.0%)
7 days post challenge	0		12 (50.0%)	0
14 days post challenge	0		11 (45.8%)	0
21 days post challenge	0		11 (45.8%)	0
28 days post challenge	0		11 (45.8%)	0
	Non-survivors		**Non-survivors**	
Terminal	24 (100.0%)	23* (95.8%)	13 (54.2%)	11* (84.6%)

*Animal L35569 on placebo arm and L35560 and L35568 on raxibacumab arm had negative terminal culture

MO comment: *Bacteremia in raxibacumab-treated survivors resolved by Day 7 post challenge or 5-6 days post treatment. The majority of non-survivors had positive terminal blood culture results. Two raxibacumab-treated culture-negative non-survivors had no bacteria noted on the microscopic exam of the tissues postmortem. L35560 had inflammatory, necrotic and hemorrhagic changes in vital organs and was found to be IHC positive for raxibacumab in the brain, while L35568 had findings consistent with Encephalitozoon rather than anthrax. Placebo-treated culture-negative non-survivor L35569 had bacteria identified in all organs including brain.*

Toxemia Results

All animals had blood samples collected for PA toxemia at a predetermined schedule throughout the study. Treatment was administered after an animal became positive by qualitative screening ECL PA assay. In addition, the same blood sample was analyzed by a quantitative PA assay. All animals were positive for PA on ECL screening assay. Animal L35560 (raxibacumab arm) was treated based on positive screening assay even though suitability criteria for the assay plate were not met. The study director made a decision to treat the animal as there was a several fold increase in signal as compared to the previous time point. Also, quantitative PA in the same blood sample was positive in this animal.

Overall results between the screen and quantitative PA assays were concordant except for one animal L35541 where PA screening assay was positive while the quantitative PA assay was negative. This animal was never bacteremic, did not develop TNA titers and had normal findings on necropsy.

Table 16: Time to PA Toxemia

	Time to PA toxemia by ECL (quantitative)		**Time to PA toxemia by ECL (screen)**	
	Placebo *N = 24*	***Raxibacumab*** *N = 23*[1]	***Placebo*** *N = 24*	***Raxibacumab*** *N = 24*[2]
Mean ± SD (h)	27.2 ± 6.1	22.8 ± 5.1	27.8 ± 5.9	23.9 ± 5.1
Median	26.7	22.6	26.8	22.6
Range	**15.8 – 39.5**	**14.2 - 34.6**	**19.7 – 39.3**	**16.3 – 34.6**

1 One animal L35541 on raxibacumab arm was below LLOQ for PA quantitative assay
2 One animal L35560 on raxibacumab arm was treated based on a positive ECL screening assay even though suitability criteria for the original and repeat assay plate were not met

MO comment: *Animals receiving raxibacumab developed toxemia on average about 4 hours earlier than animals receiving placebo.*

Reference ID: 3220416

PA toxemia levels were documented in all animals in the study. The toxemia pattern in survivors, raxibacumab-treated non-survivors and placebo treated animals was similar to that observed in previous monotherapy studies: sharp rise, plateau, followed by a second rise in controls until death, while plateau was followed by toxemia decrease in treated animals. The duration of the plateau phase in treated non-survivors was longer as compared to placebo controls. The slope of toxemia decrease was much steeper in survivors compared to treated non-survivors.

Table 17: PA Toxemia Levels at Treatment and Death for Non Survivors

	Placebo N = 24		Raxibacumab N = 24	
	At treatment N=24	At death N=24	At treatment N=24[1]	At death N=13
N samples	24	16	24	12
Mean ± SD	27.3± 29.8	13836±15277	60.2± 70.0	818± 1208
Median	15.0	5789	31	338
(Min, Max)	3-125	122-42832	0-253	0-3545

1 One raxibacumab treated animal L35541 had quantitative PA level below limit of quantitation, even though qualitative screening ECL was positive

MO comment: *Raxibacumab-treated animals on average had higher levels of PA toxemia at the time of treatment administration as compared to placebo animals. However, at the time of death placebo non-survivors had PA toxemia that was several magnitudes higher than had non-survivors on the raxibacumab arm. In raxibacumab treated animals PA toxemia reached its peak by 10 hours posttreatment and declined steadily thereafter in survivors and 7/13 non-survivors. Six non-survivors on raxibacumab arm exhibited progressively worsening PA toxemia until death.*

Majority of animals were toxemic up to 3 days post treatment. Table below denotes the timeline of survival and PA positivity.

Table 18: Positive PA (Quantitative ECL) and Survival Timeline on the Study

	Placebo N = 24		Raxibacumab N = 24	
	Survivors (%)	Toxemic (%)	Survivors (%)	Toxemic (%)
Prior to treatment1	24 (100.0%)	24 (100%)	24 (100.0%)	23 (95.8%)
5 min post tx	24 (100.0%)	24 (100%)	24 (100.0%)	23 (95.8%)
4 h post tx	24 (100.0%)	24 (100%)	24 (100.0%)	23 (95.8%)
10 h post tx	24 (100.0%)	24 (100%)	23 (95.8%)	22 (95.6%)
24 h post tx	23 (95.8%)	23 (100%)	22 (91.6%)	21 (95.4%)
2 days post tx	10 (41.6%)	10 (100.0%)	19 (79.2%)	18 (94.7%)
3 days post tx	3 (12.5%)	3 (100.0%)	15 (62.5%)	14 (93.3%)
7 days post challenge	0		12 (50.0%)	6 (50%)
14 days post challenge	0		11 (45.8%)	0
21 days post challenge	0		11 (45.8%)	1 (9.1%)
28 days post challenge	0		11 (45.8%)	0

Reference ID: 3220416

Table 19: Positive PA (Quantitative ECL) Terminal Samples in Non-Survivors

	Placebo Non-survivors N=24 (100.0%)		Raxibacumab Non-survivors N=13 (54.2%)	
	N with Sample	**Toxemic (%)**	**N with Sample**	**Toxemic (%)**
Terminal Samples	16/24	16/16 (100%)	12/13	11/12 (91.7%)

MO comment: *The majority of raxibacumab-treated animals resolved PA toxemia by Day 14 post challenge. One raxibacumab-treated survivor L35551 had a low level PA noted in Day 21 blood sample that resolved a week later. This animal was TNA positive at Day 28. One animal (raxibacumab arm, L35568) had a negative PA result in the terminal blood sample. The study pathologist did not identify lesions consistent with anthrax as a cause of death in necropsy and microscopic examination of this animal.*

Therapeutic intervention in this study commenced at the time of positive PA by screening assay in each animal.

Onset of treatment

The treatment in this study occurred after individual animal were found to be toxemic by PA screening assay. The following tables examine the actual timing of treatment relative to PA positivity, onset of bacteremia and its relationship to survival.

Table 20: Time to Treatment Initiation

	Placebo N = 24	Raxibacumab N = 24
Mean ± SD (h)	32.3 ± 5.2	28.5 ± 4.7
Median	31.4	27.7
Range	24.8 - 42.6	20.7 - 38.3

MO comment: *The timing of treatment was earlier in raxibacumab treated animals as compared to those on placebo arm, p = 0.0122, driven by the differences in the onset of PA toxemia, p= 0.0122.*

Table 21: Time from Positive PA to Treatment

Time from diagnosis (PA ECL) to treatment (h)	Placebo N = 24	Raxibacumab N = 23	
All treated			
Mean ±SD	5.1±5.0	5.4±2.7	
Median	4.4	4.5	
Range	0-26.8	1.3-13.3	
Based on outcome		**Survivors N=10**	**Non-survivors N=13**
Mean ±SD		4.5±0.6	6.3±3.4
Median		4.4	5.0
Range		3.6-5.5	1.3-13.3

Reference ID: 3220416

***MO comment**: There were no differences between the study groups in time to treatment from the onset of detectable PA toxemia. Survivors had on average 1.8-hour earlier treatment onset after onset of bacteremia compared to raxibacumab treated non-survivors.*

Table 22: Time from Positive Blood Culture to Treatment

Time from diagnosis (Blood Culture) to treatment (h)	Placebo N = 22		Raxibacumab N = 23	
All treated				
Mean ±SD	3.2±2.6		3.4±2.5	
Median	3.4		4.4	
Range	0-11.4		0-7.8	
Based on outcome			**Survivors N=10**	**Non-survivors N=13**
Mean ±SD			2.4±2.5	4.1±2.3
Median			2.1	4.9
Range			0-5.5	0-7.8

***MO comment**: All but one animal in this study were blood culture positive prior to treatment. Some animals had sustained bacteremia after initial positive blood culture, some had positive cultures intermittently. Survivors had on average 1.7-hour earlier treatment onset after the first positive blood culture compared to non-survivors.*

Other Parameters Measured:

MIC
The *B. anthracis* MIC for levofloxacin was 1 μg/mL prior to challenge. The terminal MICs ranged from 0.5 to 1.0 μg/mL for raxibacumab animals and 0.5-2.0 μg/mL for placebo animals, demonstrating that the original MICs to this antibiotic did not change with raxibacumab treatment.

CSF Culture, PA and Raxibacumab
CSF was successfully collected from 65% (24/37) of the rabbits that died on study and all (11/11) of the rabbits that survived to day 28 post-challenge.
One hundred percent (19/19) of the placebo-treated animals for which a CSF sample could be collected had positive CSF bacterial culture result. For raxibacumab-treated non-survivors for which a CSF sample could be collected, 80% (4/5) had a positive bacterial culture result whereas none (0/11) of the raxibacumab-treated survivors had a positive bacterial culture result from CSF obtained prior to sacrifice (Day 28). Animal L35568 was the sole raxibacumab treated non-survivor that had negative CSF culture result.

Concentrations of PA in CSF were variable in animals of both treatment groups, ranging from not detectable to > 966 ng/mL. These CSF PA concentrations were 0 -37% of plasma PA concentrations in raxibacumab-treated non-survivors in whom CSF to plasma concentration ratios were calculated. Rabbit L35562 on raxibacumab arm had a CSF PA concentration nearly 7-fold higher than the concurrent plasma PA concentration.

Reference ID: 3220416

Table 23: CSF PA in Non-Survivors

Terminal CSF PA concentration	Placebo N = 24	Raxibacumab N = 13
Evaluable samples	17	4
Mean ±SD	201±289	748±1148
Range	0-966	0-2439

MO comment: Raxibacumab treated non-survivors had higher on average levels of CSF PA as compared to placebo non-survivors, indicating more pronounced CNS infection at the time of death.

No animals in the placebo group had measurable raxibacumab in the collected CSF samples. In 4/13 non-survivors from the raxibacumab group, CSF raxibacumab concentrations were measured (due to inability to collect sample from all animals), and ranged from < 1 μg/mL to 545 μg/mL representing 0.2% - 88% of the concurrent plasma raxibacumab concentration. When the highest CSF raxibacumab concentration (545 μg/mL for rabbit L35559) is excluded, less than 2% of plasma circulating raxibacumab reached CSF. For comparison, CSF raxibacumab concentrations for 11 rabbits that survived ranged from not detectable to 0.331μg/mL. For the only surviving rabbit for whom the CSF to plasma raxibacumab concentration ratio could be determined, the CSF level was <2% of the concurrent plasma raxibacumab concentration. Overall there was little distribution of raxibacumab into CSF, with the exception of 1 rabbit that died (L35559).

MO comment: Interesting to note that animal L35559 with the highest raxibacumab concentration in the CSF did not have any gross or microscopic findings in the CNS despite positive brain immunohistochemistry staining for raxibacumab. It appeared to have died from severe necrotizing mediastinitis.

Three raxibacumab treated survivors L35581, L35566, and L35532 had terminal CSF samples positive for raxibacumab. Two of them (L35566 and L35532) have cleared raxibacumab from plasma prior to study Day 28.
L35581 a survivor with CSF positive for raxibacumab had the following clinical course: after a challenge dose of 197xLD_{50}, this animal was found to be toxemic and bacteremic at 23 hours post challenge, received treatment at 28 hours post challenge; febrile response in this animal was registered at 47 hours post challenge and lasted 36 hours with no other notable clinical observations; the animal had an uneventful recovery. Gross necropsy was unremarkable; however, on microscopic examination this animal had lymphoid hyperplasia in the spleen and minimal neuronal necrosis in hippocampus identified only based on positivity of Fluoro-Jade staining for acute neuronal degeneration. Positive IHC staining for raxibacumab was only intravascular. This animal was bacteremic until Day 4 post challenge. Detectable PA toxemia was last detected on Day 7 post challenge.
Animal L35566 was found to have minimal hemorrhage of meninges and brain tissue as well as minimal-mild lymphoid hyperplasia of lymph nodes and spleen present on microscopic

Reference ID: 3220416

examination. This animal was challenged with 151 x LD_{50} and developed detectable PA toxemia and bacteremia at 18 and 22 hours post challenge, respectively. This animal was treated at 22 hours and resolved toxemia and bacteremia by Day 7 post challenge.
On histopathologic examination of animal L35532 notable findings related to anthrax included minimal inflammatory changes in spleen and lymph nodes. Brain examination revealed normal findings. Incidental embryonal nephroma was found on kidney examination. This animal was challenged with 130 x LD_{50} and developed detectable PA toxemia and bacteremia at 33 and 36 hours post challenge, respectively. This animal was treated at 36 hours and resolved toxemia and bacteremia by Day 7 post challenge.

MO comment: *Raxibacumab penetration into CSF in three animals above was likely related to increase blood-brain barrier permeability during systemic infection. Animals L35532 and L35566 did not have positive raxibacumab serum concentration at the time of terminal CSF sampling and therefore false positive results due to blood contamination is not possible. Findings of resolving inflammation and hemorrhage in the brain of surviving animals treated with raxibacumab are reassuring as they suggest resolution of meningitis. Direct toxicity of raxibacumab is unlikely, particularly in the absence of positive immunohistochemistry brain staining for raxibacumab in the animals that had detectable levels of raxibacumab in the CSF. It is important to note that in the majority of raxibacumab non-survivors brain immunohistochemistry for raxibacumab was positive, but only 3 animals has concurrent positive CSF samples. There were no raxibacumab-treated non-survivors that had positive CSF raxibacumab in the absence of positive brain immunohistochemistry. This finding indicates that raxibacumab penetration into CSF likely follows meningeal seeding with bacteria. In raxibacumab-treated animals that succumbed to anthrax, low levels of raxibacumab in the CSF were not sufficient to clear the infection, while high levels noted in one non-survivor likely precluded development of significant pathology in the brain.*

Anti-raxibacumab antibody levels and TNA
Anti-raxibacumab antibody levels and TNA post-challenge were collected during the study: at screening and on Day 28 post challenge in survivors.
The plasma raxibacumab concentrations were similar among the survivor/non-survivor and antibody positive/negative subgroups during the first 6 days after raxibacumab administration. Thereafter, raxibacumab concentrations were very low or not detected in the rabbits that were positive for anti-raxibacumab antibodies compared with the sustained raxibacumab exposure in rabbits that were anti-raxibacumab antibody negative.

Table 24: Immunogenicity (Anti-Raxibacumab Antibodies)

	Placebo N=24	Raxibacumab N = 24	
	Pretreatment	Pretreatment	Day 28
Evaluable samples	24	24	11
Positive	3	1[1]	8

1 animal L35530 was positive for anti-raxibacumab antibodies both pre and post treatment with raxibacumab

Reference ID: 3220416

MO comment: Presence of anti-raxibacumab antibodies pretreatment does not appear to have an effect on survival of the raxibacumab treated animals. Their presence possibly resulted in an increased raxibacumab clearance. Among three raxibacumab-treated survivors positive for raxibacumab in Day 28 CSF samples, two (L35532 and L35566) were found to have developed anti-raxibacumab antibodies

TNA titers at Day 28 were only determined for rabbits that survived. One animal L35541 did not develop a TNA titer. This animal was febrile during the study; however, it was not toxemic by quantitative PA ELISA and did not have bacteremia.

Table 25: TNA Titers in Surviving Rabbits

	Raxibacumab N=24	
	Pretreatment	**Day 28**
Evaluable samples	11	11
Mean ±SD	0±0	2175±1870

Adapted from the sponsor's study report p.49

MO comment: Surviving animals were able to mount an immune response to anthrax in the presence of raxibacumab.

All animals that survived were monitored for 28 days post-challenge. Clinical signs and symptoms of anthrax, including body temperature elevations, resolved following resolution of bacteremia. Animals exhibited normal baseline behavior at the time of study termination. Animals found moribund were euthanized. Complete necropsies were performed on all animals, including those that died and those surviving to terminal sacrifice on Day 28 postexposure.

Pathology, Gross and Microscopic

Complete necropsies were performed on animals found dead or euthanized, including the animals surviving to terminal sacrifice on Day 28. Gross lesions in the rabbits found dead or sacrificed moribund included discoloration or foci in the appendix and brain, enlargement (with or without discoloration) of bronchial and mediastinal lymph nodes, and fluid in the body cavities and thymus. These gross lesions were considered typical of anthrax in rabbits and correlated histologically with hemorrhage, necrosis, edema, and/or acute inflammation. Lesions of the nervous system were more commonly observed in raxibacumab-treated animals than in placebo-treated animals. Brain inflammation was more common in raxibacumab-treated animals, and localized to the cerebral cortex, hippocampus, ventricular system, cerebellum, and meninges. Neuronal necrosis was also more common in raxibacumab-treated animals, and was more pronounced the cerebral cortex and cerebellum. Cerebral vasculitis was more common in raxibacumab-treated animals. Although hemorrhage was present in the brain of approximately the same number of rabbits in each group, hemorrhage was especially notable in the hippocampus, cerebellum, cerebral cortex and meninges of raxibacumab-treated animals.

Reference ID: 3220416

In the placebo treatment group lesions in all organs, except the brain, were more commonly observed and generally with greater severity than they were in raxibacumab-treated non-survivors.
Among raxibacumab-treated rabbits surviving to study termination on Study Day 28, several lesions expected in animals recovering from sepsis were identified and included inflammation, increased macrophage numbers, and lymphoid hyperplasia in lymph nodes and spleen, and chronic or chronic-active inflammation in the lungs. Acute hemorrhage in the brain (as an agonal/perimortem change in the absence of other brain lesions), acute renal inflammation and tubulo-interstitial nephritis, hemorrhage and edema in lymph nodes, and splenic hemosiderosis were considered to be incidental findings. All of the microscopic findings in the raxibacumab-treated survivors were graded as minimal to mild, except 1 instance each of hemosiderosis and of hyperplasia in the spleen which were graded as moderate.

MO comment: *Histopathologic findings of increased inflammatory changes along with elevated bacterial load in the brains of raxibacumab-treated non-survivors relative to non-survivors in the placebo groups were consistent with the findings on microscopic examination from the original monotherapy studies in rabbits and cynomolgus macaques. Taken together with the raxibacumab penetration data and the immunohistochemistry staining patterns for raxibacumab and endogenous rabbit IgG, these findings are most likely indicative of increased permeability of the blood brain barrier in the raxibacumab-treated animals and insufficient levels of the product to eliminate the infection. Exaggerated inflammatory response in these animals relative to placebo remains unexplained with a potential hypothetical mechanism of partial inhibition of lethal toxin immunosuppressive effects within the confined space of the CNS.*
However, it is also reassuring that survivors were devoid of significant brain pathology. In addition, it is reassuring to know that raxibacumab penetration into CSF in some survivors, most likely the result of blood brain barrier leak, was not detrimental and resulted in CSF and brain tissue clear of B. anthracis.

Table 26: Incidence of Pathology Findings on Necropsy

Pathology Findings/Necropsized Animals	**Placebo N=24 (%)**	**Raxibacumab N=24 (%)**		**Negative controls N=3**
Study outcome	**Died**	**Died**	**Lived**	**Lived**
	24 (100)	**13 (54.2)**	**11 (45.8)**	**3**
Brain (gross brain pathology and/or histopathology+ bacteria)	12/23[1] (52)	10 (77)	2 (18)	0
Kidney	19 (79)	5 (38)	2 (18)	0
Liver	21 (88)	8 (62)	0 (0)	0
Bronchial/Mediastinal Lymph Nodes	24 (100)	13 (100)	8 (73)	2 (67)
Lung	22 (92)	7 (54)	3 (27)	1 (33)
Spleen	23 (96)	9 (70)	7 (64)	2 (67)

[1] Brain sections from 1 placebo animal were not examined microscopically

MO comment: *Among rabbits that received raxibacumab, fewer organs contained visible bacteria than was seen in placebo rabbits. However, a higher proportion of animals that*

Reference ID: 3220416

succumbed to anthrax in the raxibacumab-treated group had brain pathology findings (77%) as compared to placebo (52%).

The reviewer applied a consistent method to compare histopathology findings between groups and between studies. The main focus as in the previous BLA submission animal efficacy studies **review was on "high grade" lesions. The reviewer** applied the following grading criteria:

- Criteria for High grade
  - Bacteria, hemorrhage, inflammation > grade 2
  - Presence of necrosis regardless of grade of bacteria, hemorrhage, inflammation
- Criteria for Low grade
  - Bacteria, hemorrhage, inflammation 0-2, necrosis 0

Table 27: Incidence of High Grade Lesions in Treated Animals

	Placebo N=24 (%)	Raxibacumab N=24 (%)		Negative Controls N=3 (%)
		Died N=13	Survivors N=11	Survivors N=3
Brain	1/23[1] (4)	9 (69)	1/11 (10)	0
Lung	12 (50)	3 (23)	0	0
Mediastinum (LN)	24 (100)	10 (77)	0	0
Liver	13 (54)	8 (62)	0	0
Spleen	22 (92)	7 (54)	0	0
Kidney	5 (21)	2 (15)	0	0

1 brain of animal L35552 was not available for microscopic examination. Grossly it was unremarkable

MO comment: *All animals that died and were necropsized had either gross or histopathology findings or both consistent with disseminated anthrax infection. Only in one non-survivor L35568) the pathologist did not document presence of the bacteria in the organs examined. Animal L35568 gross and microscopic exam findings were consistent with a common rabbit infection rather than anthrax.*

Below presented is reviewer's finding on the incidence of minimal-mild (grade 0-1) and moderate-severe pathology findings in the brains of deceased animals.

Table 28: CNS Histopathology Results by Treatment Group and Survival Status

		Placebo N=23[1] (%)	Raxibacumab Non-survivors N=13 (%)	Raxibacumab Survivors N=11 (%)
CNS Bacteria	**Grade 0**	14 (61)	6 (46)	0
	Grade 1	7 (30)	0 (0)	0
	Grade 2	1 (4)	3 (23)	0
	Grade 3-4	1 (4)	4 (31)	0
Inflammation	**Grade 0**	21 (91)	5 (39)	0
	Grade 1	2 (9)	1 (7)	0
	Grade 2	0 (0)	2 (15)	0
	Grade 3-4	0 (0)	5 (39)	0
Hemorrhage	**Grade 0**	12 (52)	4 (31)	0

Reference ID: 3220416

	Grade 1	10 (44)	0 (0)	0
	Grade 2	0 (0)	7 (54)	1 (9)
	Grade 3-4	1 (4)	2 (15)	0
Necrosis	**Grade 0**	22 (96)	6 (46)	0
	Grade 1	1 (4)	3 (23)	1 (9)
	Grade 2	0 (0)	3 (23)	0
	Grade 3-4	0 (0)	1 (7)	0

1 one placebo animal L35552 had missing brain tissue for microscopic examination

MO comment: *Moderate-severe brain pathology was more common in deceased animals treated with raxibacumab compared to those treated with placebo.*

The time to death for the non-survivors was not different between the study groups (raxibacumab group median = 89 hours, and in the placebo group median = 84 hours). However, among raxibacumab non-survivors the average time to death in rabbits with moderate-severe inflammation in the brain was 95 hours compared with 46 hours in rabbits that had no to minimal pathology findings in the brain, suggesting that the increased survival time allowed for additional pathology to develop. This finding is consistent with the observations in the original monotherapy studies in NZW rabbits and cynomolgus macaques.

Table 29: Brain Pathology and Time to Death (Hours)

Time to Death (hours)	**Placebo**	**Raxibacumab 40mg/kg**	**All non-survivors**
All treated animals died on the study	**N=24**	**N=13**	**N=37**
Mean	90.8 ± 22.6	95.9 ± 57.9	89.5 ± 37.7
Median	80.1	88.5	82.2
Min-Max	**65 – 142**	**41 - 264**	**41 - 264**
Animals with High Grade Brain Pathology on necropsy	**N=1**	**N=9**	**N=10**
Mean	73.1	94.0 ± 23.1	91.9 ± 22.8
Median	73.1	98.3	93.4
Min-Max	73.1	54 - 122	54 - 122
Animals without Brain Pathology on necropsy	**N=11**	**N=3***	**N=14**
Mean	90.8 ± 22.6	45.5 ± 8.3	81.1 ± 27.9
Median	83.9	40.8	78.6
Min-Max	**65 – 142**	**41 - 55**	**41 - 142**

MO comment: *There is a significant overlap of time-to-death ranges between raxibacumab-treated and placebo-treated non-survivors. Time to death is also similar between animals with high grade brain lesions and those without. However, within the raxibacumab group of non-survivors, animals with high grade brain lesions had longer mean and median time to death. Although plausible, it does not appear that just the duration of survival alone could account for greater pathologic findings in the brain of animals treated with raxibacumab versus placebo.*

Reference ID: 3220416

Raxibacumab and IgG Immunohistochemistry (IHC), Brain

Brains sections of survivors and non-survivors as well as of 3 unchallenged negative controls were evaluated for the presence of raxibacumab by IHC staining. These sections were also evaluated for the presence of endogenous rabbit immunoglobulin to aid in interpretation of results of the raxibacumab IHC.

Among unchallenged rabbits, plasma was moderately to strongly positive for endogenous IgG, neuropil ranged from unstained to mild in patches, and choroidal collagen was moderately positive. Cytoplasmic staining was present among occasional members of all cell types observed in the brain (astrocytes, choroid plexus epithelium, endothelial cells, ependymal cells, meninges, neurons, oligodendroglia, and intravascular heterophils) except for microglial cells. Staining was usually greatest in endothelial cells.

Spore-challenged rabbits with inflammation in the brain exhibited greater frequency and intensity of IgG staining than spore-challenged rabbits with no hemorrhage, inflammation, or necrosis in the brain. There was a good concordance of positive IgG staining and presence of inflammation and/or necrosis in brain lesions detected on H&E staining (9/10 animals).

Among non-survivors that had no diagnoses of hemorrhage, inflammation, or necrosis, raxibacumab-treated animals appeared to exhibit slightly more IgG staining in the choroid plexus, endothelium, and neuropil, suggesting greater permeability of the blood-brain barrier than in placebo-treated rabbits. However, staining was greater for placebo-treated animals in meninges and oligodendroglia.

IHC for raxibacumab was positive only in rabbits treated with raxibacumab. The staining pattern was identical to that for endogenous IgG in that plasma was moderately to strongly positive among non-survivors, choroid collagen was frequently more positive than was epithelium, and neuropil exhibited a range of staining from negative (among survivors) to strongly positive in rabbits with abundant lesions. Similarly, cytoplasm was positive occasionally in all cell types. As for endogenous IgG, rabbits with the most severe and widespread brain lesions also had the most intense and widespread staining for raxibacumab in neurons and neuropil and staining was often associated with presence of bacteria and/or inflammation. Among raxibacumab-treated rabbits surviving to Day 28, 3 (L35581, L35530, and L35531) had positive raxibacumab staining of plasma, although in 2 of these (L35530 and L35531) the staining was weak, suggesting the gradual loss of raxibacumab in circulation over time, as is confirmed by the raxibacumab PK results.

Strong correlation between microscopic brain lesions and frequency, location, and intensity of raxibacumab and endogenous rabbit IgG staining of the brain was observed in non-surviving raxibacumab treated animals. An overlapping pattern of endogenous IgG and raxibacumab staining of neurons and neuropil was often observed in regions rich with bacteria, inflammation, and/or necrosis.

MO comment: *Infection, circulating bacterial toxins, and/or inflammation (including sepsis) has been shown to enhance blood brain barrier (BBB) permeability. The pattern of ICH brain staining revealed a co-localization of raxibacumab and endogenous IgG, suggesting a non-specific leak of plasma across the damaged blood brain barrier during bacterial meningitis in the raxibacumab group. Only one placebo animal (#L35576) with severe meningitis also had positive IgG brain staining, suggesting compromise of the blood brain barrier sufficient for IgG*

Reference ID: 3220416

and raxibacumab penetration did not generally occur in the placebo group. Surviving animals also may have experienced an alteration in blood brain barrier permeability early in the disease, as greater endogenous IgG positive staining of vascular endothelium, meninges, and neuropil was observed in raxibacumab treated survivors compared to unchallenged controls. However, there was no raxibacumab staining of brain tissue in the surviving animals.

Other stains
Bielschowsky's stain was used to detect neuritic plaques, i.e, regions where chronic damage to axons or dendrites resulted in aberrant neurite outgrowth. No staining was detected in any study samples, as anthrax-related CNS lesions are typically acute.

Fluoro-Jade C often confirmed acute neuronal degeneration/necrosis in the same regions where acute neuronal necrosis was noted on H&E-stained sections. In a single case of a raxibacumab-treated survivor (L35581), a diagnosis of neuronal degeneration/necrosis was made only on the basis of Fluoro-Jade C without coincident H&E staining.

Efficacy results
The primary efficacy endpoint was survival at Day 28 post challenge. Raxibacumab was superior to placebo for the primary endpoint in the ITT population and in additional sensitivity analyses.

Table 29: Primary Efficacy Analyses

Study population	Placebo	Raxibacumab
Primary analysis ITT population		
	N = 24	**N=24**
Survival, n (%)	**0 (0)**	**11 (45.8)**
Difference (95% CI)	**45.8 (25.3, 67.2)**	
P value*	0.0002	
Modified ITT(toxemic animals)		
	N=24	**N=23**
Survival, n (%)	**0 (0)**	**10 (43.5)**
Difference (95% CI)	**43.5 (22.9 , 65.5)**	
P value*	0.0002	
Modified ITT (bacteremic animals)		
	N=22	**N=23**
Survival, n (%)	**0 (0)**	**10 (43.5)**
Difference (95% CI)	**43.5 (22.5 , 65.5)**	
P value*	0.0006	
Modified ITT (animal died from non anthrax causes excluded)		
	N=24	**N=23**
Survival, n (%)	**0 (0)**	**11 (47.8)**
Difference (95% CI)	**47.8 (26.6 , 69.4)**	
P value*	0.0001	

*P-values are based on two-sided Fisher's exact test for comparisons between the treatment and placebo

Reference ID: 3220416

MO comment: *ITT population above represents animals that were randomized, challenged, and received treatment. All ITT animals were toxemic by screening ECL prior to treatment administration.*

Secondary efficacy analysis, Survival Time
The sponsor reported a greater time of survival in animals both toxemic/bacteremic at treatment initiation with a median time to death (TTD) of 3.3 days for placebo versus 8.0 days (p-values ranging from 0.0002 to 0.0007) for the raxibacumab group (see Figure below).

Figure 2: Survival Time During 28-day Study Period in Rabbits Toxemic and/or Bacteremic at Treatment Initiation

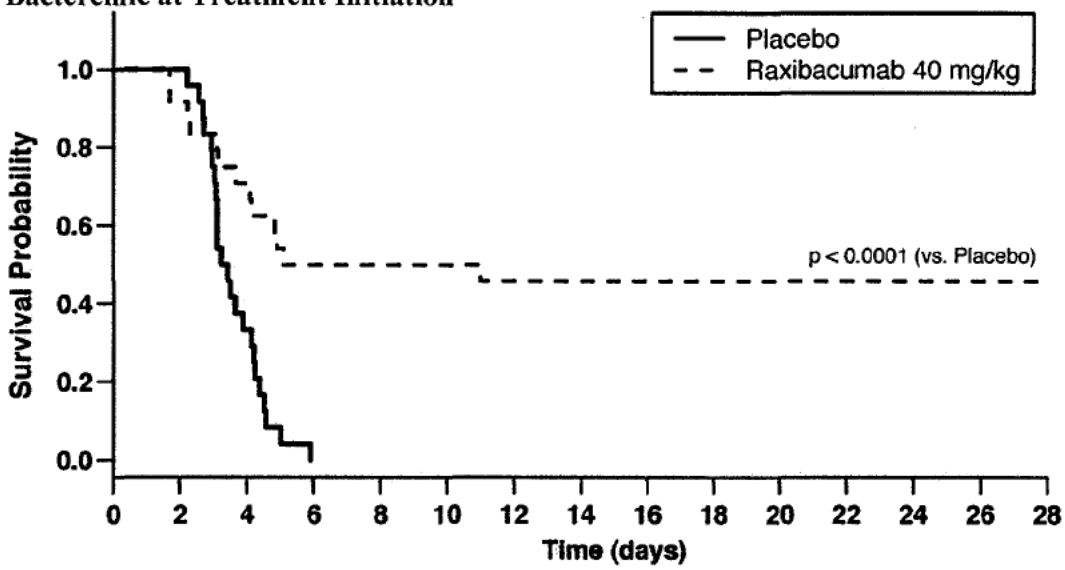
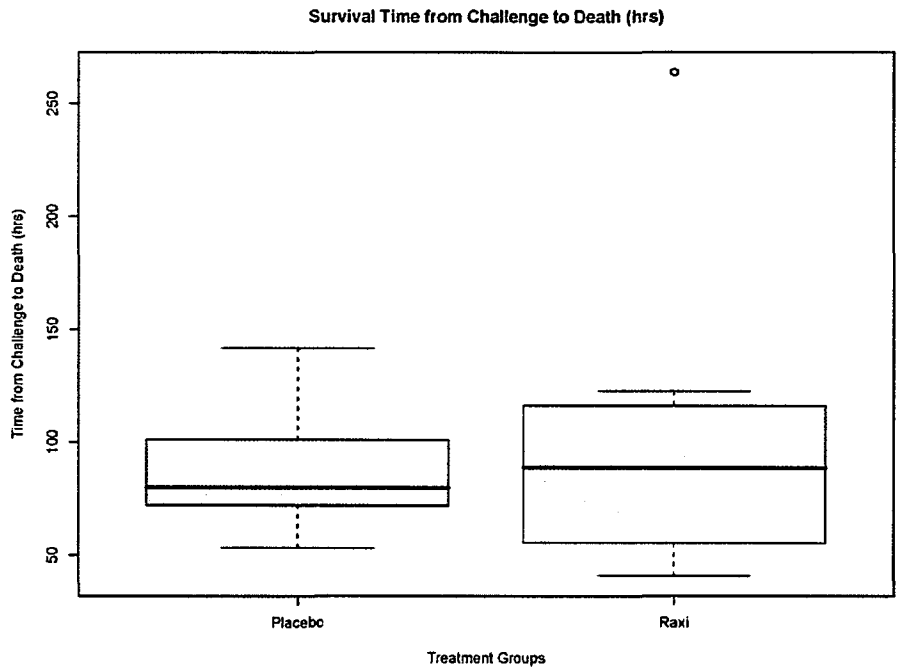


Figure 7-3 from HGS report p. 35
P-value obtained from a log-rank test comparing survival time between placebo and raxibacumab-treated groups.

The analysis of survival time performed by statistical reviewer, Dr. Lan Zeng, showed no significant difference in mean TTD post-challenge in raxibacumab and placebo treated non-survivors (mean 3.99 versus 3.59 days, respectively), with an approximate difference of only 9.7 hours. (Note: The TTD recorded is an estimate of the actual TTD post-challenge, since a majority of animals that succumbed to disease in both groups were found dead.) The figure below shows the distribution of TTD post-challenge for animals that died in the placebo and raxibacumab groups. The raxibacumab animal (#L35568) that died at 263.7 hours after anthrax challenge did not have extravascular bacteria and the cause of death was deemed unlikely to be anthrax related [note: the study pathologist indicated possible renal infection of a parasitic fungi (*Encephalitozoon cuniculi*)].

Reference ID: 3220416

Figure 3: Time from Challenge to Death in Non-Survivors



Courtesy of statistical reviewer Dr. Lan Zeng

Efficacy conclusions:

- Raxibacumab at 40 mg/kg IV bolus dose was superior to placebo in preventing inhalational anthrax induced mortality in ITT population resulting in survival of 45.8% of treated animals as compared to placebo 0% (p=0.0001).
- In the sensitivity analyses of efficacy in the populations of animals bacteremic prior to treatment or toxemic prior to treatment, raxibacumab was superior to placebo in preventing inhalational anthrax induced mortality with survival of 43.5% versus 0% on placebo arm, p=0.0006 (bacteremic animals) and p=0.0002 (toxemic animals).

Safety conclusions:

- Raxibacumab at 40mg/kg IV offered a statistically significant survival benefit to the NZW rabbits with established systemic inhalational anthrax disease evident by bacteremia and toxemia prior to therapeutic intervention.
- Penetration of raxibacumab into CSF is minimal (<2%) in the absence of meningitis. Low levels of raxibacumab in CSF were present in both survivors and non-survivors in this study. High CSF levels of raxibacumab were associated with absence of pathological changes in the CNS.
- Frequent and more extensive findings of CNS pathology in raxibacumab-treated animals on necropsy as compared to placebo are possibly associated with inability of raxibacumab

Reference ID: 3220416

achieve adequate levels in CNS to control bacterial spread in raxibacumab treated animals. However, the precise mechanism for exaggerated inflammatory response in the CNS of raxibacumab treated non-survivors remains unclear. This pathology finding should be described in the resultant label of raxibacumab if approved. Studies of lethal toxin suggest that subinhibitory concentrations of raxibacumab within the CNS might have resulted in reversal of lethal toxin-induced immunosuppression and localized findings of immuno-reconstitution.

- Co-localization of endogenous IgG and raxibacumab within the brain of raxibacumab-treated animals together with negative brain tissue cross reactivity studies suggest that direct toxic effect of raxibacumab on CNS is unlikely.

4.3.4 Nonclinical Study 1141-CG920871 Added Benefit of Raxibacumab with Levofloxacin vs. Levofloxacin as Post-exposure Treatment in the New Zealand White Rabbit Inhalational Anthrax Model

INVESTIGATOR: Gabriel Meister, PhD

STUDY CENTER: Battelle Biomedical Research Center (BBRC)

STUDY PERIOD: 10 January 2010 to 15 May 2012

OBJECTIVES:
Primary objective:

- To evaluate the added benefit against lethality of raxibacumab treatment combined with levofloxacin treatment compared with levofloxacin treatment alone, when administered at a predetermined time-point following inhalational exposure to *B. anthracis* in NZW rabbits.

METHODOLOGY: Parallel-group, randomized, blinded, single center study to evaluate efficacy of raxibacumab combined with levofloxacin compared to levofloxacin alone as a therapeutic treatment of inhalational anthrax in NZW rabbits.

NUMBER OF ANIMALS: Two cohorts of 90 animals each, shipped on 3 separate days, each shipment group randomized to 1 of 2 challenge days, survivors randomized to treatment or control at 84 hours post exposure. Total enrollment was 209 New Zealand White rabbits (180 required and 29 replacements).

STUDY DRUG: IV raxibacumab given as a single bolus infusion at a 40 mg/kg dose. Levofloxacin at 50 mg/kg gavage dose was administered to animals 1 dose at the time of raxibacumab infusion, followed by 2 additional daily doses 24 hours apart.

Reference ID: 3220416

Table 30: The Appearance, Formulation, and Dosage Strength of the Study Drug, Study 1141

Study Drugs	Appearance	Formulation	Dose Unit	Batch numbers
Raxibacumab	Clear liquid	Raxibacumab sterile solution for injection	50mg/ml	71128
Placebo (raxibacumab formulation buffer)	Clear liquid	n/a	n/a	71043
Levaquin Oral Solution	Clear liquid	Oral solution	25mg/ml	AEB2V00

MO comment: *The test article was raxibacumab (Lot #71128). Vials were labeled 1-96 according to the randomization scheme. The levofloxacin dose of 50 mg/kg once daily in NZW rabbits results in exposures (Cmax 8.7 mcg/mL and AUC 66.7 mcg*h/mL) similar to that achieved at human doses of 500-750 mg daily (Cmax 5.1-9.3 mcg/mL and AUC 47.9-101 mcg*h/mL).*

DURATION OF TREATMENT: Blinded raxibacumab treatment given as a single IV bolus dose of 40 mg/kg to NZW rabbits at 84 hours post exposure immediately after the first dose of levofloxacin. Levofloxacin was administered by oral gavage for 3 days starting at 84 hours post exposure.

TREATMENT COMPLIANCE: Study drug infusion information, including dosage and volume prescribed, and volume administered, was recorded.

STUDY DESIGN: Parallel-group, randomized, blinded, single center study to evaluate efficacy of raxibacumab combined with levofloxacin compared to levofloxacin alone as a therapeutic treatment of inhalational anthrax in NZW rabbits at 84 hours post exposure.

Background: To date the sponsor has conducted evaluation of raxibacumab in several animal species and under various conditions. Efficacy of raxibacumab was evaluated in a proof-of concept rat model of Lethal Toxin infusion in pre-exposure and post-exposure setting. In addition, when used for pre-exposure prophylaxis raxibacumab showed dose-dependent survival benefit in rabbits and monkeys. Time-related effect on survival was also observed in a post-exposure prophylaxis setting in a rabbit model. Monotherapy studies of raxibacumab administered to rabbits and cynomolgus macaques at the time of diagnosis of inhalational anthrax disease showed a statistically significant survival benefit relative to placebo treatment. Studies evaluating raxibacumab with an antibacterial relative to antibacterial alone in NZW rabbits and cynomolgus macaques demonstrated near 100 % survival in both treatment groups when therapeutic intervention was administered at the time of inhalational anthrax disease diagnosis. The complete response letter for the original BLA identified that the timing of intervention resulting in high survival rate with antibacterials as a major deficiency and asked the

Reference ID: 3220416

sponsor to conduct a combination study where survival with antibacterial alone approaches 50% to evaluate potential added benefit of raxibacumab.
The protocol for this study was reviewed under SPA and an agreement on the study design was reached on October 14, 2010.
Eighty-four hours post challenge was chosen as the timing of intervention based on the data collected by HGS and Battelle on the untreated and levofloxacin-treated animal survival as described in the tables below.

Table 31: Rabbit Survival in HGS Studies – Placebo-Treated Animals

Time post challenge	# (%) Survivors
	(N = 39)
72 h	20 (51.3%)
84 h	13 (33.3%)
96 h	9 (23.1%)

Spore challenge: 200 x LD_{50}.
Includes 39 placebo controls from 4 rabbit efficacy studies (8 rabbits in 615-N104504, 16 rabbits in 682-G005758, 3 rabbits in 723-G005835, and 12 rabbits in 781-G923701).
Adapted from Sponsor's study report

Table 32: NIH/Battelle Studies with Levofloxacin Treatment of Anthrax-Infected Rabbits

Levofloxacin Treatment Time (post challenge)		N Treated/Challenged (% Surviving to be Treated)	N (%) Survivors at Day 30 (All Animals)	N (%) Survivors at Day 30 (Treated Animals)
Study 1	48 h	8/8 (100%)	7/8 (88%)	7/8 (88%)
	72 h	7/10 (70%)	5/10 (50%)	5/7 (71%)
	96 h	6/12 (50%)	3/12 (25%)	3/6 (50%)
Study 2	96 h	5/16 (31%)	2/16 (12.5%)	2/5 (40%)
Study 3	72 h	9/16 (56%)	7/16 (44%)	7/9 (78%)

• **Spore challenge: 200 x LD50.** • **Animals were symp**tomatic for anthrax disease by sustained increased **body temperature, bacteremia and/or detectable PA.** • **Levofloxacin dose: 50 mg/kg, PO, daily for 3 days**
From Meister et al, 2010
Adapted from Sponsor's study report

At a regulatory briefing on the applicability of the animal rule to the licensing of anthrax anti-toxin monoclonal antibodies held at CDER on October 10, 2006, a consensus was reached that the efficacy of the monoclonal antibody against anthrax toxin should be demonstrated in two animal species (rabbit and NHP). Both models must show a significant benefit of the monoclonal over placebo. In addition, both models should explore the magnitude of benefit of an antitoxin therapeutic when combined with an antimicrobial over antimicrobial alone.
This submission addresses the evaluation of raxibacumab efficacy in addition to levofloxacin when compared to levofloxacin alone in one of **two proposed recommended animal models – the** NZW rabbit.
The natural history studies in a NZW rabbit model of inhalational anthrax were reviewed prior to the original BLA submission. The natural history of inhalational anthrax disease in NZW rabbits conducted thus far demonstrated reproducible and predictable clinical laboratory profile (body temperature, clinical hematology, PA toxemia, and bacteremia) that correlates with mortality

Reference ID: 3220416

(see the following table). In addition, a correlation of PA toxemia and temperature elevation of ≥2°F to onset of bacteremia has been established in the natural history as well as therapeutic efficacy studies in this animal model.

Table 33: Correlation between Survival Time and Other Event Parameters

	Spearman Correlation Coefficients (P Values)			
	Time to 1st Serum PA Detected	Time to 1st Bacteremia (Culture)	Time to 1st Bacteremia (PCR)	Time to 2°F Temperature Increase
Survival time	0.86446 (0.0056)	0.80995 (0.0148)	0.92729 (0.0009)	0.77846 (0.0229)
Time to 1st PA detected	-	0.94341 (0.0004)	0.96290 (0.0001)	0.96978 (< .0001)
Time to 1st bacteremia (culture)	-	-	0.96304 (0.0001)	0.85391 (0.0070)
Time to 1st bacteremia (PCR)	-	-	-	0.87958 (0.0040)

Inclusion criteria:

- Disease free New Zealand White (NZW) rabbits 50% male and 50% female weighing 2.5-4.5 kg supplied by [redacted] (b) (4)
- Implanted vascular access ports and telemetry (temperature) transponder
- Quarantine period of 7 days

Exclusion criteria:

- Malformations
- Clinical signs and symptoms of disease

Treatments and Study Groups:

Table 34: Animal Allocation

Challenge Day	Shipment Number	No. of Animals Allocated to Be Challenged
A	1	30
B		30
C	2	30
D		30
E	3	30
F		30
Total		**180**

Study procedures: The study was conducted under GLP.

Reference ID: 3220416

- Animals were housed in the individual cages.
- Study animals were randomized by dose group and gender prior to challenge in 6 sets of 30 animals.
- Aerosol challenge at targeted 200 x LD_{50} of *B. anthracis* Ames strain spores were to occur on Day A, B, C, D, E, F, G, H.
- Clinical observations and temperature monitoring
- Single dose IV study treatment was administered.
- Clinical observations were done every 6 hours from the time of challenge until Day 10 postexposure and twice daily thereafter
- Animals were euthanized if found moribund on the study or survived until the end of the study on Day 35 postexposure
- Necropsy and histopathology were conducted on all euthanized and all dead animals in a blinded fashion

Table 35: Blood Collection and Assay Schedule

Timepoint	Sample	Bacteremia (Culture)	PA	Raxibacumab	Levofloxacin[6]	TNA	Ig[7]
Study Day 0 (Pre-Challenge)	Day 0	X	X	X	X	X	X
12 h post-challenge and every 12 hours until treatment[1]	12hPC	X	X				
	24hPC	X[3]	X				
	36hPC	X	X				
	48hPC	X	X				
	60hPC	X	X				
	72hPC	X	X				
Immediately prior-to-1st-treatment blood sample[2]	PTT	X[3]	X	X	X	X	
5 min post raxibacumab dose	5mPTR		X	X			
2 h post 1st levofloxacin dose	2hPTL1	X	X		X		
8 h post raxibacumab dose	8hPTR		X	X			
24 h post 1st levofloxacin dose prior to 2nd levofloxacin dose[2]	24hPTL1	X[3]	X	X	X		
2 h post 2nd levofloxacin dose	2hPTL2	X	X		X		
24 h post 2nd levofloxacin dose prior to 3rd levofloxacin dose2	24hPTL2	X	X	X	X		
2 h post 3rd levofloxacin dose	2hPTL3	X	X		X		
24 h post 3rd levofloxacin dose	24hPTL3	X			X		
2 days post 3rd levofloxacin dose/ 96 h post raxibacumab dose	2dPTL3	X[3]	X	X	X		
3 days post 3rd levofloxacin dose	3dPTL3	X	X				
7 days post raxibacumab dose[4]	7dPTR	X	X	X			
14 days post raxibacumab dose[4]	14 dPTR	X	X	X			
21 days post raxibacumab dose[4]	21 dPTR	X	X	X			
28 days post raxibacumab dose[4]	28 dPTR	X	X	X		X	X

Reference ID: 3220416

Timepoint	Sample	Bacteremia (Culture)	PA	Raxibacumab	Levofloxacin[6]	TNA	Ig[7]
Terminal[4]		X[5]	X	X	X		

Adapted from Sponsor's study report p.24
1 Post-challenge pre-treatment bleed time points were calculated based on one median challenge time per challenge day.
2 Specimen was collected 30 min (± 10 min) prior to the scheduled dose.
3 Quantitative culture was performed.
4 Blood samples were not collected from the VAP.
5 Levofloxacin MIC was performed on positive terminal blood cultures collected from animals that received at least one treatment and succumbed to disease prior to 35 days post-challenge.
6 1 mL of whole blood was collected into a chilled EDTA tube, additional whole blood required for other assays was collected in an EDTA tube at room temperature. Only animals that received at least one dose of levofloxacin had samples subjected to levofloxacin analysis.
7 Immunogenicity (anti-raxbacumab antibodies)

Raxibacumab and Levofloxacin PK and PA Concentrations and TNA
The PK analyses of raxibacumab concentration-time profiles for all raxibacumab-dosed rabbits were conducted using population analysis techniques (mixed effect modeling), with the NONMEM software. Plasma raxibacumab and levofloxacin concentration-time data, serum PA concentration-time data, individual raxibacumab and levofloxacin PK parameters, and individual PA kinetic parameters were summarized using descriptive statistics. For the purpose of assessing PA kinetics, the PA concentration-time data was divided into 2 subgroups for analysis: 84 hour non-survivors and 84 hour survivors (treated animals). The TNA titers in plasma samples from surviving rabbits were determined using a cell killing assay.

Immunogenicity
Plasma samples were assessed for anti-raxibacumab activity using a 2-part ECL-based bridging assay.

Endpoints:
Primary efficacy endpoint was survival in the ITT study population on Day 28 after the last dose of levofloxacin treatment (Day 35 post exposure).

Analysis Populations:
The ITT population is defined as all randomized rabbits that received the study agent.

MO comment: *Additional sensitivity analyses will be performed by the FDA in the population of animals that were bacteremic at the treatment onset.*

Statistical Plan:
The primary efficacy endpoint was 28-day survival, defined as the proportion of animals that survive to Day 28 after the last dose of levofloxacin treatment. Based on previous experience (natural history of inhalational anthrax in NZW rabbits and results from the placebo arms in the previous efficacy studies), about 35% of animals would be expected to survive until 84 hours after spore challenge. It was estimated that 32 animals would be treated in each of the two active treatment groups. This study design had approximately 52% power to detect a 25% absolute improvement in 28-day survival from the raxibacumab/levofloxacin combination group over the levofloxacin group, assuming 40% 28-day survival in the levofloxacin group. The study would

Reference ID: 3220416

yield a p-value of 0.044 if there were 21 (65.6%) survivors and 13 (40.6%) survivors at Day 28 after the last dose of levofloxacin treatment in the raxibacumab/levofloxacin combination group and the levofloxacin group, respectively. The study was not powered to demonstrate a statistically significant difference at a medically important change in mortality, which would be less than the 25% improvement used for the sample size calculation. The primary efficacy analysis will be performed using 2-sided likelihood ratio test or Fisher's exact test. Adjustment is made for multiple comparisons.

MO comment: *Please refer to the Statistical review by Lan Zeng, Ph. D.*

PROTOCOL CHANGES:
The original protocol was submitted and reviewed by the Division on October 10, 2010. Subsequently, the protocol was amended 4 times:

Amendment 1 (24 January 2011)
- Clarifications were made to time window for blood collections at each time point.

Amendment 2 (03 June 2011)
- Unblinding of the treatment assignments will occur at the time of the completion of the in-life portion of the study to the study director but not to the pathologist to allow analyses of raxibacumab PK and immunogenicity as well as efficacy to proceed while ensuring that the pathology evaluation will proceed in a blinded fashion.

Amendment 3 (30 November 2011)
- Clarifications were made to limit inclusion of blood samples to samples from animals that survived to be treated for PK analyses of levofloxacin concentrations.
- Details of the location, and sample and document retention for (b) (4) (b) (4) which were not available at the time the original protocol was signed, were added.
- Dose confirmation for levofloxacin was removed from the protocol because commercial levofloxacin was used at the stock concentration directly from the manufacturer's vial, and not diluted prior to administration.
- Clarified that archived storage of specimens will be at the Battelle Memorial Institute, rather than EPL, for long term storage.

Amendment 4 (18 April 2012)
- Clarifications were made to the timing of TNA assay on samples from surviving rabbits on Days 0 (pre-challenge) and 28 after raxibacumab treatment

RESULTS

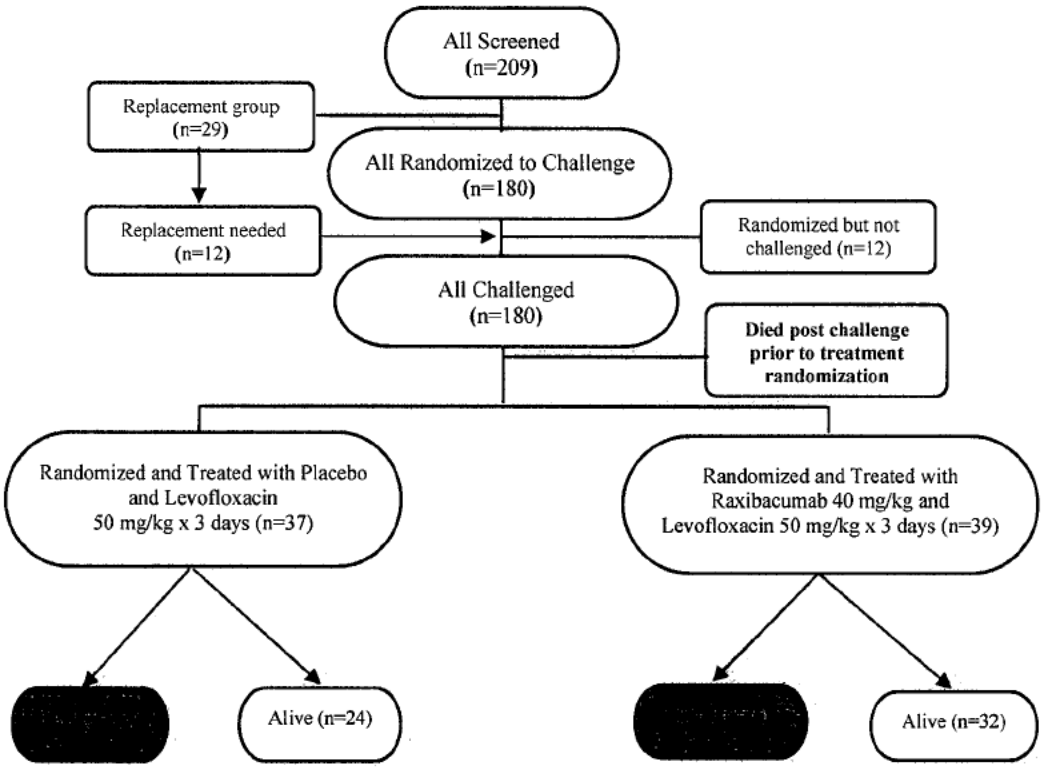
Animal Disposition
Study centers: 1 study center, (b) (4)

Total of 209 animals were available for the study, 180 were initially selected and randomized, 12 of them were replaced due to health conditions unacceptable for the study, 180 total received

Reference ID: 3220416

spore challenge; 76 of 180 (42%) survived to 84 hours post challenge and were randomized to receive study treatment (37 in the levofloxacin group and 39 in the raxibacumab/levofloxacin combination group). All rabbits received the treatment to which they were assigned.
The following flowchart and the tables summarize the composition of the applicant's and reviewer's populations for analysis, the primary reasons for exclusion and the demographics of the study population.

Figure 4: Animal's Disposition, Study 1141



APPEARS THIS WAY ON ORIGINAL

MO comment: *Animal L34828 was not bacteremic prior to being treated with raxibacumab/levofloxacin and remained culture negative following treatment, but the animal was febrile and positive for PA by quantitative ECL prior to treatment and remained febrile with high PA levels until after 24 hour post treatment. This animal will be excluded from the efficacy analysis in the population of animals bacteremic prior to treatment.*

Table 36: Treatment Assignments by Challenge Set and Day, Study 1141

Challenge Day	Number Challenged	Treatment	Number Treated
Day A		Raxibacumab + Levofloxacin	5 (M2; F3)
24 January 2011	30 (15M; 15F)	Buffer + Levofloxacin	5 (M2; F3)

Reference ID: 3220416

Challenge Day	Number Challenged	Treatment	Number Treated
Day B		Raxibacumab + Levofloxacin	7 (M2; F5)
27 January 2011	30 (15M; 15F)	Buffer + Levofloxacin	7 (M2; F5)
Day C		Raxibacumab + Levofloxacin	7 (M2; F5)
21 March 2011	30 (15M; 15F)	Buffer + Levofloxacin	7 (M5; F2)
Day D		Raxibacumab + Levofloxacin	8 (M4; F4)
24 March 2011	30 (15M; 15F)	Buffer + Levofloxacin	7 (M4; F3)
Day E		Raxibacumab + Levofloxacin	9 (M6; F3)
16 May 2011	30 (15M; 15F)	Buffer + Levofloxacin	7 (M3; F4)
Day F		Raxibacumab + Levofloxacin	3 (M2; F1)
19 May 2011	30 (15M; 15F)	Buffer + Levofloxacin	4 (M2; F2)

Demographic and Baseline Characteristics

Table 38: Demographics and Baseline Characteristics, Study 1141 (ITT Population)

	Spore Challenged N = 180	Died before Treatment1 N = 104	Levofloxacin (50 mg/kg x 3) + Placebo N = 37	Levofloxacin (50 mg/kg x 3) + Raxibacumab (40 mg/kg) N = 39	P value[1]
Sex					0.8276
Male	90 (50%)	54 (51.9%)	18 (48.6%)	48 (46.2%)	
Female	90 (50%)	50 (48.1%)	19 (51.4%)	21 (53.8%)	
Weight(kg)					0.7609
Mean ± SD	3.1 ± 0.2	3.2 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	
Median	3.1	3.1	3.1	3.1	
Range	(2.8, 3.9)	(2.8, 3.9)	(2.8, 3.5)	(2.8, 3.6)	
Age at randomization (mos)					0.8727
Mean ± SD	7.5 ± 1.6	7.5 ± 1.6	7.4 ± 1.5	7.4 ± 1.5	
Median	6.7	7.0	6.7	6.7	
Range	(6.0, 11.8)	(6.0, 11.8)	(6.0, 11.1)	(6.0, 10.9)	
Toxemic at baseline	3 (1.7%)	3 (2.9%)	0 (0.0%)	0 (0.0%)	

Modified from Sponsor's study report p.36
1 Challenged and died before treatment.
2 P-value for comparison across 2 treatment groups obtained from likelihood ratio chi-square or Fisher's exact test for categorical data, and 1-way ANOVA for continuous data.
3 Toxemic is defined as detectable PA.

MO comment: *Study animals were of the same age and were comparable in weight and sex distribution.*

Extent of Anthrax Exposure

Study animal were challenged with a target head only aerosol of 200xLD_{50} *B. anthracis* spores on one of 6 challenge days. Therapeutic intervention was administered at 84 hours post exposure to all surviving animals. A comparison of exposure dose between different days of challenge,

Reference ID: 3220416

survivors and non-survivors at 84 hours post challenge, and assigned treatment groups is presented in the following table.

Table 39: Mean Spore Challenge Dose, LD_{50} by Challenge Day, Study 1141

Challenge Day	Mean Challenge Dose (SD)			
	Treated		Untreated	All challenged
A	173 ± 31		178 ± 38	176 ± 35
B	168 ± 47		189 ± 41	179 ± 45
C	203 ± 32		214 ± 43	209 ± 38
D	174 ± 40		195 ± 34	184 ± 38
E	194 + 51		194 ± 48	194 ± 49
F	214 ± 81		175 ± 48	184 ±58
All animals			189 ± 43	187 ± 45
	Levofloxacin + Placebo	Levofloxacin + Raxibacumab		
	174 ± 43	197 ± 49		

Modified from Sponsor's study report p.37

MO comment: The challenge spore dose was within the target of 200x LD_{50} for all animals in the study regardless of challenge day, survival to treatment or treatment assignment. Animals on the combination arm received on average 23xLD_{50} higher dose than those on levofloxacin alone arm. (two-sided p value of 0.058). There was no relationship between the challenge dose received and survival to study termination, survival to treatment or time to death.

Clinical Observations

Abnormal clinical signs were first documented in the majority of animals at approximately 48-72 hours post-challenge. The most common abnormal clinical signs reported were lethargy, loss of appetite, stool abnormalities and respiratory abnormalities. Among animals that succumbed to disease 8/13 (62%) on the levofloxacin + placebo arm and 5/7 (71%) on the levofloxacin + raxibacumab arm were found dead. The rest of the non-survivors were euthanized. Criteria for euthanasia that were observed most often included respiratory distress and unresponsiveness. Among survivors normalization of clinical signs occurred by Day 10-11 post-challenge with the exception of a few animals that demonstrated inappetence and/or minor stool abnormalities. Animal L33741 (raxibacumab arm) had documented muzzle swelling through Day 12, but recovered the following day and survived through Day 35 post-challenge.

Temperature monitoring

All challenged animals demonstrated increases in body temperature during the study, most of them on Days 2 though 6 following exposure to *B. anthracis*, similar to observations in previous studies. However, not all animals had temperature elevations high enough to meet fever definition (>2 F above baseline for ≥2 hours): 50 animals that succumbed to disease prior to

Reference ID: 3220416

treatment, 8 animals on levofloxacin alone arm, and 7 animals on the combination arm did not meet fever criteria, although these animals were toxemic and bacteremic either prior to death in the case of untreated animals, or prior to treatment in the case of animals that received treatment. In the majority of treated survivors, body temperature returned to normal levels following the first week after exposure.

Table 37: Animals with Fever (>2F above Baseline for ≥2 Consecutive Measurements), Study 1141

Time to Fever	Levofloxacin + Placebo N = 37 (%)	Levofloxacin + Raxibacumab N = 39 (%)	Died Before treatment N = 104 (%)
Febrile animals on study	**29/37 (78.4)**	**32/39 (82.1)**	**54 (51.2)**
Febrile prior to treatment	29/37 (78.4)	31/39 (79.5)	n/a

MO comment: *Although per protocol temperature monitoring was ongoing continuously in all animals on study, temperature data input into the dataset was limited to morning and evening temperatures. Therefore, in this review the fever definition is more restrictive than in reviews for the other rabbit efficacy studies, as 2 consecutive measurements in this study are approximately 12 hours apart as compared to hourly measurements in other studies.*
All animals febrile on the study, except for one on the combination arm (L33771) met fever definition criteria prior to treatment. The proportion of animals febrile prior to treatment was not different between the study arms. There were a total of 9 survivors that were persistently febrile for the duration of the study: two on levofloxacin alone arm (L33713 and 34854) and seven on the combination arm (L29365, L33771, L34826, L34833, L34846, L34866, and L34868). These animals were challenged with B. anthracis spore doses ranging from 140 to 208xLD_{50} and were bacteremic and toxemic at the time of treatment. All of them cleared bacteremia by Day 7 post treatment. Seven of them resolved toxemia by Day 7 post treatment. Animal L34833 cleared toxemia on Day 14.
Animal L29365 was PA negative in Day 3 through Day 14 post treatment samples, then had PA detected at low levels on Day 21 and Day 28 post treatment.

Bacteremia Results

All animals except for L34828 (levofloxacin raxibacumab combination arm) were found to be bacteremic prior to randomization and treatment administration. Animal L34828 remained negative for bacteremia following treatment. All but 2 of the animals (L35281 and L33701) that died before 84 hours exhibited positive terminal bacteremia results. Animal L35281 was bacteremic at 24, 36, 48, and 60 hours post spore exposure and succumbed to disease prior to the 72 hours collection time point. Animal L33701 was positive for bacteremia at 36, 48, 60, and 72 hours post exposure and succumbed to disease prior to 84 hours post spore exposure.

Table 38: Time to Bacteremia, Study 1141

Time to Bacteremia	Levofloxacin + Placebo N = 37	Levofloxacin + Raxibacumab N = 38[1]	Died Before Treatment N = 104
Mean ± SD (h)	32.9 ± 14.1	35.2 ± 13.0	27.3 ± 5.5

Reference ID: 3220416

Time to Bacteremia	Levofloxacin + Placebo N = 37	Levofloxacin + Raxibacumab N = 38[1]	Died Before Treatment N = 104
Median	27.5	34.2	25.6
Range	**14.7 – 86.9**	**20.3 – 71.9**	**10.6 – 44.7**

1 Animal L34828 is excluded from the analyses as it had negative bacteremia results

MO comment: *Animals on levofloxacin alone arm had on average shorter time to onset of bacteremia as compared to the animals on combination arm.*

Within 2 hours following the first dose of levofloxacin, 38/39 (97.4%) animals treated with levofloxacin/raxibacumab were alive and 16 of these animals (42.1%) were already negative for bacteremia. Of the 34 levofloxacin/ raxibacumab/treated survivors 32 (94.1%) became blood culture negative following the first dose of levofloxacin. The 2 animals with positive blood cultures (L35259 and L35261) cleared their blood cultures at 2 hours after receiving their second dose of levofloxacin. Survivors on levofloxacin/raxibacumab arm were culture negative for the duration of the study after the second levofloxacin dose. Among seven non-survivors on raxibacumab/levofloxacin arm 4 animals (57.1%) were blood culture negative in the terminal sample.

On levofloxacin alone arm 36/37 animals (97.3%) were alive at 2 hours following the first dose of levofloxacin. Ten of them (27.8%) had negative blood cultures 2 hours following their first dose of levofloxacin. All 24 survivors on levofloxacin arm were negative for bacteremia at 7 days post treatment. No animals were bacteremic or died beyond 3 days after the last dose of levofloxacin. Of the 13 animals treated with levofloxacin alone that succumbed to disease, 11 animals (84.6%) were blood culture negative in the terminal sample.

Table 39: Positive Bacteremia and Survival Timeline, Study 1141

	Levofloxacin + Placebo N = 37		Levofloxacin + Raxibacumab N = 39	
	Survivors (%)	Bacteremic (%)	Survivors (%)	Bacteremic (%)
Prior to treatment	37 (100%)	37 (100%)	39 (100%)	38 (97.4%)
2 h post 1st levo	36 (97.3%)	26 (72.2%)	38 (97.4%)	22 (58%)
24 h post 1st levo	29 (78.4%)	0 (0%)	34 (87.2%)	2 (5.9%)
2 h post 2nd levo	28 (75.7%)	1 (3.6%)	34 (87.2%)	0 (0%)
24 h post 2nd levo	27 (73.%)	0 (0%)	34 (87.2%)	0 (0%)
2 h post 3rd levo	26 (70.3%)	1 (3.8%)	34 (87.2%)	0 (0%)
24 h post 3rd levo	25 (67.6%)	0 (0%)	33 (84.6%)	0 (0%)
2 days post 3rd levo	25 (67.6%)	0 (0%)	32 (82%)	0 (0%)
3 days post 3rd levo	24 (64.9%)	1 (4.2%)	32 (82%)	0 (0%)
7 days post 3rd levo	24 (64.9%)	0 (0%)	32 (82%)	0 (0%)

Adapted from Sponsor's study report p.45

MO comment: *Of 13 non-survivors on the levofloxacin alone arm, 12 died during the course of levofloxacin therapy. One animal on levofloxacin arm succumbed to disease 3 days after the last dose of levofloxacin. That animal was culture negative at death. A single animal was culture positive after levofloxacin treatment discontinuation. However, this animal survived. Although the duration of levofloxacin therapy might be called into question for its adequacy, the evidence from the study under review suggests that animals that succumbed to anthrax after antibiotic*

Reference ID: 3220416

discontinuation are likely to do so because of effects of anthrax toxin rather than bacteremia. Animal model and antimicrobial regimen developmental work was conducted by NIAID. The summary data presented to FDA was assuring since animals that died on the antibiotic arm did so during the course of treatment.
Animals on the combination arm cleared bacteremia faster than those on levofloxacin alone arm. The timing of death was similar between the two treatment arms.

Toxemia Results
All animals in this study became toxemic post challenge. Among animals that did not survive to treatment 100% were PA positive starting 36 hours post exposure and remained positive until time of death with progressively rising titers.

Table 40: Time to PA Toxemia

Time to PA toxemia by ECL (quantitative)	Levofloxacin + Placebo N = 37	Levofloxacin + Raxibacumab N = 39	Died Before treatment N = 104
Mean ± SD (h)	30.6 ± 13.1	29.0 ± 7.9	25.5 ± 5.0
Median	26.3	26.8	24.7
Range	**12.6 – 86.9**	**12.1 - 47.4**	**11.0 - 37.1**

Adapted from Sponsor's study report p.46

MO comment: *Onset of PA toxemia was similar between two study arms. Animals that did not survived to treatment had on average 5 hour earlier onset of toxemia compared to those that survived to treatment.*

After study treatment, animals in both treatment groups exhibited toxemia clearance: two days after the end of levofloxacin regimen majority of animals in both groups were PA negative. In levofloxacin alone treatment group all animals were toxemia free at 21 and 28 days post treatment. Sporadic positive toxemia results were noted in 3 animals at 21 and 28 days post treatment.

Table 41: Positive PA Toxemia and Survival Timeline on the Study

	Levofloxacin + Placebo N = 37		Levofloxacin + Raxibacumab N = 39	
	Survivors (%)	Toxemic (%)	Survivors (%)	Toxemic (%)
Prior to treatment	37 (100%)	37 (100%)	39 (100%)	39 (100%)
2 h post 1st levo	36 (97.3%)	36 (100%)	38 (97.4%)	38 (100%)
24 h post 1st levo	29 (78.4%)	28 (96.6%)	34 (87.2%)	33 (97.1%)*
2 h post 2nd levo	28 (75.7%)	28 (100%)	34 (87.2%)	34 (100%)
24 h post 2nd levo	27 (73%)	26 (96.3%)	34 (87.2%)	33 (97.1%)*
2 h post 3rd levo	26 (70.3%)	25 (96.2%)	34 (87.2%)	34 (100%)
2 days post 3rd levo	25 (67.6%)	4 (16%)	32 (82.1%)	13 (40.6%)
3 days post 3rd levo	24 (64.9%)	1 (4.2%)	32 (82.1%)	4 (12.5%)
7 days post 3rd levo	24 (64.9%)	1 (4.2%)	32 (82.1%)	1 (3%)
14 days post 3rd levo	24 (64.9%)	2 (8.4%)	32 (82.1%)	0 (0%)
21 days post 3rd levo	24 (64.9%)	0 (0%)	32 (82.1%)	2 (6%)
28 days post 3rd levo	24 (64.9%)	0 (0%)	32 (82.1%)	1 (3%)

* sample for animal L35274 was too small for analysis (animal was positive at the next time point)

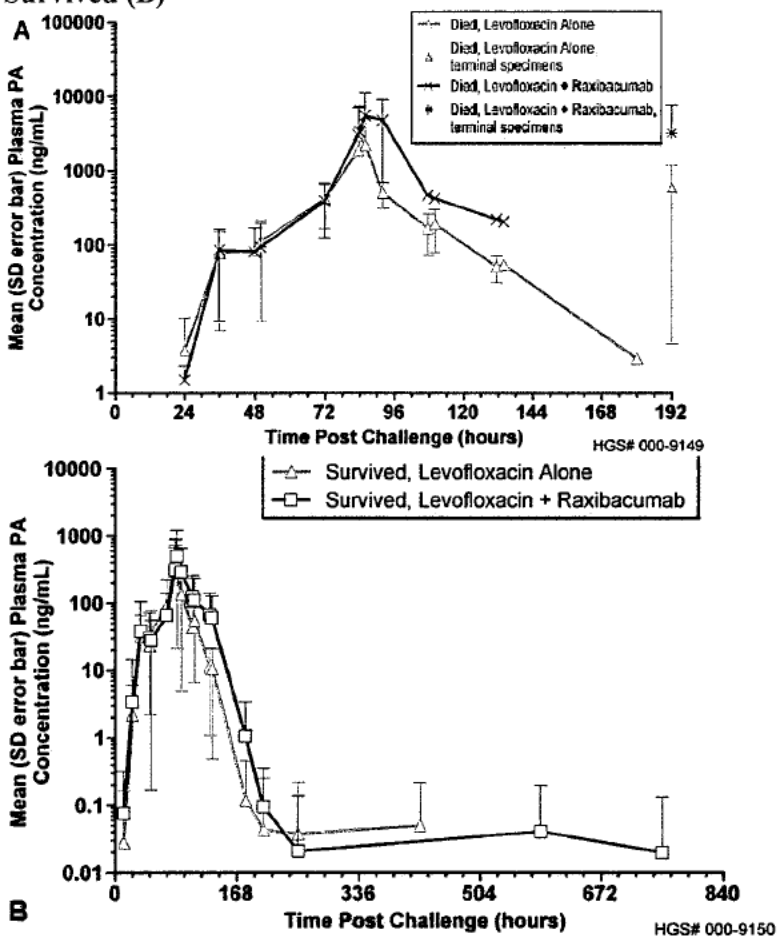
Reference ID: 3220416

MO comment: *All non-survivors in this study were positive for PA toxemia in the terminal sample. Clearance of PA from circulation was similar between two study arms. However, in the combination arm PA was identified in the blood of two animals at 14 and 21 days post treatment and one animal L29365 continued to have positive PA result at the end of the study, while all survivors treated with levofloxacin alone were PA negative at day 21 posttreatment and beyond. PA positive animals identified at 7 days posttreatment had developed toxin neutralizing antibodies at the end of the study. Sporadic PA positivity in the surviving animals is most likely explained by continuous spore germination following aerosol exposure of 200xLD_{50}.*

Mean plasma PA concentration-time profiles for the dead (Figure A) and surviving (Figure B) animals treated with levofloxacin vs. levofloxacin+40 mg/kg raxibacumab as well as PA levels recorded at the time of treatment (treated animals) or in a terminal sample (untreated animals) are shown below. Comparison of the PA kinetics for treated rabbits that died and survived showed a general overlap in PA kinetic estimates, with the exception of both the height and duration of the first and second plateau in PA levels. Animals that survived to 84 hours post-challenge had 10-fold less plasma PA than non-surviving animals at this time point. For all surviving animals in both treatment groups, plasma PA concentrations declined below the LLOQ by the end of the study.

Reference ID: 3220416

Figure 5: Plasma PA Concentration-Time Profile in Treated Rabbits that Died (A) and Survived (B)



(Adapted from figures 5-7 and 5-8 on page 402-403 of the Battelle study report)

Table 42: PA Toxemia Levels at Treatment for Treated Animals and at Death for Untreated Animals, Study 1141

	Levofloxacin + Placebo N = 37		Levofloxacin + Raxibacumab N = 39		Died before Treatment[1] N = 104
	Non-survivors	Survivors	Non-survivors	Survivors	
n	13	24	7	32	104
Mean ± SD	1888.0 ± 1899.3	394.9 ± 469.1	3058.1 ± 4023.2	300.5 ± 370.3	3920.9 ± 7413.6
Median	1356.4	227.1	1388.9	135.2	1013.3
(Min, Max)	(375.7, 7769.7)	(2.2, 1638.0)	(286.8, 11206.3)	(5.1, 1076.8)	(27.7, 41145.4)

Adapted from Sponsor's study report p.47
1 Last available PA sample prior to the terminal endpoint

MO comment: Levels of toxemia were similar between the treatment groups at the onset of treatment. However, non-survivors had much higher levels of toxemia compared to survivors, indicating that progressive toxemia at the time of treatment onset impedes positive outcome of treatment.

Onset of Treatment

Per study protocol, randomization of surviving animals to treatment groups was to occur at 84 hours post challenge. The following tables will examine the timing of therapeutic intervention post challenge, relative to onset of toxemia, and bacteremia in both treatment groups.

Table 43: Time to Randomization / Treatment Initiation, Study 1141

Inhalational Exposure to Anthrax Spores	Levofloxacin + Placebo N = 37	Levofloxacin + Raxibacumab N = 39	*P-value*
Mean ± SD (h)	85.2 ± 2.1	85.8 ± 1.9	0.4744
Median	85.1	85.9	
Range	81.8- 89.2	82.4-89.2	

Adapted from Sponsor's study report p.38

MO comment: Target timing for randomization to treatment and treatment administration was set at 84 hours post exposure. Most animals on both arms received the first dose of treatment within 2 hours of target time. There was no difference in the timing of treatment initiation between the treatment groups.

Table 44: Time from Positive PA to Treatment, Study 1141

Time from Diagnosis (PA ECL) to Treatment (h)	Levofloxacin + Placebo N = 37		Levofloxacin +Raxibacumab N = 39	
All treated				
Mean ±SD	54.7±13.1		56.8±8.1	
Median	61.4		61.3	
Range	0.4-72.5		37.8-73.8	
Based on outcome	**Survivors N=24**	**Non-survivors N=13**	**Survivors N=32**	**Non-survivors N=7**
Mean ±SD	53.0±15.5	57.8±6.2	56.2±8.6	59.6±4.8
Median	61.3	61.4	55.8	61.5
Range	0.4-72.5	48.5-62.5	37.8-73.8	48.7-61.8

MO comment: Timing of treatment relative to onset of PA toxemia was similar between the study groups and between survivors and non-survivors.

Table 45: Time from Positive Blood Culture to Treatment, Study 1141

Time from Diagnosis (Bl Cx) to Treatment (h)	Levofloxacin + Placebo N = 37	Levofloxacin +Raxibacumab N = 38[1]
All treated		

Reference ID: 3220416

Time from Diagnosis (Bl Cx) to Treatment (h)	Levofloxacin + Placebo N = 37		Levofloxacin +Raxibacumab N = 38[1]	
Mean ±SD	52.3±14.2		50.7±13.4	
Median	60.9		50.2	
Range	0.4-72.6		13.5-63.0	
Based on outcome	**Survivors N=24**	**Non-survivors N=13**	**Survivors N=31[1]**	**Non-survivors N=7**
Mean ±SD	52.0±15.1	52.9±14.0	49.1±14.2	57.9±5.8
Median	55.6	60.9	49.8	61.3
Range	0.4-62.8	13.1-72.6	13.5-63.0	48.7-61.6

1 Animal L34828 is excluded from the analyses as it had negative bacteremia results

MO comment: *All but one animal in levofloxacin/raxibacumab group were blood culture positive prior to treatment. On average there was no difference between the study groups in timing of treatment relative to bacteremia onset. It appears that non-survivors on the combination arm sustained pretreatment bacteremia on average 9 hours longer than survivors.*

Other Parameters Measured:

The *B. anthracis* MICs for levofloxacin, prior to treatment and the terminal MICs ranged from 0.25 to 1.0 µg/mL, demonstrating that the original MICs to these antibiotics did not change during the course of the study.
Anti-raxibacumab antibody levels and TNA post-challenge were collected during the study.

Table 49: Immunogenicity (Anti-Raxibacumab Antibodies), Study 1141

	Levofloxacin + Placebo N = 37		Levofloxacin + Raxibacumab N = 39	
	Pretreatment	**Day 28**	**Pretreatment**	**Day 28**
Evaluable samples	37	24	39	32
Positive	3[1]	4	3[1]	23

[1] These animals were also positive on Day 28
Adapted from the sponsor's study report p.49

MO comment: *Presence of anti-raxibacumab antibodies pretreatment did not affect survival of the animals on the combination arm.*

Table 46: TNA Titers in Surviving Rabbits, Study 1141

	Levofloxacin + Placebo N = 37		Levofloxacin +Raxibacumab N = 39	
	Pretreatment	**Day 28**	**Pretreatment**	**Day 28**
Evaluable samples	24	24	32	32
Mean ±SD	0±0	5150±3495	0±0	4741±3005

Adapted from the sponsor's study report p.49

Reference ID: 3220416

MO comment: *There were no significant differences between the study groups in the ability to mount an immune response post infection.*

All animals that survived were monitored for 28 days post-treatment. Clinical signs and symptoms of anthrax, including body temperature elevations, resolved following resolution of bacteremia. Animals exhibited normal baseline behavior at the time of study termination. Animals found moribund were euthanized. Complete necropsies were performed on all animals, including those that died prior to treatment and those surviving to terminal sacrifice on Day 35.

Pathology, Gross and Microscopic

Complete gross necropsies were performed on all animals, including those that died prior to initiation of treatment. Protocol-specified tissues (brain, bronchial lymph nodes, heart, kidney, liver, lungs, mediastinal lymph nodes, and spleen) were sampled and preserved in 10 percent neutral buffered formalin only from treated animals. Animals were examined in a blinded manner: the pathologist was unaware of the treatment assignments. Gross findings in animals died on the study included: discoloration or lesions in the brain, lungs, and large intestines; enlargement of mediastinal lymph nodes; fluid (effusion) in multiple body cavities, mesentery, thymus, and skin; and cystic structures in the lung. These gross lesions were considered typical of anthrax in rabbits and, in animals examined microscopically, were associated with hemorrhage, edema, and acute inflammation.
The only gross lesion present in any of the animals surviving to study termination was a unilateral kidney cyst in a levofloxacin/raxibacumab-treated animal, L33739, which was considered to be a common incidental finding with no relation to anthrax challenge or treatment.
All protocol-predefined tissues from all animals surviving to treatment were examined microscopically for evidence of anthrax, with the exception of the brain from raxibacumab/levofloxacin-treated animal, L34818, and the spleen from raxibacumab/levofloxacin-treated animal, L33747, which were not successfully processed to slides. All microscopic findings were graded semiquantitatively (grades 0-4) according to the following scale: minimal (Grade 1) represented the least detectible lesion; mild (Grade 2) represented an easily discernible lesion; moderate (Grade 3) represented a change affecting a large area of the represented tissue; and marked (Grade 4) represented a lesion that approached maximal.
Microscopic findings consistent with anthrax were present in nearly all animals examined that were found dead or euthanized for moribundity after the initiation of treatment but prior to scheduled Study Day 35 sacrifice in both levofloxacin-treated and raxibacumab/levofloxacin-treated groups. Lesions typical of anthrax in this study included acute inflammation, necrosis, and hemorrhage, and were generally of similar incidence and severity in both treatment groups. Findings in the brain were present in 2 animals in the levofloxacin treatment group and comprised of meningeal and parenchymal hemorrhage and meningeal vascular necrosis (L23083) and parenchymal hemorrhage and necrosis (L34864); there were no brain findings in the raxibacumab/levofloxacin-treated non-survivors. Survivors had the following findings: levofloxacin-treated L34863 had mixed infiltrate in the liver and L34834 had renal tubular

Reference ID: 3220416

atrophy and cardiac osseous metaplasia; levofloxacin/raxibacumab-treated L35231 had arterial thrombus in the lung, while L35259 had a mixed cellular infiltrate in the liver.
The reviewer compared histopathology findings between groups.

Table 47: Incidence of Pathology Findings on Necropsy, Study 1141

Pathology Findings/Necropsized Animals	Levofloxacin N=37 (%)		Raxibacumab N=39 (%)	
Study outcome	**Lived**	**Died**	**Lived**	**Died**
	24 (64.9)	**13 (35.1)**	**32 (82.1)**	**7 (17.9)[1]**
Brain (gross brain pathology and/or histopathology+ bacteria)	0 (0)	2 (15)	0 (0)	0 (0)
Kidney	1 (4)	1 (8)	0 (0)	1 (14)
Liver	1 (4)	6 (46)	1 (3)	3 (43)
Bronchial/Mediastinal Lymph Nodes	0 (0)	12 (92)	0 (0)	6 (86)
Lung	0 (0)	10 (77)	1 (3)	2 (29)
Spleen	0 (0)	7 (54)	0 (0)	3 (43)

[1] Brain sections from 1 animal and spleen sections from 2 animals were not examined microscopically

MO comment: *Findings on histological examination were incidental in survivors in both treatment groups, while in non-survivors findings consistent with inhalational anthrax were present and comparable in both treatment groups. Two animals on levofloxacin group had histological evidence of meningitis as compared to none in the combination group.*

The main focus as in the previous BLA submission animal efficacy studies review was on "high grade" lesions. The reviewer applied the following grading criteria for the consistency of evaluation across different studies:

- Criteria for High grade
  - Bacteria, hemorrhage, inflammation > grade 2
  - Presence of necrosis regardless of grade of bacteria, hemorrhage, inflammation
- Criteria for Low grade
  - Bacteria, hemorrhage, inflammation 0-2, necrosis 0

Table 48: Incidence of High Grade Lesions in Treated Animals, Study 1141

	Levo N=37		Levo + Raxi N=39	
	Died N=13 (%)	**Survivors N=24 (%)**	**Died N=7[1] (%)**	**Survivors N=32 (%)**
Brain	2 (15)	0 (0)	0 (0)	0 (0)
Lung	3 (23)	0 (0)	1 (14)	1 (3)
Mediastinum (LN)	12 (92)	0 (0)	5 (72)	0 (0)
Liver	6 (46)	1 (4)	2 (29)	1 (3)
Spleen	3 (23)	0 (0)	2 (29)	0 (0)
Kidney	1 (8)	1 (4)	1 (14)	0 (0)

[1] Brain sections from 1 animal and spleen sections from 2 animals were not examined microscopically

Reference ID: 3220416

MO comment: *Incidence of high grade lesions was comparable between treatment groups. Among two non-survivors with meningitis on levofloxacin alone arm, moderate bacteria was found in one (L23083) animal, while hemorrhage and necrosis were noted in both (L23083 and L34864) animals examined.*

In three levofloxacin-treated non-survivors (L23083, L34808, and L34816) and one raxibacumab/levofloxacin non-survivor (L33730) bacteria were present in one or more organs, including the brain, lung, bronchial and mediastinal lymph nodes, and spleen. The remaining animals lacked visible bacteria in any organs, although all of these animals had positive bacteremia results for *B. anthracis* at the blood collection prior to death and/or inflammation and hemorrhage typical of anthrax in one or more of the examined organs.

MO comment: *One animal L33722 (levofloxacin alone) had no gross or microscopic findings associated with B. anthracis infection, and the cause of death of this animal was not apparent. This animal was continuously bacteremic and toxemic starting 24 hours post challenge and converted to negative 24 hours after the first dose of levofloxacin. It never exhibited a strong febrile response to infection (a temperature elevation of at least 2 F above baseline 24 hours prior to treatment) and was the last animal that succumbed to disease among non survivors at 107 hours after the receiving treatment.*

Efficacy results

The primary efficacy analysis was survival at 28 days after the last dose of levofloxacin, Day 35 post challenge. The following table contains results for primary endpoint in the ITT population and additional sensitivity analyses performed by the reviewer.

Table 49: Primary Efficacy Analyses, Study 1141

Study population	Levofloxacin + Placebo	Levofloxacin +Raxibacumab
Primary analysis ITT population, all toxemic animals		
	N = 37	**N=39**
Survival, n (%)	**24 (64.9)**	**32 (82.1)**
Difference (95% CI)	**17.2 (-2.35 , 36.72)**	
P value*	**0.0874**	
Modified ITT (bacteremic animals)		
	N=37	**N=38**
Survival, n (%)	**24 (64.9)**	**31 (81.6)**
Difference (95% CI)	**16.7 (-3.00 , 36.43)**	
P value*	0.0998	

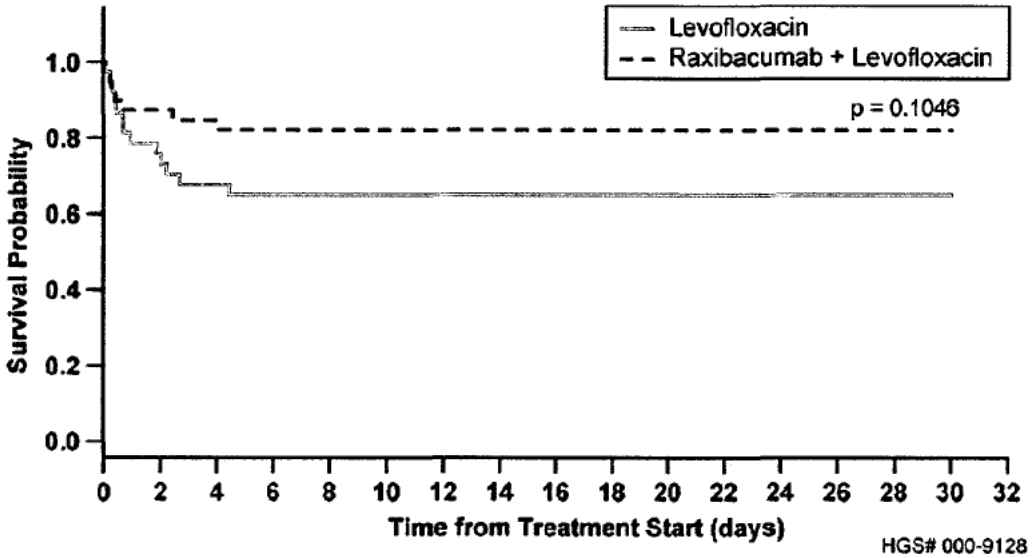
*P-values are based on two-sided Fisher's exact test for comparisons between the treatment and placebo

MO comment: *The ITT population above represents animals that were challenged, survived to 84 hours post challenge, were randomized and received treatment. All ITT animals were toxemic prior to treatment administration.*

Reference ID: 3220416

Secondary Efficacy Analysis, Survival Time
The following figure shows the survival curves for randomized animals.

Figure 6: Survival Time, Study 1141



(Figure 1 from Battelle Study Report, pg 23).

Statistical analysis of survival time in the non-survivors performed by statistical reviewer, Dr. Lan Zeng, showed that time to death (TTD) was similar between the treatment groups, 4.9 days in the levofloxacin alone compared to 4.7 days in the levofloxacin + raxibacumab treatment group. Animals that survived until Day 9 post-challenge in either treatment group remained alive until the scheduled euthanasia on Day 35. The TTD listed is an estimate of the time to death post-treatment since a majority of dead animals in both groups were found dead in their cages.

Efficacy findings:

- Study design achieved survival with levofloxacin alone of 62.9% similar to that observed in inhalational anthrax cases during the 2001 bioterrorism attack (55%).
- Raxibacumab at 40 mg/kg IV bolus dose administered at 84 hours post challenge with levofloxacin 50 mg/kg qd dose for 3 days resulted in improved survival (82.1%) as compared to levofloxacin alone (64.9%) all randomized toxemic animals. The treatment difference of 17.2% did not achieve statistical significance (p=0.0874).
- The difference in efficacy between the treatment groups in the bacteremic population of animals was 16.7% [81.6% versus 64.9% (p=0.0998)] and was similar to the ITT analysis.

Reference ID: 3220416

Safety findings:

- Raxibacumab at 40 mg/kg IV did not interfere with efficacy of levofloxacin. There was a trend of improvement in survival (17% difference, not significant) in the combination arm relative to levofloxacin alone.
- There was no difference between the treatment groups in the timing of clearance of bacteremia or toxemia.
- Animals that succumbed to disease had findings consistent with anthrax in multiple organs. The pattern of organs affected did not differ between the treatment arms.
- Pathological CNS findings were absent in levofloxacin/raxibacumab-treated non-survivors compared to findings of meningitis in 2/13 (15%) of levofloxacin-treated non-survivors.
- Survivors on both arms had incidental findings on microscopic examination unrelated to anthrax.

4.4 Clinical Pharmacology

Please refer to the final Clinical Pharmacology review for this resubmission by Dr. Ryan Owen and the review of the original BLA submission by Dr. Kimberly Bergman.
To allow selection of an effective dose in humans for therapeutic treatment of anthrax, the applicant has submitted clinical pharmacology data for raxibacumab in humans and in the two pivotal animal species, rabbits and cynomolgus monkeys. The pharmacokinetics (PK) and safety of raxibacumab administered as the product proposed for licensure has been evaluated in a Phase 1 dose-ranging study (PAM-NH-01), an antibiotic interaction study with ciprofloxacin (HGS1021-C1064), a repeat dose immunogenicity study (HGS1021-C1069) and a Phase 2/3 safety study (HGS1021-C1063). Based on a cross-species comparison of raxibacumab exposure and exposure-response information for raxibacumab dose/concentration, PA concentration and survival, the clinical pharmacology information provided by the applicant is acceptable and supports the use of the 40 mg/kg dose of raxibacumab. The dose and concentration response relationship with the endpoint of survival indicates potential to achieve higher response rates at higher doses. If future trials are conducted, higher doses (e.g. 60 or 80 mg/kg) should be explored along with 40 mg/kg.

4.4.1 Mechanism of Action

In vitro studies demonstrated that raxibacumab binds PA in supernatants from Sterne, Ames and Vollum strains of *B. anthracis*. The mechanism of action appears to be by neutralization of free PA. Studies show that PA binds the surface of human and murine macrophages, and the epitope on recombinant PA that binds the cell surface receptor appears to be the same as the one binding raxibacumab (5), thereby suggesting that some structural similarity between the antigen recognition site on raxibacumab and the cell surface receptors binding site for PA exists. Raxibacumab blocks cAMP induction by recombinant ET when PA is preincubated with

5 Xu L, Frucht DM. Bacillus anthracis: a multi-faceted role for anthrax lethal toxin in thwarting host immune defenses. Internat J. Biochem. Cell Biol 2007:39;20-4.

Reference ID: 3220416

raxibacumab prior to addition of EF. Similarly, raxibacumab inhibits killing of murine macrophage (J774-A.1) cells when PA is preincubated with raxibacumab prior to addition of LF.

4.4.2 Pharmacokinetics and Pharmacodynamics

The clinical pharmacology characteristics of raxibacumab have been defined in rabbits, monkeys and humans. Raxibacumab dose selection is supported by a cross-species comparison of exposure and exposure-response (E/R) relationships described in in vivo animal models of inhalation anthrax infection. These studies show raxibacumab demonstrates the following clinical pharmacology characteristics:

- Following single intravenous administration of raxibacumab 40 mg/kg in healthy, male and female human subjects, mean raxibacumab steady-state volume of distribution was greater than plasma volume, suggesting some tissue distribution. Clearance values were much smaller than the glomerular filtration rate indicating that there is virtually no renal clearance of raxibacumab. These findings were similar in monkeys and rabbits.
- Humans achieve similar or greater exposures to raxibacumab following a single 40 mg/kg IV dose compared to rabbits and monkeys receiving the same dose. Mean raxibacumab C_{max} in humans following a single 40 mg/kg IV dose was similar to or greater than mean C_{max} values in monkeys and rabbits. Mean raxibacumab AUC_{inf} in humans following a single 40 mg/kg IV dose was 2.4 and 4.6-fold that of the mean AUC_{inf} values in monkeys and rabbits, respectively. Mean total clearance of raxibacumab was substantially slower in humans as compared to rabbits by a factor of approximately 5 and in monkeys by a factor of approximately 3. Thus, half-life of raxibacumab was longer in humans compared to mean half-lives observed in monkeys and rabbits (20.6 ± 6.5 days versus 10.1 ± 2.4 days and 4.1 ± 0.85 days, respectively). Variability in C_{max} was similar across species; %CV values ranged between approximately 13 to 17%. Variability in AUC_{inf} was wider, ranging between 13 and 32% across species.
- Serum raxibacumab concentration-time profiles were similar following administration of raxibacumab alone and with both PO and IV ciprofloxacin. Overall, exposure to ciprofloxacin appears to have no consistent or meaningful impact on raxibacumab PK. Likewise, ciprofloxacin exposure is equivalent when ciprofloxacin is administered alone and with raxibacumab. Thus, there is no significant interaction between raxibacumab and ciprofloxacin.
- The exposure-response data suggest a relationship between raxibacumab dose, concentration, and survival. The dose and dosing regimen selected by the applicant, raxibacumab 40 mg/kg IV single dose, is consistent with the known relationship between dose-concentration-response.
- Dose and concentration response for the endpoint of survival indicates potential to achieve higher response rates at higher doses. If future trials are conducted, higher doses (e.g. 60 or 80 mg/kg) should be explored along with 40 mg/kg.
- Based on in vitro binding kinetics studies, the proposed dose of 40 mg/kg raxibacumab in humans would be expected to maintain levels required for virtually complete binding (99.9%) of PA for 7 days and those required for 99% binding for up to 42 days. Despite

Reference ID: 3220416

the theoretical importance of targeting virtually complete binding of PA, the duration of time raxibacumab concentrations remain above the threshold of 202 μg/mL (99.9% PA binding) does not appear to impact efficacy in inhalational anthrax models.

- Based on predicted human PK profiles, more than 95% of humans administered a 40 mg/kg IV raxibacumab dose can be expected to achieve serum raxibacumab concentrations that are equimolar to or in excess of the highest observed serum/plasma PA concentration in any animal that died in the therapeutic intervention studies (for 48 and 28 days relative to rabbits and monkeys, respectively). In addition, modeling and simulation indicated that a single 40 mg/kg IV raxibacumab dose in humans is adequate to bind at least 99.7% of serum PA for up to 28 days after administration, in at least 95% of patients who receive raxibacumab.
- In the pivotal animal studies, animals that were treated with raxibacumab and died exhibited a higher incidence and severity of histopathologic findings in the brain versus placebo treated animals. No clear relationship between exposure and CNS pathology can be discerned based on the limited amount of data in animals that died. Overall, the raxibacumab 20 mg/kg dose group had a higher incidence and severity of CNS findings than the 40 mg/kg group in both rabbits and monkeys. Due to the small numbers of animals that exhibited specific pathology findings, no definitive conclusions about exposure-response for bacteria, hemorrhage, inflammation, or necrosis could be made.
- Raxibacumab was non-immunogenic in humans and there were no subjects with a positive anti-raxibacumab antibody response in the human studies following single or repeat (every 14 days or following ≥ 4 months) doses.

5 Sources of Clinical Data

This resubmission does not contain any new clinical data. Therefore, only a summary from the original clinical review by Dr. Sue Lim will be presented in this review. This BLA resubmission contains the results of 2 animal studies conducted in response to the deficiencies identified during the first cycle of the review.

Reference ID: 3220416

5.1 Tables of Studies/Clinical Trials

In the BLA 125349 submission the sponsor seeks raxibacumab approval for the indication of treatment of inhalational anthrax under the regulatory provisions of the Animal Rule: 21 CFR 601.900 (Biologics). The efficacy studies submitted were performed in the animal models of inhalational anthrax, and the only clinical trials conducted were PK/safety trials of raxibacumab in healthy human volunteers.

Table 50: Studies Submitted and Reviewed during the BLA First Cycle

Type of Study	Study Identifier	Title of Report	Objective of the Study	Study Design and Type of Control	Test Product(s): Dosage Regimen: Route of Administration	Number (Sex) of Subjects per group	Healthy Subjects or Diagnosis of Patients	Duration of Study
Efficacy	682-G005758	Evaluation of raxibacumab efficacy as therapeutic treatment against inhalation anthrax in the rabbit model	Rabbit efficacy	Randomized, placebo-controlled (GLP)	Placebo (raxibacumab buffer) or raxibacumab 20 and 40 mg/kg IV: Treatment was administered based on detection of increased temperature or serum PA by individual animal	9 M, 9 F	Anthrax challenged	14 Days
Efficacy	724-G005829	Evaluation of raxibacumab efficacy as therapeutic treatment against inhalation anthrax in the cynomolgus macaque	Monkey efficacy	Randomized, double-blind, placebo-controlled (GLP)	Placebo (raxibacumab buffer) or raxibacumab 20 and 40 mg/kg IV: Treatment was administered based on detection of serum PA by individual animal	12 M, 12 F (placebo) 14 M, 14 F (treatment groups)	Anthrax challenged	28 Days
Efficacy	781-G923701	Evaluating the Efficacy of Raxibacumab in Combination with Levofloxacin for Post- exposure Treatment in the New Zealand White Rabbit Inhalational Anthrax Model	Rabbit Raxibacumab / levofloxacin Therapeutic efficacy	Randomized, double-blind, placebo-controlled (GLP)	Placebo (raxibacumab buffer) + 50 mg/mL levofloxacin PO doses q12h x 6 50 mg/mL levofloxacin PO doses q12h x 6 + 40 mg/kg raxibacumab: Treatment was administered based on detection of increased temperature or serum PA by individual animal	12 M, 12 F (placebo) 20 M, 20 F (treatment groups)	Anthrax challenged	28 Days
Efficacy	789-G923702	Evaluation of the Efficacy of Raxibacumab in Combination with Ciprofloxacin for Therapeutic Treatment in the Cynomolgus Monkey Inhalation Anthrax Model	Monkey Raxibacumab / ciprofloxacin combination study	Randomized, double-blind, placebo-controlled (GLP)	Placebo (raxibacumab buffer) + 75 mg/ ciprofloxacin PO doses q12h x 6 75 mg ciprofloxacin PO doses q12h x 6 + 40 mg/kg raxibacumab: Treatment was administered based on detection of serum PA by individual animal	12 M, 12 F (placebo) 14 M, 14 F (treatment groups)	Anthrax challenged	28 Days
Safety	HGS1021-C1063.CSR	A randomized, single-blind, placebo-controlled study to evaluate the safety and tolerability of raxibacumab mAb (human monoclonal antibody to B. anthracis protective antigen) in healthy subjects	Human safety	Randomized, single-blind, placebo-controlled (non-GLP1)	Raxibacumab or placebo (raxibacumab formulation buffer) single or double dose 40 mg/kg raxibacumab IV single dose on day 0 or double dose on days 0 and 14	165 M, 164 F 240 raxibacumab (217 single dose, 23 double dose) and 80 placebo (74 single dose, 6 double dose)	Healthy subjects	56 Days after last dose
Safety	HGS1021-C1064.CSR	An open-label study to evaluate the pharmacokinetics and safety of	Safety and PK of raxibacumab	Randomized (Groups 1 & 2),	Raxibacumab single dose and/or ciprofloxacin for 7.5 days 500 mg ciprofloxacin PO doses	43 M, 45 F	Healthy subjects	56 Days

Reference ID: 3220416

Type of Study	Study Identifier	Title of Report	Objective of the Study	Study Design and Type of Control	Test Product(s): Dosage Regimen: Route of Administration	Number (Sex) of Subjects per group	Healthy Subjects or Diagnosis of Patients	Duration of Study
		raxibacumab (human monoclonal antibody to B. anthracis protective antigen) administered in combination with ciprofloxacin in healthy subjects	in combination with ciprofloxacin	open-label (non-GLP1)	q12h x 15 doses on days 0-7 + 40 mg/kg raxibacumab IV on day 5 (Group 1) 40 mg/kg raxibacumab IV on day 0 (Group 2) 400 mg ciprofloxacin IV q12h x 2 doses + 40 mg/kg raxibacumab IV on day 0 and 500 mg ciprofloxacin PO q12h x 13 doses on days 1-7			
Safety	HGS1021-C1069.CSR	An open-label study to evaluate the immunogenicity and safety of raxibacumab (human monoclonal antibody to B. anthracis protective antigen) administered in healthy subjects	Safety of raxibacumab reinjection after wash-out	Open-label (non-GLP1)	Raxibacumab single dose 40 mg/kg IV Note: All subjects enrolled had received a single 40 mg/kg raxibacumab IV dose at least 4 months prior to receiving treatment in this study	12 M, 8 F	Healthy subjects	70 Days

Table 51: Studies Submitted and Reviewed for the Current BLA Cycle

Type of Study	Study Identifier	Title of Report	Objective of the Study	Study Design and Type of Control	Test Product(s): Dosage Regimen: Route of Administration	Number (Sex) of Subjects per group	Healthy Subjects or Diagnosis of Patients	Duration of Study
Efficacy	1141-CG920871	Added Benefit of Raxibacumab with Levofloxacin vs. Levofloxacin as Post-exposure Treatment in the New Zealand White Rabbit Inhalational Anthrax Model	Rabbit efficacy	Randomized, double blind, active control, (GLP)	levofloxacin PO doses q12h x 6 50 mg/mL levofloxacin PO doses q12h x 6 + 40 mg/kg raxibacumab: Treatment was administered at 84 hours postexposure to surviving animals	18 M, 21 F (levofloxacin) 18 M, 19 F (levofloxacin + raxibacumab)	Anthrax challenged	35 Days
Efficacy Nonclinical Safety	1103-CG923704	Evaluation of raxibacumab efficacy as therapeutic treatment against inhalation anthrax in the cynomolgus macaque	Rabbit safety and efficacy	Randomized, double-blind, placebo-controlled (GLP)	Placebo (raxibacumab buffer) or raxibacumab 40 mg/kg IV: Treatment was administered based on detection of serum PA in an individual animal	13 M, 11 F	Anthrax challenged	28 Days

Reference ID: 3220416

5.2 Review Strategy

This review will focus only on the studies submitted in response to the original BLA complete response letter. For the first cycle clinical review, please refer to the clinical reviews by Drs. Sue Lim, Susan McCune, and Yuliya Yasinskaya.

5.3 Discussion of Individual Studies/Clinical Trials

See Sections 4.3.3 and 4.3.4 of this review.

6 Review of Efficacy

First Cycle Review Efficacy Summary

The original BLA for raxibacumab was submitted to the FDA on May 14, 2009. The application contained four GLP animal efficacy studies of raxibacumab for the treatment of inhalational anthrax in New Zealand white (NZW) rabbits and cynomolgus macaques in lieu of adequate and well controlled studies in humans.

NZW rabbits and cynomolgus macaques were selected for the efficacy studies of raxibacumab for treatment of inhalational anthrax. In the original BLA, four treatment studies with raxibacumab were submitted. The two efficacy studies tested raxibacumab versus placebo and two others compared the efficacy of raxibacumab plus antimicrobial and antimicrobial alone versus placebo (two studies each in rabbits and in monkeys). The animals were challenged with aerosolized *B. anthracis* spores at 200x LD_{50}. In rabbit studies sustained elevation of body temperature above baseline for 2 hours or a positive result on PA toxemia screen assay served as trigger for intervention (average 28 hours postexposure). In monkey studies intervention commenced at the time of positive PA result (average 42 hours postexposure).
The review revealed that raxibacumab at 40 mg/kg IV single dose was superior to placebo in the rabbit and monkey raxibacumab monotherapy studies. In the monkey study, raxibacumab 20 mg/kg was also superior to placebo, but in the rabbit study, superior efficacy was limited to the 40 mg/kg arm.

Table 52: Efficacy Results in Raxibacumab Monotherapy Studies

Treatment group	Cynomolgus macaques at 28 days			NZW rabbits at 14 days		
	Number (%) of survivors	P value vs. placebo[1]	95% CI of raxibacumab-placebo[2]	Number (%) of survivors	P value vs. placebo[1]	95% CI of raxibacumab-placebo[2]
Placebo	0/10 (0%)			0/13 (0%)		
20 mg/kg raxibacumab	5/12 (41.7%)	0.0396	(7.2, 68.7)	4/16 (25.0%)	0.1067	(-2.2, 50.9)
40 mg/kg raxibacumab	9/13 (69.2%)	0.0016	(31.1, 88.9)	6/17 (35.3%)	0.0237	(7.3, 59.6)

[3] P value based on 2-sided Fisher's exact test for the comparison vs the placebo control group.
[4] 95% CIs are exact confidence intervals

Reference ID: 3220416

When given in combination with antimicrobials, the efficacy of the combination was high, but the efficacy of antimicrobials alone was also high. This raised the question whether the animal models adequately represented advanced anthrax disease in humans, where high mortality was observed despite antimicrobial treatment.

Table 53: Efficacy Results in Raxibacumab Combination Studies with Antimicrobials

Treatment group	Cynomolgus macaques at 28 days		NZW rabbits at 28 days	
	Number (%) of survivors	95% CI of Cipro/raxibacumab-ciprofloxacin[2]	Number (%) of survivors	95% CI of Levo/raxibacumab-levofloxacin[2]
Placebo	0/10 (0%)	-	0/10 (0%)	-
Antimicrobial alone[1]	13/13 (100%)	-	19/20 (95.0%)	-
Antimicrobial[1] + 40 mg/kg raxibacumab	11/13 (84.6%)	(-45.5, 11.4)	16/17 (94.1%)	(-23.9, 19.6)

1 NHP study antimicrobial was 75 mg ciprofloxacin twice daily x 3 days
NZW rabbit study antimicrobial was 50 mg/kg levofloxacin daily x 3 days
2 95% CIs are exact confidence intervals

Based on the information from the natural history of inhalational anthrax disease in man, in rabbits, and monkeys; the mechanism of action of raxibacumab; and the efficacy of raxibacumab 40 mg/kg IV in the animal studies, clinical reviewer concluded that NZW rabbit and cynomolgus macaque models of inhalational anthrax disease are reflective of human disease and the efficacy findings from the therapeutic studies in these models could be extrapolated to humans.

However, several limitations of the efficacy findings in pivotal and combination animal studies and in animal models themselves were discussed.

First, the timing of intervention is likely to differ between animals and humans. In the experimental settings, animals were closely monitored for either an elevation in temperature (rabbits) or a positive plasma PA (rabbits and monkeys), and once detected, a therapeutic intervention was delivered. Humans may have delayed presentation to the healthcare setting or be given alternative diagnoses prior to the definitive diagnosis of anthrax being made. As the effects of delayed administration of raxibacumab after the onset of clinical symptoms has not been studied in the animal models, it is unclear how long raxibacumab administration can be delayed and still provide survival benefit.

Second, the combination efficacy studies demonstrated very high efficacies (nearly 100%) of levofloxacin in NZW rabbits and ciprofloxacin in monkeys, respectively. In contrast, during the 2001 anthrax attacks 5 of the 11 people who developed inhalational anthrax died despite best available antimicrobial and supportive treatment. In addition, a question regarding efficacy interaction between raxibacumab and antimicrobial when given together for the treatment of inhalational anthrax remained unanswered. Twelve of twenty four AIDAC committee members pointed out a number of deficiencies in the combination study design that precluded definite

Reference ID: 3220416

conclusions regarding efficacy interactions, e.g. timing of intervention, antibiotic regimen, and the absence of raxibacumab alone arm. A majority of the committee members (17/24) recommended additional evaluation of the contribution offered by raxibacumab to the efficacy of antimicrobial alone.

Third, the conditions of an animal experiment do not include any supportive care that human patients with inhalational anthrax will receive in a healthcare or clinical trial setting, including but not limited to mechanical ventilation, fluid resuscitation, pleural fluid drainage, dialysis, etc.

Fourth, due to concerns about the reliability of raxibacumab and antimicrobial PK data generated for the original BLA, FDA was unable to conclude that the proposed human dosing regimen would result in the exposures similar to those seen at the efficacious dose in animal studies. Because of these concerns, it was also unclear that the antimicrobial exposures in the combination animal studies did not exceed human exposures achieved with the approved human dose regimen.

Resubmission Review Efficacy Summary

Two new animal efficacy studies included in this resubmission of the BLA were conducted to evaluate the added benefit (over antibacterials) of raxibacumab when the treatment intervention is delayed and to provide additional information on the CNS toxicity findings seen in the original BLA submission.

The design of the CNS safety study 1103 in rabbits was similar to the previously-conducted raxibacumab monotherapy study in rabbits, where animals challenged with an aerosolized dose of *B. anthracis* spores ($200xLD_{50}$) were randomized and treated in a blinded fashion either with raxibacumab 40 mg/kg IV single dose or matching placebo. The primary efficacy endpoint was survival at Day 28 post challenge. Survivors and non-survivors underwent systematic blinded histopathology examination with immunohistochemistry analysis of brain tissue for raxibacumab and endogenous IGG.

The added benefit study 1141 in rabbits evaluated efficacy of the combination levofloxacin and raxibacumab treatment relative to levofloxacin alone. Surviving animals exposed to an aerosolized dose of *B. anthracis* spores ($200xLD_{50}$) underwent randomization and treatment in a blinded manner at 84 hours post challenge to achieve survival with antimicrobial alone that is reflective of historical survival in humans with inhalational anthrax.

6.1 Indication

(b) (4)

Under the Animal Rule, animal efficacy data was used to support the licensure of raxibacumab for treatment of inhalational anthrax. Efficacy was demonstrated in more than one animal species expected to react with a response predictive for humans and the studies used an endpoint that is clearly related to the desired benefit in humans.

Reference ID: 3220416

Six animal therapeutic studies of inhalational anthrax were conducted by the applicant: two in cynomolgus macaques and four in New Zealand White rabbits. Among them, three studies evaluated efficacy of raxibacumab as monotherapy against placebo and three others efficacy of raxibacumab administered together with an antimicrobial. Two monotherapy studies and two combination therapy studies in monkey and rabbit animal models of inhalational anthrax have been reviewed by Drs. Susan McCune and Yuliya Yasinskaya during 1st cycle of the BLA review in 2009. This review will focus on studies 1103 and 1141 conducted in the rabbit model of inhalational anthrax and designed to evaluate raxibacumab effect on CNS in surviving and non-surviving animals and added benefit of raxibacumab to antimicrobials as a delayed treatment, respectively. See sections 4.3.3 and 4.3.4 for the complete review of these studies.

6.1.1 Methods

The final reports and accompanying datasets for Studies 1103 an 1141 were reviewed and the findings are described in Section 4.3.3 and 4.3.4, respectively.

6.1.2 Subject Demographics and Disposition

In study 1103 forty-eight experimentally naïve NZW rabbits of both sexes were exposed to a target aerosol of *B. anthracis* spores at 200xLD_{50} and randomized to receive either raxibacumab alone or placebo at the time of positive PA toxemia. All challenged animals were treated as randomized, twenty-four animals in each treatment group. All but one animal were bacteremic and toxemic prior to treatment.

In study 1141 one hundred and eighty experimentally naïve NZW rabbits of both sexes were exposed to a target aerosol of *B. anthracis* spores at 200xLD_{50} and the surviving animals were randomized to receive either levofloxacin alone or a combination of levofloxacin and raxibacumab treatment after an 84 hour delay post challenge. Seventy six challenged animals survived to 84 hours post challenged and were treated as randomized: 37 rabbits with levofloxacin alone and 39 with a combination therapy of levofloxacin + raxibacumab.

6.1.3 Analysis of Primary Endpoint(s)

The primary endpoint was survival at the end of the study: Day 28 for study 1103 and Day 35 for study 1141.
In study 1103 treatment with raxibacumab at 40 mg/kg IV single dose resulted in 45.8% survival compared to 0% survival with placebo, p=0.0002. Similar improvements in survival were seen in the population of animals toxemic or bacteremic prior to treatment.

Reference ID: 3220416

Table 54: Primary Efficacy Analysis, Study 1103

Study population	Placebo	Raxibacumab
Primary analysis ITT population		
	N = 24	N=24
Survival, n (%)	0 (0)	11 (45.8)
Difference (95% CI)	45.8 (25.3, 67.2)	
P value*	0.0002	
Modified ITT(toxemic animals)		
	N=24	N=23
Survival, n (%)	0 (0)	10 (43.5)
Difference (95% CI)	43.5 (22.9 , 65.5)	
P value*	0.0002	
Modified ITT (bacteremic animals)		
	N=22	N=23
Survival, n (%)	0 (0)	10 (43.5)
Difference (95% CI)	43.5 (22.5 , 65.5)	
P value*	0.0006	
Modified ITT (animal died from non anthrax causes excluded)		
	N=24	N=23
Survival, n (%)	0 (0)	11 (47.8)
Difference (95% CI)	47.8 (26.6 , 69.4)	
P value*	0.0001	

In study 1141 treatment with raxibacumab in addition to levofloxacin resulted in 17% (p=0.0874) improvement in survival over levofloxacin alone. Efficacy with levofloxacin approached survival rates reported historically in humans treated with antimicrobials. Raxibacumab did not interfere with efficacy of antimicrobial. Efficacy results in animals bacteremic prior to treatment were similar to that of all treated population.

Table 59: Primary Efficacy Analysis, Study 1141

Study population	Levofloxacin + Placebo	Levofloxacin +Raxibacumab
Primary analysis ITT population, all toxemic animals		
	N = 37	N=39
Survival, n (%)	24 (64.9)	32 (82.1)
Difference (95% CI)	17.2 (-2.35 , 36.72)	
P value*	0.0874	
Modified ITT (bacteremic animals)		
	N=37	N=38
Survival, n (%)	24 (64.9)	31 (81.6)
Difference (95% CI)	16.7 (-3.00 , 36.43)	
P value*	0.0998	

Reference ID: 3220416

6.1.4 Analysis of Secondary Endpoints(s)

Time to death was the secondary analysis for both studies. Kaplan-Meier survival curves are displayed below.

6.1.5 Analysis of Secondary Endpoints(s)

Figure 7: Survival Time During 28-day Study Period in Rabbits Toxemic and/or Bacteremic at Treatment Initiation, Study 1103

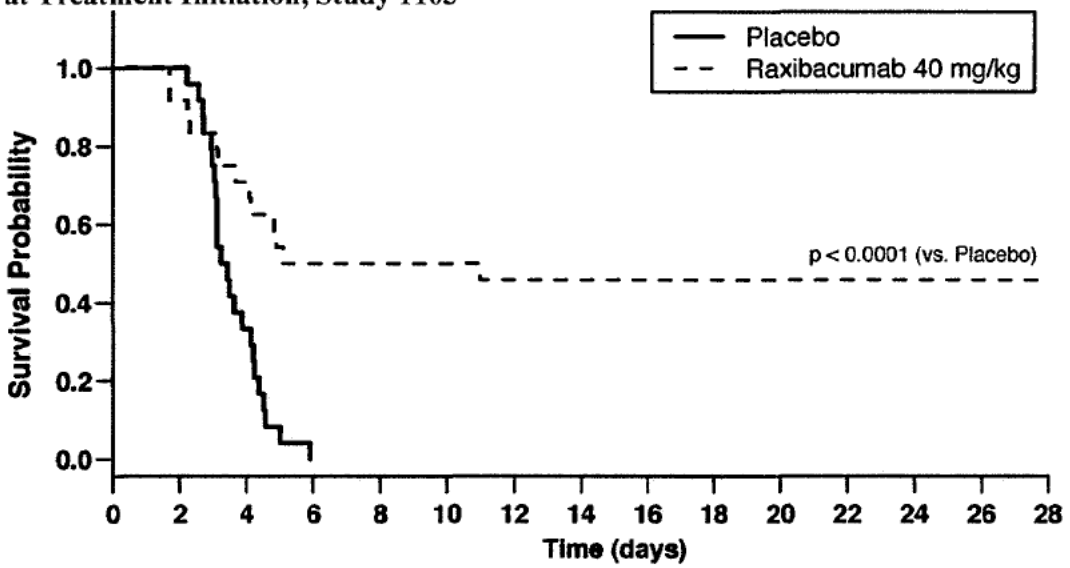
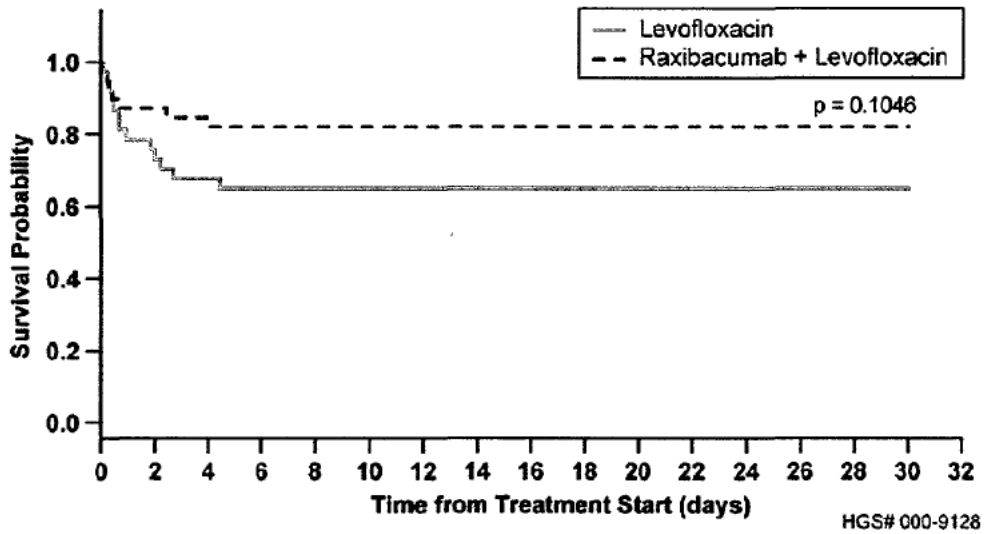


Figure 7-3 from HGS report p. 35
P-value obtained from a log-rank test comparing survival time between placebo and raxibacumab-treated groups.

Reference ID: 3220416

Figure 8: Survival Time During 35-day Study Period in Rabbits Survived to Treatment, Study 1141



(Figure 1 from Battelle Study Report, pg 23).

6.1.6 Other Endpoints

Not applicable

6.1.7 Subpopulations

Sensitivity analyses were performed in the populations of animals bacteremic or toxemic prior to treatment in addition to the primary efficacy analysis in ITT population of animals.

6.1.8 Analysis of Clinical Information Relevant to Dosing Recommendations

A dose-dependent improvement in survival was observed in raxibacumab monotherapy efficacy studies in monkey and rabbit animal models of inhalational anthrax. A tested dose of 40 mg/kg achieved the highest therapeutic efficacy in both animal models. Raxibacumab exposures (Cmax and AUC) at a 40 mg/kg IV single dose in humans are similar or greater than those achieved at a 40 mg/kg dose in animals.

6.1.9 Discussion of Persistence of Efficacy and/or Tolerance Effects

The survival benefit of raxibacumab as a single IV dose of 40 mg/kg persisted until the end of the therapeutic animal studies 14 to 35 days post exposure. By day 14 animals are able to mount

Reference ID: 3220416

an immune response as evident by positive Toxin Neutralizing Antibody (TNA) titers. No late deaths had occurred in raxibacumab treated animals.

6.1.10 Additional Efficacy Issues/Analyses

Raxibacumab has limited penetration into CNS and does not prevent or adequately treat anthrax meningitis and should be used in addition to antimicrobials that penetrate the blood-brain barrier. See Section 4.3.3.

7 Review of Safety

Safety Summary

A total of 326 healthy volunteers received the recommended dose of raxibacumab of 40 mg/kg manufactured by the M11 process (the to-be-marketed product) and were evaluated for safety of raxibacumab. Of these, 303 subjects received a single dose (Study HGS1021-C1064 and Study HGS1021-C1063), 23 subjects received a second dose two weeks after their first dose (Study HGS1021-1063), and 20 subjects from Study HGS1021-C1064 received a 2nd dose more than 4 months after their initial dose (Study HGS1021-C1069). In Study HGS-1021-C1064, 48 subjects who received raxibacumab also received ciprofloxacin as part of this drug interaction study.

Studies HGS1021-C1064, HGS1021-C1063 and HGS1021-C1069 are briefly summarized in the Table 7.2-1 below.

7.1 Methods

7.1.1 Studies/Clinical Trials Used to Evaluate Safety

Table 55: Studies in Healthy Volunteers Included in the Safety Analysis

Reference ID: 3220416

Study ID	Design Control Type	Group: Entered/ Completed	Indication	Sex M/F; Median age (range); Race	Duration	Study and Control Drug, Route, Regimen, Dosing frequency
HGS-1021-C1063	P3, R, SB, PC, RD	Double dose raxibacumab:24/23 Double dose placebo: 8/5 Single dose raxibacumab: 216/206 Single dose placebo: 72/68	Safety and PK in healthy subjects with single and repeat injection	156M/164F; 39.8 (18-88); 259W, 30B, 24A, 13NL, 10MR, 5NH	Single-dose (day 0) Double-dose (days 0 and 14) with 56 day follow-up	Raxibacumab, IV Placebo, IV; Single dose (day 0), double dose (days 0 and 14)
HGS-1021-C1069	P2/3, SG. OL, RD	All 20 subjects treated with a single 40 mg/kg IV raxibacumab dose at least 4 months after an initial 40 mg/kg. Single dose administered IV during study HGS-1021-C1064	Safety and PK in healthy subjects with repeat injection	12M/8F; 38.5 (22-61); 13W, 7B, 4HL	Single-dose with 56 day follow-up	Raxibacumab, IV; single dose
HGS-1021-C1064	P2/3, R, OL, single-dose	PO cipro, IV raxi : 32/26 IV raxi: 28/22 IV cipro, IV raxi :28/22	Safety and PK in healthy subjects with concomitant use with ciprofloxacin	43M/45F; 34.4 (18-61); 53W, 31B, 3NL, 1BNH, 21HL	7 days with 56 day follow-up	Raxibacumab IV; Ciprofloxacin PO; Ciprofloxacin IV Raxibacumab IV single dose; Cipro PO Q12h, 15 doses, Cipro IV 2 doses 12h apart

A, Asian; B, Black; BNH, Black and Native Hawaiian; HL Hispanic or Latino; MR, Multiracial; NH Native Hawaiian; NL Not listed; W, White
CPL, Cipro, Ciprofloxacin
OL, Open label; P1, Phase 1; P2/3, Phase 2/3; P3, Phase 3
PC, Placebo-controlled; R, Randomized; RD Repeat dose; SB, Single blind; SG, Single Group;
Modified from original BLA Integrated Summary of Safety Table 1-1 pp. 18-19

Demographics for all 326 subjects are shown in the table below. Most subjects were white, within a median age of 38 years (range 18 to 88 years) with a similar number of males and females enrolled.

Reference ID: 3220416

Table 56: Demographics of Subjects Enrolled Across Studies

	Single-Dose (HGS1021-C1063 + HGS1021-C1064[1]) N = 283	Double-Dose ≥ 4 Months Apart (HGS1021-C1069) N = 20	Double-Dose 14 Days Apart (HGS1021-C1063) N = 23	All Raxibacumab Treated (HGS1021-C1064/ HGS1021-C1069[2] +HGS1021-C1063) N = 326
Sex				
Male	130 (45.9 %)	12 (60.0 %)	10 (43.5 %)	152 (46.6 %)
Female	153 (54.1 %)	8 (40.0 %)	13 (56.5 %)	174 (53.4 %)
Race[3]				
White	207 (73.1 %)	14 (70.0 %)	21 (91.3 %)	242 (74.2 %)
Asian	18 (6.4 %)	-	-	18 (5.5 %)
Black or African American	48 (17.0 %)	6 (30.0 %)	1 (4.3 %)	55 (16.9 %)
American Indian or Alaska Native	1 (0.4 %)	-	-	1 (0.3 %)
Native Hawaiian or Other Pacific Islander	5 (1.8 %)	-	-	5 (1.5 %)
Not Listed	14 (4.9 %)	-	1 (4.3 %)	15 (4.6 %)
Multiracial	9 (3.2 %)	-	-	9 (2.8 %)
Hispanic or Latino origin	46 (16.3 %)	3 (15.0 %)	1 (4.3 %)	50 (15.3 %)
Age (years)[4]				
n	283	20	23	326
Mean ± SD	39.0 ± 15.4	40.5 ± 13.3	48.5 ± 14.6	39.8 ± 15.3
Median	36.5	38.5	50.5	37.4
Range	(18.1, 87.9)	(22.4, 60.6)	(22.1, 76.2)	(18.1, 87.9)
Age group				
< 65 years	264 (93.3 %)	20 (100.0 %)	21 (91.3 %)	305 (93.6 %)
> = 65 years	19 (6.7 %)	-	2 (8.7 %)	21 (6.4 %)
Weight (kg)				
n	283	20	23	326
Mean ± SD	76.3 ± 17.3	78.4 ± 13.9	82.6 ± 19.2	76.9 ± 17.3
Median	74.7	77.2	79.9	75.6
Range	(44.6, 155.9)	(56.5, 98.6)	(54.8, 121.6)	(44.6, 155.9)

1 Excluded the 20 subjects who received a 2nd Raxibacumab dose in HGS1021-C1069.

2 Subjects participating in both HGS1021-C1064 and HGS1021-C1069 were counted once only with race, age, and weight based on HGS1021-C1064.

3 Subjects who checked more than one race category were counted under individual race categories as well as the multiracial category.

4 Age was calculated from the date of birth to the date of randomization.

Adapted from original BLA submission, ISS, p.54

Reference ID: 3220416

7.1.2 Categorization of Adverse Events

Usual definitions were used in the identification and categorization of adverse events. An adverse event was defined as any unfavorable or unintended sign, symptom, or disease that is temporally associated with the use of a study agent but is not necessarily caused by the study agent. This included worsening (e.g., increase in frequency or severity) of pre-existing conditions. All AEs that were identified from the start of study agent administration on Day 0 through Day 56 (single-dose subjects) or Day 70 (double-dose subjects) were to be recorded on the AE CRF. The record of each AE on the CRF included AE description or diagnosis, start date, end date, whether the AE was ongoing, seriousness, severity, action taken with study agent, relationship to study agent (causality), and outcome. It was required that all data fields on the AE CRF be completed.

The severity of an AE was graded using the modified DMID toxicity tables. For AEs not included in these toxicity tables, the following guidelines applied:
Mild - causing no limitation of usual activities
Moderate - causing some limitation of usual activities
Severe - causing inability to carry out usual activities
Life-threatening - potentially life threatening or disabling

A serious adverse event was defined as any AE resulting in any of the following outcomes:
- **Death**
- **Was life threatening (i.e., was an immediate threat to life)**
- **Inpatient hospitalization**
- **Prolongation of an existing hospitalization**
- **Persistent or significant disability or incapacity**
- **Congenital anomaly or birth defect**
- **Medically important**

The causality assessment was to be made by the investigator based on information available at the time that the SAE worksheet was completed.

7.1.3 Pooling of Data Across Studies/Clinical Trials to Estimate and Compare Incidence

All single dose exposures were pooled for analysis. This included subjects from studies HGS1021-C1064 and HGS1021-C1063. Double dose subjects were presented both separately (study HGS1021-1063, where subjects received a second dose 2 weeks after their initial dose, and study HGS1021-C1069, where subjects received a second dose more than 4 months after their initial dose) and pooled for analysis.

Reference ID: 3220416

7.2 Adequacy of Safety Assessments

7.2.1 Overall Exposure at Appropriate Doses/Durations and Demographics of Target Populations

Three hundred twenty-six subjects received a 40 mg/kg IV dose of raxibacumab manufactured with the process intended for licensure. With a safety database of >300 subjects a serious or fatal adverse event of frequency of 1% or greater could be excluded with 95% confidence. Elderly subjects comprised 6.4% of the safety database. Pediatric subjects and pregnant or nursing mothers were not studied.

7.2.2 Explorations for Dose Response

One dose escalation study was performed in the raxibacumab development program. Study PAM-NH-01, which used the M10 formulation of the product, was a phase 1 single-blind, placebo-controlled, single-injection study which evaluated the safety and pharmacokinetics of 3 different single doses of PA mAb [0.3 mg/kg, 1 mg/kg and 3 mg/kg given intramuscularly (IM in **2 different sites – gluteus and vastus** lateralis)] and 5 different single doses of PA mAb [1 mg/kg, 3 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg given intravenously (IV)] in healthy subjects. While this first in man study predominantly used doses well below the putative therapeutic dose and assessed the safety of a developmental product rather than the to-be-marketed scale-up product, it did not find any dose-related AEs. Dose response exploration for the M11 formulation was limited only to subjects who received 2 doses of raxibacumab 14 days apart (Study HGS1021-C1063), but would not include subjects enrolled in Study HGS1021-1069 who received their second dose more than 4 months after the first dose, as washout was complete by this time.

The table below shows the number of treatment emergent AEs in the groups relative to the number of doses received. Note that placebo rates are not shown

Table 57: Number of Raxibacumab-Treated Subjects with Treatment Emergent AEs

	Placebo C1063[1] N=80 (%)	Single Dose C1063+C1064[2] N=283 (%)	Double Dose C1063 Post 1st dose prior to 2nd dose N=23 (%)	Double Dose C1069 Post 1st dose prior to 2nd dose N=20 (%)	Double Dose C1063 Post 2nd dose N=23 (%)	Double Dose C1069 Post 2nd dose N=20 (%)	Total Raxibacumab Subjects[3] N=326 (%)
At least 1 AE	38 (47.5)	143 (50.5)	4 (17.4)	6 (30.0)	9 (39.1)	8 (40.0)	170 (52.1)
At least 1 SAE	-	1 (0.4)	0	0	1 (4.3)	0	2 (0.6)
At least 1 severe AE	3 (1.4)	5 (1.8)	0	0	1 (4.3)	0	6 (1.8)

[1] Includes both single (n=74) and double (n=6) dose placebo subjects.
[2] Excluded the 20 subjects who received a 2nd raxibacumab dose in HGS1021-C1069.
[3] Subjects participating in both HGS1021-C1064 and HGS1021-C1069 were counted only once.

Reference ID: 3220416

The number of subjects reporting at least 1 AE was higher in the single dose group (50.5%) compared to either of the two double-dose groups prior to their 2nd dose (17.4% and 30%). This observation may be related to the small study numbers in the double dose groups. The number of subjects reporting at least 1 AE post 2nd dose was also lower (approximately 40% in both groups), which again could be related to small study numbers, or the absence of a dose-adverse event relationship when a second raxibacumab dose is administered 2 weeks or later following the first dose. Overall, the numbers of SAEs and AEs of severe intensity were infrequently reported.

7.2.3 Special Animal and/or In Vitro Testing

Three relevant nonclinical studies were reviewed in detail by the Pharmacology/Toxicology team and are briefly summarized here:

The initial *ex vivo* tissue cross-reactivity study in human and cynomolgus monkey tissue showed specific cytoplasmic (non-membrane) binding of raxibacumab (M10 manufacturing process) in skeletal muscle and thyroid (both species), endometrium (human only) and breast and prostate (monkey only). These tissues were identified as possible target organs for toxicity.

The second *ex vivo* tissue cross-reactivity study was performed on tissues of NZW rabbits, cynomolgus macaques, and man and tested raxibacumab produced by the to-be-licensed M11 manufacturing process. This study showed no thyroid histopathology or abnormal levels of circulating thyroid hormones. Please refer to **Dr. Terry Miller's summary in Section 4.3** Preclinical Pharmacology/Toxicology for additional information on these studies.

Regarding safety, three non-clinical studies were reviewed in detail for the purposes of the CDC IND and are briefly summarized here:

The 120-day monkey study evaluated raxibacumab 40 mg/kg given on days 0, 13, and 25, either IM or IV and again at day 69 by IM route. No difference in weight or feeding between the placebo and raxibacumab arms, and no gross pathological changes in the study arms were reported. Local inflammation resulting from trauma at site of IM injection was noted. Some animals had microcysts in the thyroid. Some animals had chronic inflammation in the prostate. These findings were consistent with the tissue cross-reactivity study above. CPK and thyroid hormones were reported to be unremarkable. Although the histological findings were not judged to be significant, these organs were mentioned as possible targets of toxicity. No anti Fab or Fc antibodies were found in these animals. There were no gross or histopathological CNS changes reported.

The rabbit embryo-fetal toxicity study tested raxibacumab 0, 40, 120 mg/kg IV on gestation days 7 and 14. No adverse effects on the fetus or mother were identified, and the NOAEL was determined to be 120 mg/kg, the highest dose tested. Minor skeletal variations were noted, but these were not considered as adverse effects, and were stated to be within the rate for such variations seen in rabbits. It was briefly noted that in this study, 4 animals (2/20 in 40 mg/kg

Reference ID: 3220416

arm, 2/20 in 120 mg/kg arm) with anti-raxibacumab antibodies had reduced serum raxibacumab levels compared to animals without such antibodies. However, caution should be exercised when interpreting the significance of these findings, as detection of the anti-raxibacumab antibodies is hindered when the raxibacumab concentration exceeds 150 μ/mL.

7.2.4 Routine Clinical Testing

Routine physical exams, vital signs, and laboratory tests were collected for all studies.

7.2.5 Metabolic, Clearance, and Interaction Workup

Please refer to the Clinical Pharmacology first cycle BLA review by Dr. Kimberly Bergman of for details.

7.2.6 Evaluation for Potential Adverse Events for Similar Drugs in Drug Class

Not applicable

7.3 Major Safety Results

7.3.1 Deaths

There was one death reported among the 326 subjects. Subject US001-002 was enrolled in study HGS1021-C1063 and randomized to the placebo double-dose group. This subject died from injuries sustained in a motor vehicle accident.

7.3.2 Nonfatal Serious Adverse Events

Three subjects (0.9%) experienced a serious adverse event, which are shown in Table below.

Table 58: Serious Adverse Events Reported Across All Studies

Study	Treatment received	Subject ID	A/S /R	MedDRA system organ class	Preferred Term	AE Start Day/ Duration (days)	Outcome	Relationship to study drug	Action taken with study drug
HGS1021-C1063	Placebo double dose	US001-002	45/ F/W	Injury, poisoning and procedural	Injury	39/1	Fatal	Not related	None
HGS1021-C1063	Raxibacumab double dose	US002-017	52/ F/W	Hepatobiliary disorders	Cholecystitis	24/3	Recovered/ resolved	Possibly related	None
HGS1021-C1064	Raxibacumab single dose	US001-005	48/ B/ M	Psychiatric disorders	Schizophrenia	5/ ongoing	Not recovered/ not resolved	Probably not related	None

A/S/R: age/sex/race

Reference ID: 3220416

The narratives of the nonfatal Serious Adverse events are presented below:

Subject US001-017

A 52-year-old white female in the raxibacumab double-dose group, developed upper abdominal pain, nausea, and low grade fever 10 days after her 2nd raxibacumab dose (Day 24). The subject was hospitalized and underwent a cholecystectomy on Day 26; the post-operative diagnosis was confirmed to be cholecystitis. Dosing was completed and no action was taken with regard to raxibacumab. The investigator indicated that the cholecystitis (severe) was possibly related to raxibacumab, and most likely caused by progression of concurrent disease as the subject had multiple risk factors for cholelithiasis including female gender, age, obesity (body weight of 111.5 kg), elevated serum glucose and elevated triglycerides.

Subject US001-005

A 48-year-old black male received his 1st dose of ciprofloxacin (500 mg PO) on Day 0 (31Jan 2007) and his raxibacumab dose (40 mg/kg IV) on Day 5 per protocol. Relevant medical history which was not disclosed at screening included diagnoses of schizophrenia, depression, and bipolar disorder. There were no concomitant medications taken. The subject completed ciprofloxacin dosing as planned on Day 7. At the Day 47 follow-up visit, the subject reported auditory hallucinations occurring 2 to 3 times per week. He stated that the auditory hallucinations began after receiving raxibacumab; however, he did not report this to the clinical staff at that time. The subject denied suicidal ideation. The subject denied prior history of symptoms before entering the study and denied any history of psychiatric illness for himself or his family. A physical examination was within normal limits with no evidence of agitation, anxiety or depression. The subject left the clinical unit prior to completing further psychiatric evaluation. The subject later returned for evaluation and disclosed a past medical history of depression, bipolar disorder, and schizophrenia dating back to his early 20s. On Day 61, the subject was diagnosed with schizophrenia and prescribed olanzapine. As the subject received all 15 ciprofloxacin doses and the single raxibacumab dose per study schedule prior to disclosing the SAE, no action was taken with regard to study agents. The investigator revised the event term to schizophrenia with a severity grading of life-threatening, and indicated that it was probably not related to raxibacumab or ciprofloxacin. The schizophrenia was ongoing at the end of the study.

Both cases of cholecystitis and schizophrenia were deemed unlikely to be related to raxibacumab based on temporal presentation (long duration between raxibacumab dosing and onset of the AE) and the presence of pre-existing conditions which could account for the SAE.

7.3.3 Dropouts and/or Discontinuations

There were no subject withdrawals due to adverse reactions (AR). However, there were four subjects (1.2%) who experienced AR and discontinued treatment, as shown in Table 7.2-4.

Reference ID: 3220416

Table 59: Subjects with AR Resulting in Discontinuation of Treatment

Study	Treatment received	Subject ID	A/S /R	Preferred Term	Serious/ Severity	Study Day	Relationship to study drug	Notes
HGS1021-C1064	Cipro/ raxibacumab	US003-006	36/ F/W	Urticaria	No/mild	5	Probably related	No premedication with diphenhydramine
HGS1021-C1064	Cipro/ raxibacumab	US003-028	56/ F/W	Clonus	No/mild	5	Probably related	**"Left arm and leg clonic muscular contractions" and "facial flushing", both mild and** resolved in 1 day; no change in vital signs
HGS1021-C1064	Raxibacumab single dose	US003-002	33/ F/W	Urticaria	No/mild	1	Probably related	No premedication with diphenhydramine
HGS1021-C1063	Raxibacumab single dose	US002-011	26/ M/ B	Dyspnea	No/mode rate	0	Probably not related	**"Difficulty breathing" of** moderate severity during infusion; no sx anaphylaxis or rash, no changes in vital signs; investigator attributed the **subject's symptoms as most** likely due to esophageal spasm caused by rapid eating prior to the infusion and/or anxiousness
HGS1021-C1063	Placebo double dose	US001-022	59/ M/ W	Skin infection	No/mode rate	12	-	

A/S/R: age/sex/race

7.3.4 Severe Adverse Events

Adverse events of severe intensity were reported in 1.9% of raxibacumab-treated subjects and are shown in Table below.

Table 60: Severe Adverse Events across Studies (FDA Analysis)

MedDRA system organ class	MedDRA Preferred Term	Single Dose - C1063+C1064 N=283 (%)	Double Dose - C1063 N=23 (%)	All raxibacumab subjects* N=326 (%)
Hepatobiliary disorders	Cholecystitis	0	1 (4.4)	1 (0.2)
Infections and infestations	Influenza	1 (0.4)	0	1 (0.2)
Investigations	Blood amylase increased	1 (0.4)	0	1 (0.2)
	Blood creatine phosphokinase increased	1 (0.4)	0	1 (0.2)
	Prothrombin time prolonged	1 (0.4)	0	1 (0.2)
Psychiatric disorders	Schizophrenia	1(0.4)	0	1 (0.2)

*There were no AR of severe intensity reported in the double dose separated by ≥ 4 months group (Study HGS1021-1069) although they are included in the Total Subject count

There were 2 severe and serious adverse events in raxibacumab-treated subjects (cholecystitis, schizophrenia) which were described previously. In placebo-treated subjects, there was a single event of severe leukocytosis reported. All other severe adverse events were unlikely to be related to raxibacumab.

Reference ID: 3220416

7.3.5 Submission Specific Primary Safety Concerns

CNS Adverse Events
Based on the increased CNS findings in animals treated with raxibacumab that died of anthrax in the pivotal efficacy studies, CNS adverse events were examined more closely in the human safety database.
In the nervous system disorders SOC, headache was by far the most frequently reported adverse events. It was reported in 8.8% of single dose subjects, 14% of double dose subjects, and 10% of placebo subjects. The following nervous system disorders adverse events were reported (Table 7.3.5-1).

Table 61: Nervous System Disorders across All Studies (FDA Analysis)

MedDRA system organ class	MedDRA Preferred Term	Placebo* – C1063 N=80 (%)	Single Dose - C1063+C1064 N=283 (%)	Double Dose - C1063 N=23 (%)	Double Dose - C1069 N=20 (%)	All raxibacumab subjects N=326 (%)
Nervous system disorders	Clonus	0	1 (0.4)	0	0	1 (0.2)
	Dizziness	1 (1.3)	3 (1.1)	0	0	3 (0.6)
	Headache	8 (10)	25 (8.8)	1 (4.3)	5 (25)	31 (9.5)
	Lethargy	1 (1.3)	0	0	1 (5)	1 (0.2)
	Paresthesia	0	1 (0.4)	0	0	1 (0.2)
	Somnolence	0	4 (1.4)	1 (4.3)	0	5 (1.5)
	Syncope vasovagal	0	2 (0.7)	0	0	2 (0.4)
	Tension headache	2 (2.5)	0	0	0	0

* includes both single (n=74) and double (n=6) dose placebo subjects

Headache was reported in similar percentages of placebo and raxibacumab treated subjects (10% and 9.5%, respectively). There were an additional two reports of tension headache in the placebo group.

There were no reports of meningismus across human safety studies. A single raxibacumab treated subject (US004-000030) enrolled in study HGS1021-C1063 reported fever (mild) on day 41 in conjunction with vomiting (mild), both of one day duration with no action taken. Similarly, there was one placebo subject US005-000046 enrolled in Study HGS1021-C1063 with pyrexia and headache reported on Study day 36, **again of one day's duration with no** intervention. It is unlikely that either of these represented cases of meningitis.

Infections/Infestations SOC
In contrast, within the infections and infestations SOC, upper respiratory tract infection occurred in 11.6% of double dose raxibacumab subjects, compared to 3.9% of single dose raxibacumab subjects and 5.0% of placebo subjects. However, other infections were not reported more frequently in the active treatment groups to suggest a uniform immunomodulatory/suppressive effect of raxibacumab. All AEs in the raxibacumab treated subjects ranged in frequency from 0.2% to 4.9%.

Reference ID: 3220416

Gastrointestinal SOC
In the gastrointestinal disorders SOC, rates of abdominal pain, diarrhea, nausea and vomiting were all higher in the placebo group compared to all raxibacumab subjects.

Dermatologic (Skin and subcutaneous tissue) SOC
Lastly, in the skin and subcutaneous tissue disorders SOC, adverse reactions reported by the raxibacumab-treated subjects ranged from 0.2% to 1.8% in frequency. Pruritis and rash were reported in all raxibacumab subjects at 2.1% and 1.8% respectively, and were comparable to placebo rates (pruritis 0%, rash 1.3%). In Study HGS1021-C1064 evaluating raxibacumab PK in combination with ciprofloxacin, five of the first 25 subjects dosed with raxibacumab developed dermatologic events following administration. Three subjects responded to oral diphenhydramine, and two discontinued the infusion with resolution (one with diphenhydramine and one spontaneous). As a result of these findings, the protocol was amended so that all subsequent subjects received diphenhydramine before raxibacumab infusion. At the completion of HGS1021-C1064 a total of 8/88 (9.1%) subjects reported rash. In comparison, rash occurred in 6/27 (22%) of a subset of subjects who did not receive diphenhydramine premedication. Furthermore, to closely match the anticipated use of raxibacumab in human inhalational anthrax, diphenhydramine pretreatment was also given to the monkeys participating in the inhalational anthrax efficacy studies.

7.4 Supportive Safety Results

7.4.1 Common Adverse Events

The most frequently reported system organ classes (SOC) for AR in the raxibacumab-treated subjects were nervous system disorders (13.2%), infections and infestations (11.3%), and gastrointestinal disorders and skin and subcutaneous tissue disorders (both 9.2%). These were all higher in the raxibacumab-treated subjects compared to placebo.

7.4.2 Laboratory Findings

Laboratory tests with toxicity grade greater than or equal to 3 (severe) were infrequently reported. Overall, the incidence of laboratory abnormalities was comparable between raxibacumab-treated and placebo-treated subjects (only active treatment AR reported here). The incidence was not higher after a second raxibacumab administration than after a single administration. Most lab abnormalities were of low grade (Grade 2 or less), with grade 3 and 4 laboratory abnormalities infrequently reported, isolated and transient.

7.4.3 Vital Signs

Three subjects treated with a single raxibacumab dose in study HGS1021-C1063 had AEs of hypertension, which were judged to be not related to raxibacumab. The hypertension was ongoing at the end of the study for two subjects. Two additional subjects in the raxibacumab

Reference ID: 3220416

single-dose group had an AE of mild elevated blood pressure, which resolved. The elevated blood pressure for one subject was recorded as possibly related to study agent.

There were two reports of fever in study HGS1021-C1063 which were not related to study agent and resolved on study. One case was likely related to a viral infection, as fever was also reported with vomiting and influenza. The remaining case was reported concurrent with headache. Both AEs lasted two days and resolved without intervention.

Two AEs of mild tachycardia were reported in Study HGS1021-C1063. These AEs resolved with no intervention.

.4.4 Electrocardiograms (ECGs)

Electrocardiograms were obtained prior to enrollment in all studies, and subjects with clinically significant abnormalities were excluded from participation. Telemetry monitoring was performed in Study HGS1021-C1064 prior to dosing of raxibacumab, during the infusion, and for 2 hours following the infusion. No significant arrhythmias during this period were noted. There were no protocol-required subsequent electrocardiograms obtained after the day of raxibacumab infusion in any study.

7.4.5 Special Safety Studies/Clinical Trials

Not applicable.

7.4.6 Immunogenicity

Nonclinical Evaluation
No immunologic response above background levels was detected in healthy cynomolgus macaques treated with raxibacumab in the 120-day study. Antibodies against the Fab and Fc portion of the PA mAb antibody in raxibacumab-treated animals failed to increase above control levels determined from normal sera from 50 untreated monkeys.

The overall incidence of anti-raxibacumab antibody response in pregnant NZW dams above background levels was very low (5/138).

Monotherapy Animal Studies:
No immunogenicity assessments were performed in pivotal animal (both rabbits and NHP) efficacy studies.

Combination Animal Studies:
Anti-raxibacumab immunogenicity assessments were added to Study 781-G923701 (NZW rabbits) as a protocol amendment to allow for determination of the impact of any observed anti-raxibacumab immunogenicity on plasma raxibacumab levels and survival.

Reference ID: 3220416

Fifteen of 18 rabbits (83%) in the levofloxacin/raxibacumab group tested positive for anti-raxibacumab antibodies on Day 28. In addition, 6/19 (31.6%) rabbits in the levofloxacin group also tested positive for anti-raxibacumab antibodies on Day 28. HGS accounted for this finding by noting that although the levofloxacin only treatment group did not receive raxibacumab, the levofloxacin-treated rabbits mounted an anti-PA response which may have included antibodies that cross-reacted in the anti-human anti-PA assay. Given the modest incidence of anti-raxibacumab immunogenicity at low titers compared with the raxibacumab-treated animals, this was felt to be a possible explanation. Furthermore, HGS pointed out that the emergence of potential anti-raxibacumab immunogenicity did not negatively impact survival.

Clinical Evaluation
All four human safety studies looked for the development of an anti-raxibacumab antibody response, including PAM-NH-01 which used the developmental M10 formulation of the product. In Studies HGS1021-C1064, HGS1021-C1063 (second raxibacumab dose 2 weeks following first dose) and HGS1021-C1069 (second dose ≥ 4 months following first dose, there were no subjects who had a positive anti-raxibacumab antibody response following single or repeat (every 14 days or following ≥ 4 months) doses of raxibacumab.

7.5 Other Safety Explorations

7.5.1 Dose Dependency for Adverse Events

Not applicable

7.5.2 Time Dependency for Adverse Events

Not applicable

7.5.3 Drug-Demographic Interactions

For the effect of age, race, and gender please see Clinical Review by Dr. Sue Lim conducted for the first cycle of BLA 125349.

7.5.4 Drug-Disease Interactions

Clinical trials were conducted in healthy human volunteers.
Non-clinical
In both rabbit and monkey raxibacumab monotherapy efficacy studies, raxibacumab-treated non-survivors had more pronounced anthrax meningitis/meningoencephalitis consisting of hemorrhage, necrosis, inflammation and bacteria in the brain compared to placebo non-survivors. A number of hypotheses were considered investigated, although no clear explanation could be

Reference ID: 3220416

found. Longer survival time in itself did not account for the increased CNS findings observed primarily in raxibacumab-treated non-survivors.
In study 1103, immunohistochemistry staining for raxibacumab and endogenous IgG were performed on the brain tissue. Both raxibacumab and endogenous rabbit IgG-stained neuropil, neurons, and glial cell types were seen in the brains of non-surviving raxibacumab-treated animals. Co-localizing staining patterns suggests non-specific leakage of antibodies across a damaged blood brain barrier. Intense staining of the parabrachial neurons with both raxibacumab and IgG suggested exposure to greater concentrations of circulating immunoglobulins due to their proximity to the ependyma where the BBB is less well-developed. High bacterial load detected in the brains of non-surviving raxibacumab-treated animals suggest a greater proliferation of *B. anthracis* **within the brain compared to other organs. It's suspected** that raxibacumab cannot reach CNS in the early stages of the infection until the circulating toxins and bacteria disrupt endothelial cell tight junctions and compromise the integrity of the BBB. In contrast, high plasma concentrations of raxibacumab immediately after treatment significantly diminished bacterial load in all organs other than brain, with marked reduction in incidence and severity of pathologic findings compared to placebo. An in vitro study described in the literature showed that purified anti-PA antibodies can stimulate phagocytic uptake of spores by macrophages; and inhibit spore germination[6]. This might explain significant reduction of the bacterial load in organs other than CNS, and high bacterial load in the brains of treated non-survivors. Also, LT-induced immunosuppression may contribute to bacterial invasion and dissemination in all tissues including the brain[7]. Reversal of LT immunosuppression with raxibacumab treatment in the brain and subsequent immune reconstitution cells may explain the greater CNS inflammatory response in the brains of non-surviving raxibacumab-treated animals compared to placebo. Surviving animals in monotherapy studies lacked CNS pathology.
In 3 combination studies of raxibacumab with antimicrobials in both rabbit and monkey animal models of inhalational anthrax, only a few non-survivors had findings of anthrax meningitis on the microscopic examination, and of these two animals have received antimicrobial alone and one animal was treated with a raxibacumab+antimicrobial combination. Survivors had unremarkable CNS microscopic examination.
No dose/exposure response was found for the CNS findings.
Brain tissue cross-reactivity studies in rabbits, cynomolgus macaques and humans were negative.
In summary the CNS findings in raxibacumab-treated non-survivors do not appear to be a direct toxic effect of raxibacumab and should be taken in the context of:

- reproducible (3 studies, 2 species) survival benefit of raxibacumab monotherapy (35%-69%) as compared to placebo (0%)
- the absence of dose-response with respect to the frequency and severity of the CNS pathology findings
- normal brain tissue cross reactivity studies in both species and humans

6 Welkos, S., Little, S., Friedlander, A., Fritz, D., and Fellows, P. (2001). The role of antibodies to *Bacillus anthracis* and anthrax toxin components in inhibiting the early stages of infection by anthrax spores. *Microbiology.* **147**, 1677-1685.
7 Sun, C., Fang, H., Xie, T., Auth, R.D., Patel, N., Murray, P.R., Snoy, P.J., Frucht, D.M. (2012). Anthrax lethal toxin disrupts intestinal barrier function and causes systemic infections with enteric bacteria. *Plos One.* 7(3); e33583-e33583.

Reference ID: 3220416

- normal macro and microscopic examination of the brains in raxibacumab-treated survivors
- normal macro and microscopic examination of the brains in raxibacumab/antimicrobial treated survivors and non-survivors
- normal macro and microscopic examination of the brain of the animal with the highest CSF level of raxibacumab
- immunohistochemical co-distribution of raxibacumab and endogenous rabbit IgG within the brains of affected animals suggesting plasma leak across compromised blood-brain barrier.

Potential mechanism for CNS finding in raxibacumab treated non-survivors is unknown and is hypothesized to be related to the inadequate levels of raxibacumab in CNS for infection eradication and the reversal of lethal toxin induced immune suppression systemically.

7.5.5 Drug-Drug Interactions

There were no safety interactions or effects on pharmacokinetics of either product when raxibacumab was administered with ciprofloxacin (oral or IV).

7.6 Additional Safety Evaluations

7.6.1 Human Carcinogenicity

Not applicable

7.6.2 Human Reproduction and Pregnancy Data

Not evaluated

7.6.3 Pediatrics and Assessment of Effects on Growth

As noted previously, there were no studies that included pediatric age human subjects.
As exposure of healthy children to raxibacumab is not ethical, the pediatric dosing was based on a pharmacometric bridging approach. Pediatric PK data on clearance and body weight relationship from other monoclonal antibodies (canakinumab, infliximab, pertuzumab and trastuzumab) was analyzed. It was consistently observed that clearance of monoclonal antibodies is strongly related to body weight. Specific dosing in children was derived from simulations matching plasma exposure ranges (AUC and C_{max}) in healthy adults after 40 mg/kg dose.
The dose for pediatric patients is based on weight as shown in the table below.

Pediatric Dose	Dose
>50 kg	40 mg/kg
> 15 kg to ≤ 50 kg	60 mg/kg
≤ 15 kg	80 mg/kg

Reference ID: 3220416

For detailed information regarding the modeled pediatric dose, please see the clinical pharmacology review by Dr. Ryan Owen and pharmacometrics review by Dr. Jingyu Yu. For the discussion of the inclusion of modeled pediatric dose in the raxibacumab labeling please also see consult reviews by Drs. Robert M. Nelson and Michelle D. Roth-Cline, as well as by Dr. Jeanine Best from the Office of Pediatric Therapeutics.

7.6.4 Overdose, Drug Abuse Potential, Withdrawal and Rebound

There is no clinical experience with overdosage of raxibacumab. In toxicity studies in rabbits and cynomolgus monkeys receiving multiple injections of raxibacumab at 3 to 4 times the recommended dose, there was no raxibacumab-related toxicity. In case of overdosage, it is recommended that the patient be monitored for any signs or symptoms of adverse reactions or effects and appropriate symptomatic treatment instituted immediately.

The product abuse and dependence potential of raxibacumab has not been examined in nonclinical or clinical studies but is expected to be negligible.

Withdrawal and rebound are not applicable to raxibacumab as the product is intended for single dose administration.

7.7 Additional Submissions / Safety Issues

Not applicable

8 Postmarket Experience

Not applicable

Reference ID: 3220416

9 Appendices

9.1 Literature Review/References

Not applicable

9.2 Labeling Recommendations

To be attached when finalized and agreed upon with the applicant

9.3 Advisory Committee Meeting

An Anti-Infective Advisory Committee Meeting was held on November 2, 2012. Questions posed to the committee, vote tally, and the discussion are summarized below.

Questions to the Committee:

Considering the information described for raxibacumab in the rabbit and monkey animal model studies and safety trials in healthy human volunteers:

1. **VOTE:** Do the results from the therapeutic studies of raxibacumab with and without antimicrobials in two animal models of inhalational anthrax provide substantial evidence that raxibacumab (40 mg/kg IV single dose in adults) is reasonably likely to produce clinical benefit for the treatment of humans with inhalational anthrax?

   YES: 16 ***NO: 1*** ***ABSTAIN: 1***

   Committee Discussion: *The majority of the committee agreed that the results from the therapeutic studies of raxibacumab with and without antimicrobials in two animal models of inhalational anthrax provided substantial evidence that raxibacumab (40 mg/kg IV single does in adults) is reasonably likely to produce clinical benefit for the treatment of humans with inhalational anthrax. The committee agreed that the results of the animal studies were statistically significant when comparing raxibacumab monotherapy to placebo. Although the results of the combination studies (raxibacumab + antibiotic vs. antibiotic alone) were not statistically different (p-value 0.0874), the committee agreed that these results were of clinical significance. The committee members stated that 0.05 is an arbitrary cut-off and that this drug has a 92% chance of having added benefit. Given the lack of an antagonistic effect on antibiotics and the lethality of anthrax disease, the majority of the committee agreed that this drug had clinical value especially for patients infected with multi-drug resistant anthrax and patients with multiple antibiotic allergies. The committee member who voted "no" stated that if this product was approved, providers will think that this drug has added benefit,*

Reference ID: 3220416

despite not demonstrating statistically significant evidence of this. This committee member further added that in the case of an emergency, this product would still be available via an Emergency Use Authorization (EUA) or treatment IND despite not being approved by the FDA. Please see the transcript for details of the committee discussion.

a. **DISCUSSION:** If yes, do you have any recommendations regarding labeling of the product, or if not, what additional studies are needed?

Committee Discussion: *The committee recommended several additions to the labeling if this product were to be approved. These additions include 1) clear guidance and protocols on which patients should receive this product; 2) guidance on the logistical use of this product during a large-scale emergency event; and 3) labeling emphasizing that this product will not protect against central nervous system infections such as meningitis due to its inability to penetrate the blood-brain barrier. The committee suggested additional trials be conducted to study the following: 1) potential interactions with anthrax vaccinations; 2) shorter infusion times or more concentrated infusions; 3) dosing in obese patients or those with large amounts of edema/ fluid retention (dosing based on actual body weight vs. ideal body weight); 4) efficacy of doses greater than 40 mg/kg and 5) effective dosing during different stages of disease progression. Please see the transcript for details of the committee discussion.*

2. **VOTE:** Do the results from raxibacumab safety trials in healthy volunteers and studies in animals support an acceptable risk benefit profile given the benefits of the therapy discussed in Question 1?

YES: 18 ***NO: 0*** ***ABSTAIN: 0***

Committee Discussion: *The committee unanimously agreed that the results from the raxibacumab safety trials in healthy volunteers and studies in animals support an acceptable risk benefit profile given the benefits of the therapy discussed in question #1. The committee agreed that the deficiencies in the previous BLA submission have been sufficiently addressed and the lack of dose response regarding the central nervous system events suggest that this product is safe and does not contribute to the death of patients Please see the transcript for details of the committee discussion.*

a. **DISCUSSION:** If yes, do you have any recommendations regarding labeling of the product, or if not, what additional studies are needed?

Committee Discussion: *The committee stated that labeling should reinforce the fact that the safety of this drug was evaluated in healthy humans rather than patients with inhalational anthrax. Additionally, the committee recommended further studies to evaluate the following: 1) safety of doses greater than 40 mg/kg; 2)) higher concentration and shorter infusion times; and 3) effect of raxibacumab on pre-exposure or*

Reference ID: 3220416

postexposure vaccination. Please see the transcript for details of the committee discussion.

3. **DISCUSSION:** Please discuss any recommendations regarding the proposed dosing of 40 mg/kg for adults and the pediatric proposed dosing based on body weight:

WT (Kg)	IV Dose (mg/kg)
≤15	80
>15 to ≤50	60
>50	40

Committee Discussion: *The majority of the committee agreed with the proposed dosing of 40 mg/kg for adults. However, some of the committee members stated that this dose may not be the maximum effective dose. Therefore, the committee recommended additional studies to evaluate the efficacy and safety of doses higher than 40 mg/kg. The committee suggested that these additional studies would also provide information about the safety and efficacy of the proposed weight-based dosing in pediatric patients that is higher than proposed for adults. When using the proposed pediatric dosing, the committee noted that pediatric patients who have weights that approach 15 kg or 50 kg will receive doses larger than patients with weights greater than these values while acknowledging that the simplicity of pediatric dosing is an important factor under emergency. In addition, the committee suggested evaluating alternative body surface area (BSA) or lean body weight (LBW) based dosing for pediatric patients. Please see the transcript for details of the committee discussion.*

9.4 Field Study Protocol Synopsis/Review

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Reference ID: 3220416

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

YULIYA I YASINSKAYA

11/21/2012

John, I have left out AVA-DDI study from PMR/PMC section until it is formalized. I will be able to check in an addenda to the review that will include PMR/PMC and final lableing proposal

JOHN J ALEXANDER

11/21/2012

CLINICAL FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 125349 **Applicant: HGS** **Stamp Date: 6/15/2012**

Drug Name: Raxibacumab **NDA/BLA Type: priority type II resubmission**

On initial overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	NA	Comment
FORMAT/ORGANIZATION/LEGIBILITY					
1.	Identify the general format that has been used for this application, e.g. electronic CTD.	X			
2.	On its face, is the clinical section organized in a manner to allow substantive review to begin?	X			
3.	Is the clinical section indexed (using a table of contents) and paginated in a manner to allow substantive review to begin?	X			
4.	For an electronic submission, is it possible to navigate the application in order to allow a substantive review to begin (*e.g.*, are the bookmarks adequate)?	X			
5.	Are all documents submitted in English or are English translations provided when necessary?	X			
6.	Is the clinical section legible so that substantive review can begin?	X			
LABELING					
7.	Has the applicant submitted the design of the development package and draft labeling in electronic format consistent with current regulation, divisional, and Center policies?	X			
SUMMARIES					
8.	Has the applicant submitted all the required discipline summaries (*i.e.*, Module 2 summaries)?	X			
9.	Has the applicant submitted the integrated summary of safety (ISS)?	X			
10.	Has the applicant submitted the integrated summary of efficacy (ISE)?	X			
11.	Has the applicant submitted a benefit-risk analysis for the product?	X			
12.	Indicate if the Application is a 505(b)(1) or a 505(b)(2). If Application is a 505(b)(2) and if appropriate, what is the reference drug?			X	BLA
DOSE					
13.	If needed, has the applicant made an appropriate attempt to determine the correct dosage and schedule for this product (*i.e.*, appropriately designed dose-ranging studies)? Study Number: 682 and 761 Study Title: Evaluation of Raxibacumab Efficacy as Therapeutic Treatment Against Inhalation Anthrax in the Rabbit Model and NHP Model Sample Size: 53 and 40 Arms: 3 Location in submission: previously submitted	X			
EFFICACY					
14.	Do there appear to be the requisite number of adequate and well-controlled studies in the application? Pivotal Studies #1-2 and 1103 and 1141	X			

File name: 5_Clinical Filing Checklist for NDA_BLA or Supplement 010908

Reference ID: 3164422

CLINICAL FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	NA	Comment
	Indication: anthrax Pivotal Studies #3-4 682 and 761 Indication: anthrax Study # 5-6 724 and 789 Indication: anthrax				
15.	Do all pivotal efficacy studies appear to be adequate and well-controlled within current divisional policies (or to the extent agreed to previously with the applicant by the Division) for approvability of this product based on proposed draft labeling?	X			
16.	Do the endpoints in the pivotal studies conform to previous Agency commitments/agreements? Indicate if there were not previous Agency agreements regarding primary/secondary endpoints.	X			
17.	Has the application submitted a rationale for assuming the applicability of foreign data to U.S. population/practice of medicine in the submission?			X	
SAFETY					
18.	Has the applicant presented the safety data in a manner consistent with Center guidelines and/or in a manner previously requested by the Division?			X	
19.	Has the applicant submitted adequate information to assess the arythmogenic potential of the product (*e.g.*, QT interval studies, if needed)?			X	
20.	Has the applicant presented a safety assessment based on all current worldwide knowledge regarding this product?	X			
21.	For chronically administered drugs, have an adequate number of patients (based on ICH guidelines for exposure[1]) been exposed at the dose (or dose range) believed to be efficacious?			X	
22.	For drugs not chronically administered (intermittent or short course), have the requisite number of patients been exposed as requested by the Division?	X			
23.	Has the applicant submitted the coding dictionary[2] used for mapping investigator verbatim terms to preferred terms?			X	No additional clinical data are submitted
24.	Has the applicant adequately evaluated the safety issues that are known to occur with the drugs in the class to which the new drug belongs?			X	No additional clinical data are submitted
25.	Have narrative summaries been submitted for all deaths and adverse dropouts (and serious adverse events if requested			X	No additional clinical data are submitted

[1] For chronically administered drugs, the ICH guidelines recommend 1500 patients overall, 300-600 patients for six months, and 100 patients for one year. These exposures MUST occur at the dose or dose range believed to be efficacious.

[2] The "coding dictionary" consists of a list of all investigator verbatim terms and the preferred terms to which they were mapped. It is most helpful if this comes in as a SAS transport file so that it can be sorted as needed; however, if it is submitted as a PDF document, it should be submitted in both directions (verbatim -> preferred and preferred -> verbatim).

Reference ID: 3164422

CLINICAL FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	NA	Comment
	by the Division)?				
OTHER STUDIES					
26.	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X			
27.	For Rx-to-OTC switch and direct-to-OTC applications, are the necessary consumer behavioral studies included (*e.g.*, label comprehension, self selection and/or actual use)?			X	
PEDIATRIC USE					
28.	Has the applicant submitted the pediatric assessment, or provided documentation for a waiver and/or deferral?		X		Product has orphan designation; original BLA cites the pre-BLA meeting agreement that waiver or deferral of peds studies is not required. The resubmission however includes a proposal for a field study (b) (4)
ABUSE LIABILITY					
29.	If relevant, has the applicant submitted information to assess the abuse liability of the product?			X	
FOREIGN STUDIES					
30.	Has the applicant submitted a rationale for assuming the applicability of foreign data in the submission to the U.S. population?			X	
DATASETS					
31.	Has the applicant submitted datasets in a format to allow reasonable review of the patient data?	X			
32.	Has the applicant submitted datasets in the format agreed to previously by the Division?	X			
33.	Are all datasets for pivotal efficacy studies available and complete for all indications requested?	X			
34.	Are all datasets to support the critical safety analyses available and complete?	X			
35.	For the major derived or composite endpoints, are all of the raw data needed to derive these endpoints included?	X			
CASE REPORT FORMS					
36.	Has the applicant submitted all required Case Report Forms in a legible format (deaths, serious adverse events, and adverse dropouts)?			X	
37.	Has the applicant submitted all additional Case Report Forms (beyond deaths, serious adverse events, and adverse drop-outs) as previously requested by the Division?			X	
FINANCIAL DISCLOSURE					
38.	Has the applicant submitted the required Financial Disclosure information?	X			
GOOD CLINICAL PRACTICE					

CLINICAL FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	NA	Comment
39.	Is there a statement of Good Clinical Practice; that all clinical studies were conducted under the supervision of an IRB and with adequate informed consent procedures?			X	No additional clinical studies have been submitted in this resubmission

IS THE CLINICAL SECTION OF THE APPLICATION FILEABLE? YES

If the Application is not fileable from the clinical perspective, state the reasons and provide comments to be sent to the Applicant.
Not applicable

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.
None

Yuliya Yasinskaya, MD — July 9, 2012
Reviewing Medical Officer — Date

Reference ID: 3164422

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

YULIYA I YASINSKAYA
07/25/2012

Office Director Decisional Memo

Date	11/14/2009
From	Edward M. Cox, MD, MPH
Subject	Office Director Decisional Memo
BLA #	125349
Applicant Name	Human Genome Sciences, Inc. (HGS)
Date of Receipt	May 14, 2009
PDUFA Goal Date	November 14, 2009
Proposed Proprietary Name / Established (USAN) Name	none raxibacumab
Dosage Forms / Strength	Injection for intravenous use, 50 mg/mL
Proposed Indication	Treatment of patients with inhalational anthrax due to *B. anthracis*
Action:	**Complete Response**

Raxibacumab is a fully humanized monoclonal antibody that binds to the protective antigen of *B. anthracis*. HGS is developing raxibacumab as a treatment for patients with inhalational anthrax. Inhalational anthrax is a life threatening condition with a high mortality rate once disease has developed even with antibacterial drug treatment. The currently approved drug therapies labeled for the treatment of patients with *B. anthracis* are penicillin G potassium, penicillin G sodium, and penicillin G procaine, and the tetracyclines. For the indication of post-exposure prophylaxis of inhalational anthrax the previously listed antibacterial drugs and also ciprofloxacin and levofloxacin are approved.

During the bioterrorism-related anthrax cases in 2001, there were 11 cases of inhalational anthrax, 5 of which were fatal.[1] *B. anthracis* produces toxins that contribute to much of the pathophysiology of the disease. In inhalational anthrax disease, it is hypothesized that targeting not only the bacteria with antibacterial drugs, but also targeting the toxins produced by *B. anthracis* may be a means to improve survival rates in patients with anthrax disease. Another potential role for therapies acting via a mechanism different than that of an antibacterial drug is for treatment of patients with strains of *B. anthracis* that are resistant to antibacterial drugs.

Protective antigen (PA) binds to the cell surface and forms a heptameric complex that facilitates the entry of lethal and edema toxin into the cell cytosol. Protective antigen also combines with lethal factor and edema factor to form lethal toxin (LT) and edema toxin (ET), respectively. The intended role of an antibody directed against protective antigen is to interrupt the pathophysiologic role of protective antigen in this process.

The review team has reviewed the issues in detail in their respective disciplines. For a detailed discussion of the discipline specific reviews, the reader is referred to the individual discipline

[1] Jernigan DB, Raghunathan PL, Bell BP, et. al. Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. Emerg Infect Dis. 2002 Oct;8(10):1019-28.

specific reviews, the Cross-Discipline/Medical Team Leader Review, and the Division Director Review. This memorandum will focus on selected issues from the application including the animal studies and the information that they provide regarding effectiveness in humans with inhalational anthrax disease.

The evaluation from the Product Quality standpoint for raxibacumab finds that, in general, the manufacturing is well controlled. There was, however, a deficiency from the Product Quality microbiology perspective that the endotoxin for the product needed to be re-assessed and (b) (4) There were also a number of recommendations for further work on validation procedures and updating testing protocols.

The pharmacology/toxicology review notes no significant adverse nonclinical findings and also notes the finding of binding of raxibacumab to thyroid in human and non-human primate ex-vivo tissue. The review also notes the limitations of the performance of the assay to evaluate immunogenicity in the animal studies that raises questions about conclusions that can be drawn from animal studies to evaluate immunogenicity. The recommendation from Pharmacology/Toxicology regarding Pregnancy Category is Category B. Given the findings from the inspections of the bioanalytic methods from the human pharmacokinetic studies, this raises questions about the analytic results from the toxicokinetic samples from Study 6962-140 and Study 6962-173. Therefore HGS will be asked to re-assay the samples from these studies.

The Microbiologist's review describes the in vitro and animal model studies done with raxibacumab. Of note from the studies evaluating the binding of raxibacumab to free PA or PA bound on the cell surface, it appears that raxibacumab binds to free PA, but not to PA bound to cellular receptors suggesting the binding site for raxibacumab may not be available after PA has bound to its cellular receptor. In contrast, the rabbit anti-PA polyclonal anti-sera used in these experiments bound to PA after it was bound to the cell surface receptor.

The applicant's proposed dose in humans is a single dose of 40 mg/kg administered intravenously over 2 hours and 15 minutes after diphenhydramine pre-medication. The findings from the inspections of the analytic methods and procedures for the human pharmacokinetic studies raise important concerns about the quality and reliability of human pharmacokinetic data in this application. The issues with the reliability of the pharmacokinetic data will need to be resolved, as they serve as the means to link raxibacumab human pharmacokinetic exposure to animal pharmacokinetic exposure for dose selection. Further evaluation of the available information, procedures, and samples will be needed to determine whether re-analyses from these studies can be relied upon or whether additional studies will be needed to address this deficiency.

Animal Studies Evaluating Activity and Effectiveness

For a complete discussion of the animal studies evaluating activity and effectiveness the reader is referred to the Microbiology, Statistical, and Medical Officer Reviews. The sections that follow provide an overview of the findings from these studies.

The activity of raxibacumab was evaluated in rat toxin models. The studies evaluated the effect of raxibacumab (an earlier formulation) on survival of rats administered LT. The

studies showed that intravenous administration of raxibacumab administered prior to or for a limited time after an infusion of lethal toxin was able to improve survival compared to placebo. The studies provide proof of concept for the role of raxibacumab in interrupting the lethality of LT in the rat models that were studied.

In a rabbit study (Experiment #1 in the table below) of 12 rabbits in each of 5 groups, rabbits received either placebo or 40 mg/kg of raxibacumab at 0, 12, 24, 36 hours post exposure/ challenge with aerosolized *B. anthracis* 100 LD_{50}. Administration of raxibacumab at 0 or 12 hours post exposure resulted in 12 of 12 animals surviving; survival rates declined at later time points.

A second rabbit study (experiment #2) evaluated different doses of raxibacumab administered either 24 or 36 hours post exposure. Survival rates for doses of raxibacumab administered 24 hours after challenge were between 3/12 animals and 5/12 animals for the doses between 5 mg/kg and 40 mg/kg. At 36 hours, at a dose of 20 mg/kg 0/12 animals survived. Bacteremia status was not assessed in this study.

Table. Summary of Survival Data in NZW Rabbits Treated with Raxibacumab at Various Times After Aerosol Exposure to Spores from the Ames Strain of *B. anthracis*

Treatment Group (Raxibacumab dose)	Time of Treatment (hours PI)	Survivors at 14 Days PI*	Median Time to Death Days PI (range)	Bacteremic at Time of Treatment
Experiment 1:				
1 (Placebo)	0	1/12 (8 %)	(2 – 3)	0/12 (0 %)
2 (40 mg/kg)	0	12/12 (100 %)	NA	0/12 (0 %)
3 (40 mg/kg)	12	12/12 (100 %)	NA	0/12 (0 %)
4 (40 mg/kg)	24	6/12 (50 %)	(1 – 3)	1/12 (8 %)
5 (40 mg/kg)	36	5/12 (42 %)	(2 – 4)	9/12 (75 %)
Experiment 2:				
1 (Placebo)	0	0/12 (0 %)	3 (1-4)	Not measured
2 (5 mg/kg)	24	3/12 (25 %)	3.5 (1-4)	Not measured
3 (10 mg/kg)	24	4/12 (33 %)	2.5 (1- 3)	Not measured
4 (20 mg/kg)	24	5/12 (42 %) *	6.0 (2 - 8)#	Not measured
5 (40 mg/kg)	24	4/12 (33 %)	2.5 (2 -8)	Not measured
6 (20 mg/kg)	36	0/12 (0 %)	3.0 (1- 9)	Not measured

Note: No rabbits died during the additional 28 day observation period in experiment 1
*Statistically different from placebo; p = 0.0373
#Statistically different from placebo; p = 0.0020

Studies evaluating raxibacumab administered subcutaneously as pre-exposure prophylaxis (two days prior to aerosol challenge) in cynomolgus monkeys found 0/10 animals surviving in the placebo group and 9/10 surviving in the raxibacumab 40 mg/kg dose.

Table. Survival Results in Cynomolgus Monkeys at Day 28 PI following SC Prophylactic Treatment of Inhalational Anthrax with Raxibacumab*				
Treatment	**Dose (mg/kg)**	**LD_{50} Dose (mean ± SD)**	**Survivors / Total Infected**	**Time to Death for Animals That Died (Days)**
Placebo	0	180 ± 72	0/10	2-5
Raxibacumab	10	187 ± 52	6/10	4-6
Raxibacumab	20	177 ± 61	7/10	4-6
Raxibacumab	40	194 ± 79	9/10	3
All treatments administered by the SC route two days prior to aerosol challenge with *B. anthracis* spores. *Survivors were observed for an additional 60 days. There were no additional deaths during this 60-day observation period.				

These studies show activity of raxibacumab, based upon differences in survival rates in animal models when given prior to or soon after exposure to aerosol challenge with *B. anthracis* spores.

Studies in cynomolgus monkeys and New Zealand white (NZW) rabbits were performed evaluating the effect of raxibacumab monotherapy at 20 mg/kg and 40 mg/kg following aerosol exposure to *B. anthracis* spores. Animals were exposed to aerosolized *B. anthracis* spores and followed for development of PA in serum (monkeys) or PA in serum along with elevated temperature (rabbits) at which time raxibacumab was administered intravenously. The dose groups were placebo, 20 mg/kg, and 40 mg/kg. Survival rates at the 40 mg/kg dose in cynomolgus monkeys was 9/13 (69%) compared to 0/10 (0%) for placebo recipients. For NZW rabbits the survival rates in the 40 mg/kg group was 6/17 (35%) compared to 0/13 (0%) for the placebo group. The differences in survival rates for the raxibacumab 40 mg/kg dose groups were significantly better than placebo with p-values less than 0.05. The studies show an effect of raxibacumab in increasing survival in cynomolgus monkeys and rabbits with inhalational anthrax in these animal studies.

Treatment Group	**Cynomolgus Monkeys at 28 days**			**NZW Rabbits at 28 days**		
	Number (%) of survivors	**P value vs. placebo[1]**	**95% CI of raxibacumab – placebo[2]**	**Number (%) of survivors**	**P value vs. placebo[1]**	**95% CI of raxibacumab – placebo[2]**
Placebo	0/10 (0%)			0/13 (0%)		
20 mg/kg raxibacumab	5/12 (41.7%)	0.0396	(7.2, 68.7)	4/16 (25.0%)	0.1067	(-2.2, 50.9)
40 mg/kg raxibacumab	9/13 (69.2%)	0.016	(31.1, 88.9)	6/17 (35.3%)	0.0237	(7.3, 59.6)

[1] P value based on 2-sided Fisher's exact test fro the comparison vs. the placebo control group
[2] 95% CIs are exact confidence intervals

Studies in cynomolgus monkeys and NZW rabbits evaluated the effect of an antibacterial drug (either ciprofloxacin or levofloxacin), an antibacterial drug plus raxibacumab, and placebo in animals exposed to aerosolized *B. anthracis* spores. Treatment was initiated at the time of a positive serum assay for (monkeys) or PA in serum along with elevated temperature (rabbits). The FDA analysis population is animals that were bacteremic at the time that therapy was started. In cynomolgus monkeys 13/13 (100%) animals that received antimicrobial alone

survived compared to 11/13 (85%) that received antimicrobial agent and raxibacumab. The survival rate in the placebo group was 0/10 (0%). In NZW rabbits 19/20 (95.0%) survived that received antimicrobial agent alone and 16/17 (94.1%) survived that received antimicrobial agent plus raxibacumab. In the placebo group 0/10 (0%) survived.

Treatment group	**Cynomolgus Monkeys at 28 days**		**NZW Rabbits at 28 days**	
	Number (%) of Survivors	95% CI of Cipro/raxibacumab-Cipro[2]	**Number (%) of Survivors**	95% CI of Levo/raxibacumab-Levo[2]
Placebo	**0/10 (0%)**	-	**0/10 (0%)**	-
Antimicrobial Alone[1]	**13/13 (100%)**	-	**19/20 (95.0%)**	-
Antimicrobial[1] + raxibacumab 40 mg/kg	**11/13 (84.6%)**	(-45.5, 11.4)	**16/17 (94.1%)**	(-23.9, 19.6)

[1] NHP study antimicrobial was 75 mg ciprofloxacin twice daily x 3 days
NZW rabbit study antimicrobial was 50 mg/kg levofloxacin daily x 3 days
[2] 95% CIs are exact confidence intervals

In the antimicrobial drug alone group survival was 13/13 in monkeys and 19/20 in rabbits. The very high survival rates for antibacterial drug therapy alone in these studies do not allow for any added benefit of raxibacumab to be shown above what the antimicrobial agent alone provides. During the bioterrorism event of 2001 in humans presenting with established anthrax disease antimicrobial therapy alone achieved an approximately 50% survival rate. Given the high survival rate in the animal studies with antimicrobial agent alone, it appears that intervention may be too early and that the animal models do not adequately model the human disease state where use is anticipated. One important goal of adjunctive anti-toxin therapy is to improve on the survival achieved with antibacterial drug alone. These studies lack capacity to determine if raxibacumab will provide added benefit to antimicrobial therapy alone.

Studies Evaluating Safety

Animal

In animal toxicology studies, there was no evidence of CNS toxicity in monkeys dosed with 120 mg/kg raxibacumab. A finding noted in the animal models of infection was that non-surviving animals that received raxibacumab that died of anthrax had a greater rate and severity of CNS disease than animals that received placebo. The reasons for this greater rate and severity of CNS disease are not clear and the potential relevance to humans is not clear. In the rabbit combination study where animals received antimicrobial alone or antimicrobial plus raxibacumab, animals were sacrificed and examined for CNS lesions. There were no brain lesions seen in the surviving animals that received raxibacumab plus levofloxacin, or that received levofloxacin alone. Additional study will be needed to further understand this finding.

Human

The safety of raxibacumab 40 mg/kg IV was evaluated in 326 normal volunteers, and compared to adverse events reported in 80 normal volunteers who received placebo. Rash was

seen initially in 6/25 (24%) of volunteers. Then diphenhydramine pre-treatment was initiated for all and the observed rash rate decreased to 2/61 (3%). Diphenhydramine pretreatment will be recommended, and was used in the monkey efficacy study. Other adverse effects include infusion site pain and headache. Headaches were reported in 9.5% of raxibacumab volunteers (31/326) and 10% of placebo volunteers (8/80).

Division of Scientific Investigations (DSI) Inspections
Inspections of the bioanalytical assay methods and conduct of the clinical trials found deficiencies in the testing done (bioanalytic methods) to measure levels of raxibacumab and ciprofloxacin in the human pharmacokinetic studies. This information is of critical importance because it is the means by which exposure in the animals where the efficacy of raxibacumab is evaluated is linked to the exposure in humans to arrive at an appropriate human dose. Additional information about the testing methods, the assay, use of the appropriate number of QC samples to calibrate the concentration curve and re-analysis of samples will be requested from HGS as a possible mean to address the noted deficiencies. If this additional information and re-analysis cannot address deficiencies, new studies may be needed. Given the findings from the human PKL studies additional information on the bioanalytical assay method used in the animal efficacy studies and two animal toxicology studies will also be needed. The animal studies (Study 724-G005829 and Study 682-G005758) were inspected in 2008 and found to be acceptable.

Anti-Infective Drugs Advisory Committee
The application for raxibacumab was presented to the Anti-Infective Drugs Advisory Committee on October 27th, 2009. The Committee was made aware of the questions on the reliability of the human PK data based upon inspectional findings, that the data should be disregarded, and that no question on approval/licensure would be asked of the Committee. The concerns regarding the human pharmacokinetic data were also posted publicly in advance of the meeting in an addendum to the FDA briefing material.

The Committee was asked if the animal studies predict efficacy in humans. The vote on this question was Yes 16; No 7; Abstain 1. During the discussion of this question, a reason cited among those that voted "No" was that the time of intervention for the animal models appeared to be early in disease, noting the 95-100% survival rates with antimicrobial drug alone. On the question of whether raxibacumab will not interfere with antibacterial drug, the Committee voted, Yes 10; No 11; Abstain 3. Some expressed concerns that the ciprofloxacin dose was too high and therefore the study was not a sensitive test to detect interference with antibacterial drugs. On the question of whether a study should be done to look for added benefit of raxibacumab plus antibacterial over antibacterial alone, the Committee voted Yes 17; No 5; Abstain 1. During the discussion of this question some Committee members commented that the study could be done post-approval and some noted the study could be done in rabbits only. The Committee also expressed interest in additional work to understand the CNS findings seen with raxibacumab in animal effectiveness studies and additional safety data in the very young (the idea of doing juvenile animal studies was briefly mentioned) and elderly.

Discussion
Based upon the available information in the application, the application cannot be approved at in its present form. The deficiencies that need to be addressed are enumerated in the Complete Response Letter. As noted, the inspectional issues regarding the bioanalytical methods will need to adequately addressed, re-analyses performed, and if the re-analyses are not able to adequately address the noted deficiencies, new studies may be needed.

The demonstration of effectiveness for a therapy for anthrax disease is based upon findings from animal studies because studies for this life threatening illness cannot be performed in humans. The animal rule can be relied upon when the results of adequate and well-controlled animal studies establish that the biologic product is reasonably likely to produce clinical benefit in humans. As noted in the Complete Response letter, in the animal efficacy studies that evaluated antimicrobial drug alone, antimicrobial drug plus raxibacumab, or placebo, the survival rates were 100% in cynomolgus monkeys and 95% in New Zealand white rabbits with antimicrobial drug alone. The high survival rate implies that the timing of intervention was too early to adequately model established anthrax disease in humans. In patients presenting with inhalational anthrax disease in 2001 and treated with antimicrobial therapy, the survival rate was approximately 50%. The animal model should reflect the human disease state for which the product under study in intended. The animal models and the 100% survival rate in cynomolgus monkeys and the 95% survival rates in New Zealand white rabbits with antimicrobial drug alone do not allow for the contribution of the monoclonal antibody to be assessed. In addition, the anticipated use of raxibacumab in humans would typically be in combination with antimicrobial therapy. The available data do not provide sufficient information to adequately predict response in humans with inhalational anthrax in the manner in which the product is likely to be used. Although the product was superior to placebo when used as monotherapy, this finding does not reflect how the product would typically be used. In addition, the findings in the combination studies where it appears that the intervention may have been given too early, raises questions about the how the time of intervention in the monotherapy studies relates to the point at which treatment would typically be initiated in humans with anthrax disease.

There is a range of opinions across the review team on the adequacy of the data from the animal effectiveness studies. These issue included questions of whether there was an adequate degree of similarity to the human condition and the manner therapy would be used in that setting. I have also discussed the topic of the available data and their adequacy to predict response in humans with OND Office Directors. In the Complete Response letter, I am asking for an additional study to evaluate the effect of raxibacumab in an animal model that better models the condition and the manner in which the product is likely to be used in humans.

A study should be performed in an animal model of inhalational anthrax in which there is a level of mortality in the antimicrobial alone arm to allow a demonstration of added benefit of raxibacumab when used in combination with an antimicrobial drug, for example, by showing that the outcome in the antimicrobial plus raxibacumab arm is higher than the outcome in the antimicrobial alone arm. In order to perform such a study, additional animal model developmental work will be needed.

Another finding of note is the increased rate and severity of CNS findings in non-surviving animals that received raxibacumab compared to those that received placebo. The reasons for this greater rate and severity of CNS disease are not clear and the potential relevance to humans is not clear. There appears to be a drug/disease interaction, a disease manifestation more likely to occur in the setting of raxibacumab therapy, or potentially a toxic effect. This finding should be further evaluated by performing a study to further characterize the effect of raxibacumab on the CNS in an animal model of inhalational anthrax and characterize the clinical course and histological appearance of the CNS in animals that survive and animals that die of anthrax.

As noted in the Completer Response letter, the bacterial endotoxin specification for the raxibacumab product should be re-assessed and (b) (4). In the Complete Response letter we have also provided other recommendations regarding additional product quality and clinical issues.

Deficiencies from the Complete Response Letter
The section from the Complete Response Letter enumerating deficiencies is provided below.

SAFETY AND EFFICACY

1. In the animal efficacy studies that evaluated antimicrobial drug alone, antimicrobial drug plus raxibacumab, or placebo, the survival rates were 100% in cynomolgus monkeys and 95% in New Zealand White (NZW) rabbits with antimicrobial drug alone. The high survival rate implies that the timing of intervention was too early to adequately model established anthrax disease in humans. In patients presenting with inhalational anthrax disease in 2001 and treated with antimicrobial therapy, the survival rate was approximately 50%. The animal model should reflect the human disease state for which the product under study is intended. The animal models and the 100% survival rate in cynomolgus monkeys and the 95% survival rates in NZW rabbits with antimicrobial drug alone do not allow for the contribution of the monoclonal antibody to be assessed. In addition, the anticipated use in humans would be using raxibacumab in combination with antimicrobial therapy. The available data do not provide sufficient information to adequately predict response in humans with inhalational anthrax in the manner in which the product is likely to be used.

   To address this deficiency, we recommend that you conduct a study in an animal model of inhalational anthrax to demonstrate the added benefit of raxibacumab when used with an antimicrobial drug, for example, by showing that the outcome in the antimicrobial plus raxibacumab arm is higher than the outcome in the antimicrobial alone arm. We recognize that additional animal model developmental work will be needed to perform such a study and recommend that you discuss the proposed study with the division.

2. In animal studies, non-survivors that received raxibacumab and died of anthrax had a greater rate and severity of central nervous system (CNS) disease than animals that

received placebo. The reasons for this greater rate and severity of CNS disease are not clear and the potential relevance to humans is not clear.

To address this deficiency, we recommend that you conduct a study to evaluate the effect of raxibacumab on the CNS in an animal model of inhalational anthrax and characterize the clinical course and histological appearance of the CNS in animals that survive and animals that die of anthrax.

CLINICAL PHARMACOLOGY

3. During the inspection of the bioanalytical sites responsible for analysis of serum raxibacumab and plasma ciprofloxacin concentrations in humans, the Division of Scientific Investigations (DSI) noted several deficiencies in the analytical procedures used for each of these products. Therefore, before we can rely on the pharmacokinetic data generated for raxibacumab and ciprofloxacin in humans, you will need to do the following:

    a. Revise the analytical procedures for raxibacumab to address these DSI findings, re-assay the pharmacokinetic samples obtained from human studies HGS1021-C1063, HGS1021-C1064, and HGS1021-C1069, and re-calculate the pharmacokinetic parameters for raxibacumab from these re-analyses. Depending on the outcome of these re-analyses, additional pharmacokinetic studies may be required.

    b. Revise the analytical procedure for ciprofloxacin to address the DSI findings, re-assay the plasma pharmacokinetic samples obtained from human study HGS1021-C1064, and re-calculate the pharmacokinetic parameters for ciprofloxacin from this re-analysis in humans. Depending on the outcome of this re-analysis, additional pharmacokinetic studies may be required.

4. The DSI findings noted above also bring into question whether we can rely on the pharmacokinetic data generated for raxibacumab from the animal efficacy studies in NZW rabbits and cynomolgus monkeys because these bioanalytical procedures are similar to those used for the human studies. Depending on the outcome of the re-analysis of raxibacumab in humans, re-assay of the pharmacokinetic samples from animal studies 682-G005758 and 724-G005829 and re-calculation of pharmacokinetic parameters for raxibacumab from these re-analyses in rabbits and monkeys may be required.

5. The DSI findings noted above also bring into question whether we can rely on the pharmacokinetic data generated for ciprofloxacin from the combination animal efficacy study in cynomolgus monkeys because the bioanalytical procedure is similar to that used for the human studies. Depending on the outcome of the re-analysis of ciprofloxacin in humans, re-assay of the ciprofloxacin pharmacokinetic samples from animal study 789-G923702 in monkeys and re-calculation of pharmacokinetic parameters for ciprofloxacin from this re-analysis may be required.

PHARMACOLOGY/TOXICOLOGY

6. The DSI findings above also raise questions about the reliability of analytic results from the toxicokinetic samples from Study 6962-140 and Study 6962-173.

   To address this deficiency re-assay plasma toxicokinetic samples for raxibacumab from Study 6962-140 "120-day toxicity study with PA mAb in cynomolgus monkey" and Study 6962-173 "Intravenous study for effect on embryo-fetal development and toxicokinetics with raxibacumab in rabbits" after revising the analytical procedures for raxibacumab to address the DSI findings.

PRODUCT QUALITY

7. The bacterial endotoxin specification for the raxibacumab final product is inadequate.

   To address this deficiency, re-assess and (b) (4) the bacterial endotoxin specification for the raxibacumab final product. The re-assessment should consider the endotoxin contribution from the saline solution used to dilute raxibacumab prior to administration. The revised specification should provide a safety factor (e.g., 2-4 fold) when the endotoxin limits from both the saline and the product are considered.

Edward Cox 11/14/09

Edward Cox, M.D., MPH
Director
Office of Antimicrobial Products
Office of New Drugs
Center for Drug Evaluation and Research

Division Director Review of BLA

APPLICANT:	Human Genome Sciences, Inc.
DRUG:	raxibacumab
TRADE NAME:	ABthrax (proposed trade name will not be used at this time)
BLA:	**125349/0**
Date of Submission:	May 13, 2009
Date of Receipt:	May 14, 2009
PDUFA Goal Date:	November 14, 2009
Formulation:	intravenous
Indication:	Treatment of inhalational anthrax disease
Review Designation:	Priority
Related INDs:	HGS IND 11,069 CDC IND 102,964

Material Reviewed:
The action package for this application and related documents submitted by the following were examined:
Project Management: Rebecca Saville McKinnon, Diana Willard (CPMS)
Clinical Reviews: Yuliya Yasinskaya (CDTL), Susan McCune, Sue Lim, Eileen Navarro (TL) (HGS IND 11,069, CDC IND 102,964)
Microbiology Reviews: Maureen Davidson, Lynette Berkeley, Shukal Bala (TL)
CDRH Review: Beena Puri, Freddie Poole
Clinical Pharmacology Review: Kimberly Bergman, Phil Colangelo, Pravin Jadhav (pharmacometrics), John Lazor
Statistics Reviews: Hongling Zhou, Lan Zeng, Karen Higgins (TL)
Pharmacology/Toxicology Review: Terry Miller, William Taylor
Division of Neurology Consult: Barbara Wilcox, Susanne Goldstein, Eric Bastings (TL)
Biologics Consult: Marc Walton
Product Quality Assessment (Chemistry) Review: David Frucht, Patrick Swann, Kathleen Clouse (TL)
Office of Compliance/Division of Manufacturing Product Quality (DMPQ/MAPCB/ BMT): Mary Farbman (drug substance), Colleen Thomas (drug product) Patricia Hughes (TL)
Division of Scientific Investigations (DSI):
- **DSI/Clinical**: Susan Thompson, Jeanne Mulinde, Tejashri Purohit-Sheth
- **DSI/Bioequivalence**: John Kadavil, Hyojong Kwon, Martin Yau, Jacqueline O'Shaughnessy, Michael Skelly, C.T. Viswanathan

Advisory Committee Briefing Document and meeting on October 27, 2009 before the Anti-Infective Advisory Committee, including quick notes by Minh Doan

Preliminary review of labeling and PMR/PMCs was conducted, and will be resumed when HGS responds to the CR letter to be issued for this application. The following consults pertain to labeling:
DMEPA: Kimberbly Rains, Scott Dallas (carton and container labeling)
DRISK: Shawna Hutchins (patient labeling)
DDMAC: Kathleen Klemm (labeling)
SEALD: Iris Masucci (package insert)
MHT: Jeannine Best (pregnancy and lactation labeling)

Table of Contents

1. OVERALL RECOMMENDATION and OUTSTANDING ISSUES: 3
1.1 Complete Response Issues: 3
1.2 Other Issues: 3
2 BACKGROUND 6
2.1 Raxibacumab and Anthrax 6
2.2 BLA and Main Efficacy and Safety Studies 7
3. REVIEW SUMMARY 7
3.1 Product Manufacture and Quality Assessment 7
3.1.1 Inspection of Manufacturing Facility and CMC issues 8
3.2 Pharmacology Toxicology 8
3.3 Microbiology 8
3.3.1 Mechanism of Action of Raxibacumab 8
3.3.2 Mechanism of Action of Raxibacumab vs. Antimicrobials 9
3.4 Clinical Pharmacology 9
3.4 Division of Scientific Investigation (DSI) findings 10
3.5 Efficacy Studies in Animal Models of Infection 11
3.7 Animal Rule 13
3.8 Brain Findings 15
3.8.1 Deaths due to Anthrax 15
3.8.2 Histologic findings (Systemic vs. CNS findings) 16
3.8.3 Hypotheses regarding the CNS lesions include: 17
3.8.4 PA and disease 19
3.9 Raxibacumab Treatment Doses and Timing 20
3.10 Clinical Safety 22
3.12 Immunity 23
4 ADVISORY COMMITTEE MEETING 23
5. DISCUSSION: 25
5.1 Clinical Considerations in Treatment 25
5.1.1 Infections where antimicrobial and antitoxin therapy is used 25
5.1.2 Supportive measures in anthrax treatment 25
5.1.3 Animal Efficacy Studies 26
6. RECOMMENDATIONS: 27

1. OVERALL RECOMMENDATION and OUTSTANDING ISSUES:

Raxibacumab as a single dose of 40 mg/kg infused intravenously over 2 hours 15 minutes following diphenhydramine pre-treatment is the regimen proposed by HGS for the treatment of inhalational anthrax. Human safety data on 326 normal volunteers, and four animal efficacy studies along with a broad range of nonclinical studies were submitted to support approval of the product under the Animal Rule.

1.1 Complete Response Issues:

DSI identified deficiencies in the **bioanalytical assay** conducted to measure raxibacumab at HGS, and ciprofloxacin at (b) (4)[1] therefore the application cannot be approved until the DSI inspection findings and related clinical pharmacology issues are resolved. As discussed internally and communicated by a facsimile on November 6, 2009, the bioanalytical assays used for the animal raxibacumab concentrations, ciprofloxacin concentrations, and levofloxacin concentrations need to be re-analyzed after revising the bioanalytical procedures to correct the deficiencies identified. Serum samples in the monkey and embryo-fetal toxicology study should be rerun. An amendment submitted by HGS on November 10, 2009 was examined by DSI and found to be deficient; during the telecom with HGS on November 12, 2009, DSI reiterated a number of their concerns regarding the procedures used for the assay (one example was the need to use 3 QC samples instead of only 2 as used by HGS).

In addition, review of the **CMC microbiology** portion of the application left a few unanswered questions regarding validations procedures and endotoxin measurement in the final product. These issues were initially considered post-marketing issues, but DMQP has recommended that the reassessment of bacterial endotoxin specifications in the final product be requested of HGS before the product is licensed.

As part of approval under the Animal Rule, a confirmatory field study is required. A protocol for such a study should be submitted; a study synopsis was submitted on November 5, 2009.

- A complete response letter should be issued, requesting that the bioassay issues and CMC microbiology issue be resolved.
- A protocol for a field study to collect data and study raxibacumab in the event of intentional exposure should be submitted.

1.2 Other Issues:

Although a number of reviewers concluded that the safety data and efficacy data submitted to date (conditional on the PK issues being resolved) are sufficient to meet the criteria for approval under the Animal Rule, others have expressed concern that additional data are warranted.

Three areas of discussion regarding animal efficacy studies are noted:

[1] Review by DSI, October 7, 2009

(1) The need for studies to show absence of antagonism and/or **added benefit** of raxibacumab over antimicrobial-only:

Some reviewers have recommended that a study that (a) shows there is no antagonism between the fluoroquinolone and raxibacumab and (b) shows an added benefit of raxibacumab over fluoroquinolone should be submitted preapproval; others recommend that such a study be conducted post approval. In keeping with 21st century timelines and before the DSI inspection results became known, the Division sent proposed PMR/PMC studies to HGS, including a combination study (raxibacumab plus antimicrobial study), further brain examination, and testing of higher doses for efficacy. HGS replied on November 5, 2009 to these recommendations, noted that an established model to show the added benefit of raxibacumab had not yet been developed, committed to identifying such a model, and discussed the sample size needed to show superiority [2] and challenges of such a study.

Before a study showing added benefit can be conducted, an **animal model** needs to be developed in which antimicrobial survival approximates 50%, instead of the 95-100% survival seen in the BLA studies (rabbit levofloxacin-raxibacumab study, monkey ciprofloxacin-raxibacumab study). Therefore, my recommendation is that HGS commit to develop or to work with another group to develop such a model and submit a protocol for such a study as part of the complete response. This would be consistent with the recommendation that would have been made for a post-approval study, if the product had been licensed in the first review cycle. However, if the bioanalytical assay deficiencies cannot be resolved and the clinical pharmacology studies, including the pharmacokinetic portions of the animal efficacy studies, need to be repeated, then a study (or studies) to address (a) and (b) could be under way or completed and results submitted as part of the complete response.

(2) A better understanding of the **brain lesions** found in animals that had anthrax, were treated, and **died:**

In the raxibacumab monotherapy studies, animals that died in the raxibacumab arms of the studies had more severe meningeal/brain lesions, consisting of inflammation, hemorrhage, edema, necrosis and bacterial growth compared to the placebo animals (see section 3.8 Brain Findings for discussion). Various hypotheses have been offered but the mechanism and significance of these findings has not been determined. This finding may represent efficacy failure of the specific treatment dose (further, higher doses should be tested), may be a drug/disease interactions, or may be toxicity. The issue has been discussed extensively, and further studies were recommended by reviewers and consultants including:

a. Repeat efficacy study and evaluate neurological function/behavior in animals that die and survive.
b. Conduct necropsy on all animals (including survivors) and histological examination of tissues particularly the brain and meninges
c. Conduct a cross-reactivity study on rabbit, monkey, and human meningeal tissue.

[2] "To demonstrate a 20% absolute improvement in survival (to 45%) with raxibacumab over a 25% survival rate with antibiotics would require 89 animals per treatment arm to have 80% power to detect the difference at the 5% significance level...Execution of a 4-arm study (placebo, raxibacumab, antibiotic and raxibacumab + antibiotics) with over 350 animals may not be feasible under GLP at a BL3 facility or ethical from the point of the Institutional Animal Care and Use Committee. Consequently, the evaluation may be constrained to examine trends and not statistically significant added benefit."

d. Conduct histochemical staining to see whether raxibacumab is present in CSF and brain, if feasible.

In their November 5, 2009 amendment, HGS committed to the first three of these items (the fourth was not sent on October 14, 2009. Concerns were raised about immunohistochemical staining of frozen tissue that may harbor *Bacillus anthracis* as a safety hazard.)

(3) The evaluation of higher doses of raxibacumab in animal models given that survival in rabbits was 35% and monkeys 69%:

A protocol to evaluate the efficacy of higher doses should be developed, and a study initiated. Monkeys tolerated 120 mg in the toxicology study.

- A protocol for the study showing added benefit of raxibacumab over antimicrobials should be submitted and the study started, when an appropriate animal model that closely approximates the survival seen in 2001 has been developed.

- A better understanding of the brain findings in the animals that died of anthrax should be sought. The DNP consult recommends that animal efficacy studies be repeated and complete histopathology obtained to evaluate the brain lesions seen in animals that died of anthrax. In addition, studies should include measurement of raxibacumab in the CSF and brain tissue, and monitoring for anti drug antibodies is recommended (these not detected in immunogenicity studies). Animals should be treatment naïve. Raxibacumab binding to human, monkey and rabbit meninges should be evaluated. If possible, immunohistochemical staining of brain tissue from the rabbit and monkey monotherapy studies is recommended.

- A protocol for the study of higher dose of raxibacumab (e.g, 60 mg/kg or 80 mg/kg) should be developed.

Labeling incorporating the findings from the remaining studies needs to be submitted, along with any clinical pharmacology, safety, and efficacy information obtained as part of the requested studies.

In summary, a **complete response** letter should be issued outlining the following deficiencies:

- DSI/Clinical Pharmacology: resolve the bioanalytical assay and PK issues
- Compliance/Manufacturing Inspections: resolve the listed deficiency on endotoxin measurement
- Field Study: A protocol to be used in the event of an exposure for study and data collection on raxibacumab use and patient course and outcome should be submitted

In addition, HGS should be requested to design protocols and commit to initiate the following studies:

- Animal Study: A protocol to study the combination of raxibacumab and antimicrobial (e.g., fluoroquinolone) to show an added benefit of raxibacumab over antimicrobial. If a raxibacumab-alone arm is included in this study, all animals at the end of the study should be sacrificed and examined histologically, with special attention to the brain.
- Conduct a cross-reactivity study to study raxibacumab bindings in human meninges, and rabbit and monkey meninges.

- Conduct, if feasible, immunohistochemical staining of brain and meningeal tissue for the presence of raxibacumab, and measure raxibacumab levels in animals with inhalational anthrax.
- Design and conduct an efficacy study exploring higher doses of raxibacumab (e.g., 60 mg/kg or 80 mg/kg)
- Address the remaining CMC microbiology issues related to methods validations and endotoxin measurements.

2 BACKGROUND

2.1 Raxibacumab and Anthrax

Raxibacumab is a fully-humanized IgG1λ monoclonal antibody that binds domain 4 of protective antigen (PA) of *Bacillus anthracis*. *B. anthracis* is a Gram-positive bacterium found in nature, historically associated with disease in the agricultural setting and later in industrial settings (ragpickers or woolsorters disease [3]. Disease can be acquired by the cutaneous, gastrointestinal or inhalational route of entry. Cutaneous anthrax is generally characterized by a local eschar, and responds well to antimicrobial treatment. Gastrointestinal and inhalational anthrax are generally associated with bacteremia, toxemia, and even with antimicrobial treatment are often fatal. *B. anthracis* is considered a CDC Category A potential agent for bioterrorism. Natural disease was very rare in the 20th century, with the exception of two aerosolized exposures. In 1979, accidental release of *B. anthracis* spores from a factory in Sverdlovsk, Russia led to 66 deaths dues to inhalational anthrax (IA)[4], and in 2001 *B. anthracis* was disseminated through the US mail resulting in 22 cases of anthrax, including 11 cases of cutaneous anthrax and 11 cases of inhalational anthrax (IA). Mortality in the US IA cases was 45% (5/11). In addition to the 11 cases reported in 2001, 2 IA cases were reported in 2006 (Pennsylvania, Scotland) and one IA case in 2008 (London). Overall survival for these cases was 7/14 (50%) in these patients.[5]

B. anthracis carries two plasmids. The pX01 plasmid encodes for the protective antigen (PA), edema factor (EF) and lethal factor (LF). Plasmid pX02 encodes for the encapsulation B protein. The gene encoding for protective antigen is designated pagA and the gene encoding for capsular protein B is designated capB. EF is a calmodulin-dependent adenylate cyclase that increases cAMP and is responsible for edema. LF is a metalloprotease that cleaves the amino termini of the mitogen-activated protein kinase kinases (MAPKK) 1 and 2, thus inactivating them, and disrupting cellular processes. Based on research conducted by Dr. David Frucht, LT inhibits lymphocyte activation and proliferation, thereby preventing an inflammatory response to the bacterium – this is one proposed mechanism of action to account for the minimal inflammatory response seen in the placebo treated animals in the raxibacumab monotherapy studies described in the Efficacy section.

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[3] Brachman PS. Inhalational anthrax. *Ann NY Acad Sci* 1980; 353:83-93.

[4] Abramova FA et al. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak in 1979. *Proc Natl Acad Sci USA* 1993; 90:2291-4.

[5] http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5510a4.htm (2006 NYC/PA)
http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3025 (2006 Scotland)
Anaraki et al. Investigations And Control Measures Following A Case Of Inhalational Anthrax In East London In A Drum Maker and Drummer, October 2008. EUROSURVEILLANCE 13(51);1-3.· 18 December 2008
(information kindly provided by Dr. Susan McCune, OCTEC)

2.2 BLA and Main Efficacy and Safety Studies

Before submission of raxibacumab BLA 125349, FDA and HGS met on October 21, 2008 for a pre-BLA meeting.[6] The BLA was submitted May 14, 2009, given a priority review (the product has both fast-track designation and orphan designation), and scheduled for Advisory Committee meeting held October 27, 2009.

In addition to preclinical (nonclinical) toxicology and microbiology studies conducted, the following major efficacy and safety studies were reviewed:

Human Studies	• Study HGS1021-C1064 – 88 normal volunteers, • Study HGS1021-C1063 –240 normal volunteers, 80 placebo • Study HGS1021-C1069 – 20 normal volunteers Total: 326 raxibacumab and 80 placebo volunteers
Complete reports for animal efficacy studies	• Monkey: raxibacumab vs. placebo, Study 724-G005829 • Rabbit: raxibacumab vs. placebo, Study 682-G00575 • Monkey: raxibacumab plus ciprofloxacin vs. ciprofloxacin vs. placebo, Study 789-G923702 • Rabbit: raxibacumab plus levofloxacin vs. levofloxacin vs. placebo, Study 781-G923701
Histology of Brain	Examination of animals that died in the four animal efficacy studies; in Study 781-G923701, surviving rabbits were also sacrificed and examined

3. REVIEW SUMMARY

A brief summary of the various discipline reviews is presented below, for complete information on these issues, the primary and secondary reviews should be consulted.

3.1 Product Manufacture and Quality Assessment

Raxibacumab is expressed in a mouse myeloma cell line (NS0), and the secreted raxibacumab monoclonal antibody is recovered from the cell culture production medium and purified using a series of chromatographic and filtration steps. The clinical product is a single-use liquid product stored at 2-8°C, and it is protected from light until use. Each vial contains 50 mg/mL raxibacumab in 0.13 mg/mL citric acid, 2.8 mg/mL sodium citrate, 10 mg/mL sucrose, 18 mg/mL glycine, and 0.2 mg/mL polysorbate 80, pH 6.5.

Raxibacumab is supplied at a concentration of 50 mg/mL in a 50 mL sterile single use vial, containing 35.1 mL of liquid formulation per vial. It must be stored between 2°C and 8°C, and is not stable in light. The developmental version of the product was made by the "M10" manufacturing process, key human and animal efficacy studies conducted with the to-be-licensed "M11" process. (b) (4) (b) (4) (b) (4) (b) (4)

[6] Submission SN 0080, September 19, 2008

3.1.1 Inspection of Manufacturing Facility and CMC issues

The HGS production facility was initially inspected in July 2007, at which time the product was considered acceptable for shipment to the SNS. Initial recommendations from plant inspection was that results should not preclude approval, but recommended one PMR and 6 PMCs addressing process validation and more information about endotoxin limits and detection. However, once deficiencies in other areas were identified, based on the review from Patricia Hughes (November 3, 2009) the originally-proposed PMCs and PMR should now be included as deficiencies in the CR letter; these deal with validation of buffers, bioburden control, endotoxin control, limits, container closure, and statibility testing, and endotoxin levels.

Comment: Following discussion during the wrap up meeting, DMPQ recommended the bacterial endotoxin specifications in the final product should be listed as a deficiency, and the other items are listed separately as requested studies.

In addition, although not part of the HGS application or requirement for approval, an inspection of the Strategic National Stockpile **(SNS)** has been requested of CDC and is pending.

3.2 Pharmacology Toxicology

Preclinical reviews showed specific binding in skeletal muscle and thyroid (both species), endometrium (human only) and breast and prostate (monkey only). These was no binding reported in brain tissue, an observation that is important in context of the evidence of increased brain inflammation, hemorrhage, edema, bacteria and necrosis seen in rabbits and monkeys who had anthrax infection and were treated with raxibacumab.

Comment: Based on these results, the toxicology reviewers considered raxibacumab relatively safe, and mentioned the possible target organs for toxicity. Of note, there were no gross or histopathological changes reported in the CNS of the monkeys in the toxicity study.

3.3 Microbiology

Raxibacumab binds protective antigen (PA) produced by the Ames, Sterne, and Vollum strains. Therefore, it is expected that raxibacumab prevents the binding of PA to cells, and interferes with the ability of PA to form heptamers on the cell surface, creating a pore through which EF and LF can access the interior of the cell, resulting in cell damage. In efficacy studies, diagnosis relied on a positive blood culture because there were some concerns about the qualitative electroluminescence assay for PA.

3.3.1 Mechanism of Action of Raxibacumab

Raxibacumab binds to domain 4 of protective antigen (PA) with high affinity and inhibits binding of PA to anthrax toxin receptors ATR1 and CMG2 on cells. Because domain 4 is also the portion of PA that binds to cells, the domain is not available once PA is already bound to cell surface, therefore raxibacumab only binds free PA. Whether it binds PA on spore surface is unknown, however PA is expressed on the surface of spores.

The schematic that summarizes the sequence of events how PA binds to the cell surface, is cleaved, forms a heptamer pore and allows LF and EF to enter, forming LT and EF, and acting on MAPPKs and cAMP, to disrupt signal transduction and cause edema, respectively, is presented below.

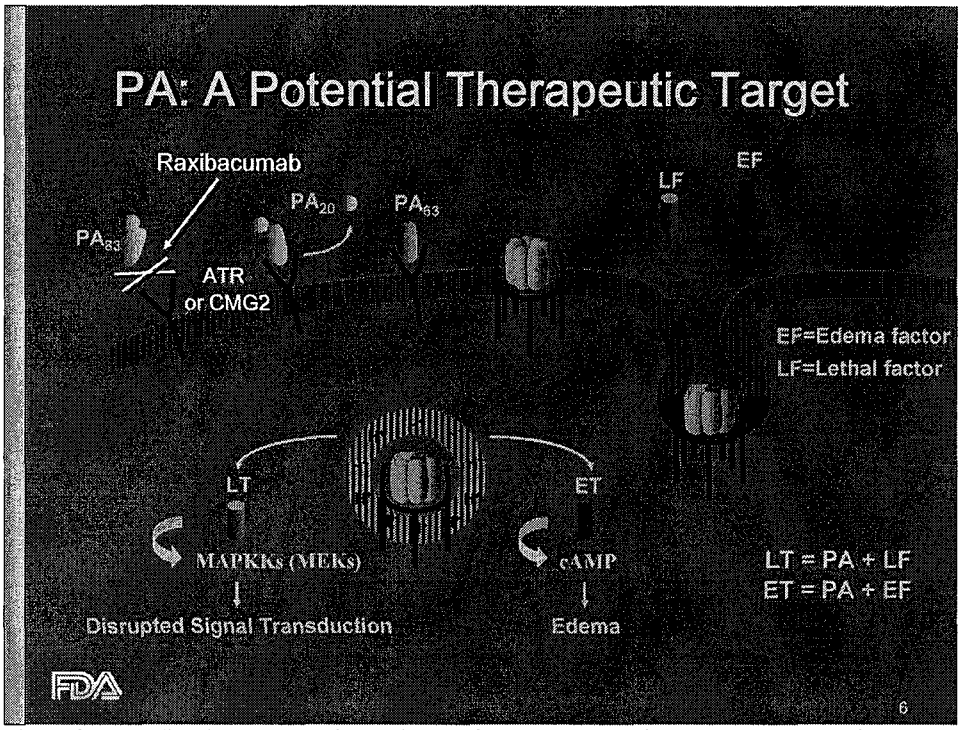


Figure from Dr. Frucht's presentation at the October 27, 2009 Advisory Committee meeting

3.3.2 Mechanism of Action of Raxibacumab vs. Antimicrobials

The mechanism of action of raxibacumab differs from small molecule antimicrobials:

- Raxibacumab targets a secreted toxin that causes immunosuppression and directly damages host tissues.
- Ciprofloxacin and Levofloxacin (approved for anthrax post-exposure prophylaxis) inhibit DNA gyrase and topoisomerase IV activity and are bactericidal.
- Doxycycline (approved for anthrax treatment) inhibits protein synthesis and is bacteriostatic.
- Penicillin (approved for anthrax treatment) inhibits cell wall synthesis and is bactericidal.

3.4 Clinical Pharmacology

The proposed dose for treatment was 40 mg/kg, and while HGS' study results supported the dose, the Division of Scientific Investigation determined that there were problems with the results of the bioanalytical assay inspection (Review dated October 7, 2009) and therefore HGS was asked to revise their procedures, correct the deficiencies, and re-assay sample to address these findings. Depending on the results, additional studies may be needed.

When the DSI inspection deficiencies were brought to the attention of the review team, there was discussion of cancelling the October 27 2009 AC meeting, but after discussion with OND and CDER the decision was made that the AC should continue, and animal efficacy models discussed. An update to the AC briefing document was issued summarizing the situation and explaining that the FDA presentations and questions would cover the efficacy and safety of the animal results, and principles of determining a human dose based on animal studies, but that FDA would not present PK data, or ask a question regarding licensure. HGS stated they were confident in their PK data and presented the information at the AC.

The principles of determining an effective human dose are:

1. Determine a regimen in animals that is safe and effective in the species chosen for the indication studied.
2. Determine the corresponding pharmacokinetic exposure (AUC, C_{max}).
3. Determine a dosing regimen in humans that will give similar or greater exposure to that observed in the animal.

The inability to discuss the PK data limited the FDA's ability to explain the doses of ciprofloxacin administered to monkeys and levofloxacin given to rabbits in the two combination studies: monkey study 789-G923702 and rabbit study 781-G923701. In her review, Dr Bergman concludes that levofloxacin dosing in rabbits at 50mg/kg by gavage q24h and that ciprofloxacin dosing in monkeys of 75mg bid by gavage were appropriately humanized before these studies were started.

> The regimen in monkeys: intragastric ciprofloxacin at a dose of 75 mg or intragastric placebo (sterile water for injection), according to treatment group assignment. This was followed by intragastric ciprofloxacin or placebo doses administered every 12 hours (q12h) for a total of six doses (three days of treatment). The recommended oral ciprofloxacin dose in humans for post-exposure of inhalational anthrax is 500 mg q12h for 60 days. An oral dose of 500 mg ciprofloxacin in humans yields a Cmax of 2.4 µg/mL. In the current study, the mean ciprofloxacin Cmax values following the first intragastric dose of 75 mg in monkeys with and with out raxibacumab co-administration were 1.12 and 0.675 µg/mL, respectively. Thus, the ciprofloxacin dose of 75 mg in monkeys was not equivalent to the human oral dose of 500 mg approved for post-exposure inhalational anthrax. Ciprofloxacin administered at the labeled oral dose of 500 mg in humans is expected to achieve higher exposures than those observed in this study conducted in monkeys with inhalational anthrax.
>
> The regimen in rabbits: intragastric levofloxacin 50 mg/kg or placebo (sterile water for injection), according to treatment group assignment. Intragastric 50 mg/kg levofloxacin or placebo doses were then administered every 24 hours (qd) for a total of 3 doses (3 days of treatment). The recommended oral levofloxacin dose in humans for post-exposure of inhalational anthrax is 500 mg q24h for 60 days. An oral dose of 500 mg levofloxacin in humans yields a Cmax of 5.1 µg/mL. In the current study, mean levofloxacin Cmax values following intragastric dosing of 50 mg/kg rabbits with and with out raxibacumab co-administration were slightly lower than the human Cmax with a 500 mg oral dose (mean Cmax range in rabbits was approximately 3.94 to 4.98 µg/mL). Thus, levofloxacin administered at the labeled oral dose of 500 mg in humans is expected to achieve slightly higher exposures than those observed in this study conducted in rabbits with inhalational anthrax.

3.4 Division of Scientific Investigation (DSI) findings

Initially the inspections of efficacy studies (monkey Study 724-G005829, rabbit Study 682-G005758) were acceptable in 2008; brain lesions were identified and promptly brought to the attention of DSPTP.

During the August and September 2009 inspection of the (b) (4) facilities where human studies C1201-1064 and C1201-1063, minor deviations were found but these were judged to have negligible impact on the results. Drs Thompson and Purohit-Sheth determined that studies C1063 and C1064 were acceptable (NAI letter September 14, 2009).

The bioanalytical assays done at HGS for human study C1201-1064 and C1201-1063 and at (b) (4) (b) (4) for ciprofloxacin in humans raised issues about the acceptability of the results and reliability of human clinical pharmacology data. These findings were brought to the attention of the review team around October 20, and resulted in an update being issued to the AC members and a revision to the FDA presentations at the AC meeting, as well as omission of any discussion of clinical pharmacology issues (See also 3.4 Clinical Pharmacology).

3.5 Efficacy Studies in Animal Models of Infection

The comparison of the *Natural History of Inhalational Anthrax* is summarized in the table below, and shows what are considered extensive similarities in the characteristics of the infection and outcome in the three species. Based on this kind of information, the rabbit and monkey model were selected for the efficacy studies.

Data Element	NZW rabbit	Cynomolgus macaque	Human
Challenge agent	Ames strain *B. anthracis* spores	Ames strain *B. anthracis* spores	Many strains: Ames strain in 2001 bioterrorism attack
Pathogenic determinants	Pathogenesis, mechanism of toxicity similar to humans	Pathogenesis, mechanism of toxicity similar to humans	Capsule, anthrax toxins
Route of exposure	Aerosol challenge with 200x the LD_{50} of *B. anthracis*	Aerosol challenge with 200x the LD_{50} of *B. anthracis*	Inhalation
Host susceptibility and response to etiologic agent	Susceptible to *B. anthracis* infection through aerosolized spores' exposure. Organ pathology generally similar to inhalational anthrax pathology in humans: thoracic lymph nodes, lungs, spleen, liver, less brain	Rhesus, African green, and cynomolgus monkeys susceptible to *B. anthracis* infection through aerosolized spores' exposure. For the most part, all three species demonstrate organ pathology lesions (thoracic lymph nodes, lungs, spleen, liver, brain) similar to each other and to human with subtle differences.	Susceptible Organs involved: thoracic lymph nodes, lungs, spleen, liver, brain

Data Element	NZW rabbit	Cynomolgus macaque	Human
Time to onset of disease/ condition	More rapid than humans (median time to bacteremia 24h)	Similar to that seen in humans but depends on the challenge dose. (median time to bacteremia 35h)	Incubation median 4 days (2001 attacks), could be as long as 43 days (Sverdlovsk)
Time course of progression of disease/ condition	More rapid than humans (median time to death 3 d)	Similar to that seen in humans but depends on the dose used for the challenge. At ≥ 2 x 105 CFU, death more rapid than humans (median time to death 3-4 d, up to 8 days)	Onset of symptoms 4-6 days. Prodromal phase 1-6 days; fulminant phase < 24 hours. Overall mortality of 85% with a median time from symptom onset to death of 6 days. In Sverdlovsk incident, death in 1-4 days.
Manifestations (signs and symptoms)	Lethargy, not eating, rapid respirations/ respiratory distress, seizure and/ or moribund	Few clinical signs until 1-2 hours prior to death, then progressively less responsive to external stimuli. Lethargy, tachycardia, and fever, although abnormal signs were individualized and did not present long before death.	Flu-like symptoms for several days followed by severe respiratory/circulatory collapse

(tables from AC presentation by Dr. Yasinskaya)

The results of the efficacy studies in rabbits and monkeys, including the design and procedures followed, were thoroughly reviewed and are summarized in the clinical, statistical, and microbiology reviews. The summary results of these studies are presented below.

The **raxibacumab monotherapy studies** evaluated saline placebo, 20 mg/kg raxibacumab and 40 mg/kg, all administered intravenously to animals that had been exposed to aerosolized *B. anthracis* spores and were bacteremic when treatment was started. Monkeys were pretreated with diphenhydramine to simulate the human regimen. Survival was assessed in each arm and is shown in the table below. The 40 mg/kg dose was superior to placebo in both arms:

Treatment group	**Cynomolgus macaque at 28 days**			**NZW rabbits at 14 days**		
	Number (%) of survivors	P value vs. placebo[1]	95% CI of raxibacumab-placebo[2]	**Number (%) of survivors**	P value vs. placebo[1]	95% CI of raxibacumab-placebo[2]
Placebo	**0/10 (0%)**			**0/13 (0%)**		
20 mg/kg raxibacumab	**5/12 (41.7%)**	0.0396	(7.2, 68.7)	**4/16 (25.0%)**	0.1067	(-2.2, 50.9)
40 mg/kg raxibacumab	**9/13 (69.2%)**	0.0016	(31.1, 88.9)	**6/17 (35.3%)**	0.0237	(7.3, 59.6)

[1] P value based on 2-sided Fisher's exact test for the comparison vs the placebo control group.

[2] 95% CIs are exact confidence intervals

(table from AC presentation by Dr. Yasinskaya)

In the **raxibacumab plus antimicrobial studies,** animals were randomized to one of three arms: saline placebo, antimicrobial (levofloxacin in rabbits, ciprofloxacin in monkeys), and a combination of 40 mg/kg raxibacumab IV plus antimicrobial (given by gavage). Antimicrobial doses were humanized as summarized under section 3.4 Clinical Pharmacology. The efficacy of the antimicrobial regimen was 95-100%. Both treatment arms were superior to placebo and there was no significant difference between the active treatment arms.

Treatment group	**Cynomolgus macaque at 28 days**		**NZW rabbits at 28 days**	
	Number (%) of survivors	95% CI of Cipro/raxibacumab-ciprofloxacin[2]	**Number (%) of survivors**	95% CI of Levo/raxibacumab-levofloxacin[2]
Placebo	**0/10 (0%)**	-	**0/10 (0%)**	-
Antimicrobial alone[1]	**13/13 (100%)**	-	**19/20 (95.0%)**	-
Antimicrobial [1] + 40 mg/kg raxibacumab	**11/13 (84.6%)**	(-45.5, 11.4)	**16/17 (94.1%)**	(-23.9, 19.6)

[1] NHP study antimicrobial was 75 mg ciprofloxacin twice daily x 3 days
NZW rabbit study antimicrobial was 50 mg/kg levofloxacin daily x 3 days

[2] 95% CIs are exact confidence intervals

(Table from AC presentation by Dr. Yasinskaya)

Comment: In these animal studies, the efficacy of the antimicrobials was high. Although there was some concern that antagonism is shown because one monkey died of anthrax in the ciprofloxacin/raxibacumab arm, in the rabbit studies, one rabbit died of anthrax on levofloxacin but none on levofloxacin/raxibacumab (one rabbit died of gavage accident). One of two monkeys in the

raxibacumab/ciprofloxacin arm died of a gavage accident, and the one rabbit in the raxibacuman/levofloxacin arm also died of a gavage accident. The deaths due to anthrax were as follows:

Deaths due to anthrax	***Monkey***	***Rabbit***
Placebo	*10*	*10*
Fluoroquinolone arm	*0*	*1*
Raxibacumab plus fluoroquinolone arm	*1*	*0*

3.7 Animal Rule [7]

Because inhalational anthrax is exceedingly rare, is not acquired naturally, and is highly fatal, intentional exposure would be unethical. Therefore, efficacy data on raxibacumab was obtained from animal models of infection, and HGS requested approval of the BLA under Subpart H of the regulations 21 CFR 601.90, *Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible.* This rule applies when adequate and well-controlled studies in humans cannot be ethically conducted and field efficacy studies are not feasible. In such circumstances, products intended to reduce or present serious or life-threatening conditions may be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals and additional supporting data.

To obtain guidance whether approval should be based on the demonstration of superiority of monoclonal antibody over placebo or whether animal efficacy studies should also show the contribution of monoclonal over antimicrobial to survival, this issue was presented at a Regulatory Briefing in October 2006. Following presentations and discussions, the senior leadership group recommended that statistical superiority must be shown of the monoclonal over placebo, and studies evaluating the monoclonal and antimicrobials should be requested. However, recognizing the challenge to conducting these studies, the group did not recommend that approval be withheld if an additive effect of each component could not be demonstrated. Furthermore, it was noted that the two products have different mechanisms of actions.

The animal rule further provides four elements that must be met for animal studies of efficacy to support approval under this rule. HGS conducted the four efficacy studies summarized above, and demonstrated that raxibacumab is superior to placebo in two species, but did not demonstrate that the monoclonal offered an added benefit over antimicrobials in the two studies where this was tested. However, the information provided by HGS in the preclinical, natural history, and efficacy studies can be used to address the elements below. In addition, before DSI inspections identified deficiencies in the bioanalytical assay, HGS had provided PK data to address the fourth element.

(1) There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;

[7] Animal Rule published May 31, 2002, titled Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible, Biologics 21 CFR 601.90 (Subpart H), Drugs 21 CFR 314.600 (Subpart I)

Studies evaluating PA and raxibacumab were conducted, and the information is summarized in section 3.3 Microbiology, showing that raxibacumab blocks the binding of PA to the cell surface receptors, thereby blocking the cascade by which LT and ET are formed and interfere with normal cellular function, leading to edema and death.

In rat lethal toxin infusion model, raxibacumab prevented mortality compared to no treatment, supporting the direct toxic effect of LT in the rat model, and demonstrating that raxibacumab prevented mortality due to LT.

(2) The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;

Efficacy studies were done in rabbits and monkeys, both of which appear to share similarities in disease pathogenesis and outcome compared to humans. In these animal models two doses were tested. In both species the 40 mg/kg was superior to placebo; in the monkey the 20 mg/kg dose was also superior to placebo.

(3) The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and

In both humans and animals, the desired benefit is survival, and this endpoint was evaluated in all four animal studies.

(4) The data or information on the pharmacokinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

The 40 mg/kg dose presented by HGS was also considered to be the appropriate dose in humans, however, the DSI inspection findings have put further discussion of the PK data on hold.

The four elements do not specifically address the need to show a benefit over existing therapy, however, the preamble to the animal rule states:

> "We have decided to eliminate the requirement that 'products would be expected to provide meaningful therapeutic benefits to patients over existing treatments,' as well as the limitation that the toxic agent be 'without a proven treatment' (proposed §§ 314.600 and 601.60). Recent events involving the multiple exposures to anthrax in our population, and deaths resulting from those infections, have indicated a need for a wide range of therapeutic options that, in some instances, might be inappropriately limited by requiring new products to have a therapeutic benefit over existing treatments, or to be used only in the absence of a proven treatment. Availability of a variety of drug and biological products is important because, for example, patient recipients may be allergic to one product and require another, may be intolerant of a product because of side effects, or may respond more favorably to one product than another. We also believe that a wider variety of therapeutic choices will limit potential problems with availability, accessibility, and distribution of products. We have modified the final rule to address these concerns and help ensure the availability of more than one therapeutic option."

The language in the preamble portion of the rule has been interpreted differently by reviewers for raxibacumab. One interpretation (shared by this reviewer) is that under the animal rule, demonstration of superiority can serve as basis of approval without the need to demonstrate an added benefit – a study to demonstrate such a benefit, however, should be requested as a post-marketing commitment. During development, FDA did ask HGS to conduct a study examining raxibacumab plus antimicrobials, but the treatment model yielded 95% to 100% survival following antimicrobials, and further animal development is needed to more closely parallel the outcome seen during 2001 (55% survival). On the other hand, the preamble can be interpreted to mean that if the studies are designed so that the "standard treatment" arm becomes the de facto "placebo" arm, then efficacy of the monoclonal would be demonstrated by showing that it is superior to the de facto "placebo" arm. Although the antimicrobial and raxibacumab arm did not show a benefit over antimicrobials, one piece of information was gained from this study. In the rabbit study, all animals were necropsied and brains examined – there were **no brain lesions** seen in any of the raxibacumab plus antimicrobial treated animals, providing initial evidence that suggests that raxibacumab in the setting on anthrax may not be toxic to the brain, albeit in this study it was used in combination with antimicrobials.

The animal rule also requires that products approved require

- ***Postmarketing studies***
  - ***such as field studies, to verify and describe the biological product's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical***

  A protocol has been requested and will be submitted by HGS

- ***Approval of product with restrictions to ensure safe use***
  - ***e.g., certain facilities, practitioners, procedures, recordkeeping***

  This is an intravenous product intended for the treatment of a potentially fatal disease, and there are no safety issues that would preclude making the product immediately available, therefore, no restrictions are anticipated at this time.

- ***Information to be provided to patient recipients***
  - ***Labeling that explains the product was approved based on animal efficacy studies***
  - ***Indication, directions for use, safety information, other relevant information***
  - ***Must be available to patient prior to administration, if possible***

  Patients labeling information was proposed in the original labeling and will be addressed during the resubmission.

3.8 Brain Findings

3.8.1 Deaths due to Anthrax

The finding of severe brain lesions in animals that had anthrax, received treatment and died caused a concern first identified by the DSI review staff in 2008. This led to a meeting and discussion of these findings before the SNS decision was made. These findings were then confirmed after thorough review of the application. However, put in context of the entire study and pathophysiology, one also needs to consider that there was a significant benefit in terms of survival in the monotherapy studies:

- Survival was 0% 25% and 35% in rabbits at 0, 20, and 40 mg/kg of raxibacumab
- Survival was 0% 44% and 69% in monkeys at 0, 20, and 40 mg/kg of raxibacumab

3.8.2 Histologic findings (Systemic vs. CNS findings)

Four efficacy studies were done, two monotherapy and two where raxibacumab was given with fluoroquiniolone (levofloxacin in rabbits and ciprofloxacin in monkeys).

In the monotherapy studies, only the animals that died were examined grossly and histologically. The data, as seen in table below, show that in placebo treated animals, lesions in thoracic and abdominal organs is more severe than in raxibacumab treated animals, whereas the brain lesions are more severe in the raxibacumab treated animals than the placebo animals (see shaded rows)

Raxibacumab Monotherapy Studies: High Grade pathology in Non-Survivors

Organ/animal	Placebo (%)	Raxibacumab 20 mg/kg (%)	Raxibacumab 40 mg/kg (%)	P-value (20 vs. p)*	p-value (40 vs. p)*
Cynomolgus Monkey (As treated)					
Mortality	**12/12 (100)**	**7/14 (50)**	**5/14 (35.7)**	**0.006**	**0.001**
CNS	**1/12 (8)**	**6/7 (86)**	**3/5 (60)**	**0.002**	**0.053**
Liver	3/12 (25)	1/7 (14)	0/5 (0)	1.0	0.515
Spleen	11/12 (92)	5/7 (71)	4/5 (80)	0.523	0.515
Mediastenitis	10/11 (91)	4/7 (57)	4/5 (80)	0.245	1.0
Pneumonia	5/12 (42)	0/7 (0)	1/5 (20)	0.106	0.6
Nephritis	2/12 (17)	0/7 (0)	0/5 (0)	0.509	1.0
NZW Rabbit (As treated)					
Mortality	**16/16 (100)**	**12/17 (70.5)**	**11/19 (57.9)**	**0.044**	**0.004**
CNS	**2/16 (12)**	**9/12 (75)**	**6/11 (55)**	**0.001**	**0.033**
Liver	6/16 (38)	3/12 (25)	3/11 (27)	0.687	0.692
Spleen	11/16 (69)	2/12 (17)	2/11 (18)	0.009	0.018
Mediastenitis	15/16 (94)	7/12 (58)	8/11 (73)	0.057	0.273
Pneumonia	10/16 (63)	0/12 (0)	0/11 (0)	0.001	0.001
Nephritis	4/16 (25)	1/12 (8)	2/11 (18)	0.355	1.0
Cardiac	0/16 (0)	0/12 (0)	2/11 (18)	ns	0.157

- two-sided Fisher's exact test
- Table from Dr Yasinskaya's review

Referring to the table of histologic findings, the animals in the placebo group are likely to have died because of systemic effects (lung, cardiac) while animals that were treated were beginning to show a systemic response (less pathology systemically) but perhaps because of the inability of raxibacumab to gain access to brain tissue, bacteria continued to proliferate in this protected site, and there is evidence of an inflammatory response to the bacteria. Fundamentally, however, the mortality in these animals was attributable to anthrax, with probably variable progression of disease in different organs, depending probably on treatment and dose. The idea that raxibacumab may be toxic to the brain has been raised, and may be considered, however, the explanation that LT blocks T cell and B cell proliferation, and PML activation, and this effect is then blocked by raxibacumab once it binds PA, may be an alternative explanation for this finding. As noted, the apparent dose effect (severity of brain lesions is lower in the 40 mg/kg arm than 20 mg/kg arm), also would suggest an efficacy component to this finding.

In the monkey/ciprofloxacin study, only the animals that died were evaluated grossly and histologically. The placebo animals has low grade bacteria present in CNS, no ciprofloxacin treated

monkey died. Two animals died in the ciprofloxacin/raxibacumab arm. The one that died of gavage accident had no brain lesions, while the one that died of anthrax had CNS lesions consistent with anthrax.

In the rabbit/levofloxacin study, placebo treated animals had low grade bacteria in the CNS, one levofloxacin treated rabbit that died of anthrax and had brain lesions, the levofloxacin treated rabbits that lived had no CNS abnormalities. One levofloxacin /raxibacumab treated rabbit died of a gavage accident after having bacteremia and starting treatment, but had no brain findings. The rabbits that survived had no pathological findings in the brain on autopsy.

Comment: Therefore, preliminary evidence from rabbits and one monkey suggest that animals that survive treatment with raxibacumab plus antimicrobial do not develop CNS lesions.

Given that bacterial meningitis is generally a symptomatic, rapidly progressive condition in the absence of appropriate treatment, the likelihood is that surviving animals have normal brain histology. HGS reported that there were no abnormal behaviors in the monkeys that survived and were observed for up to 3 months before being returned to the colony.

The reason for the brain lesions is unknown and has not been further investigated at this stage of development. Monoclonal antibodies generally are not known to penetrate the CNS, and it is not even clear whether they can penetrate the CNS when there is meningitis. In the case of raxibacumab, CNS levels have not been measured in either infected animals or as part of routine toxicology studies in normal animals. However, given that there was no evidence of inflammation, hemorrhage or edema in the toxicology studies, the effect is probably not independent to raxibacumab but in some way represents a disease/drug interaction. Tissues taken at necropsy were not stained to see whether raxibacumab penetrated the CNS.

3.8.3 Hypotheses regarding the CNS lesions include:

(1) Increased Duration of Survival

Increased duration of survival in the animals that have CNS findings: various analyses of time to death in animals that died was done and showed no statistical differences among the arms. Numerically, however, the animals that had the more severe CNS lesions lived 4 days, while those without severe lesions lived less than 3 days, but only one placebo monkey and no placebo rabbits fell into this category. Therefore, this hypothesis would need to be further studied.

Table 1: NHP Median time to death by brain inflammation status

	m/M* brain inflammation	Not m/M* brain inflammation	Wilcoxon, p-value
Pooled 3 groups (sponsor's analysis)	4.5 (n = 9)	3.2 (n = 15)	P = 0.017
Raxibacumab (pooled 20 and 40)	4.3 (n = 8)	2.5 (n = 4)	P = 0.022
Raxibacumab 40 mg	4.9 (n = 2)	2.7 (n = 3)	P = 0.149
Raxibacumab 20 mg	4.0 (n = 6)	2.3 (n = 1)	P = 0.211
Placebo	4.5 (n = 1)	3.3 (n = 11)	P = 0.385

m = moderate, M = marked inflammation

Table 2: NZW rabbits Median time to death by brain inflammation status

	m/M* brain inflammation	Not m/M* brain inflammation	Wilcoxon, p-value

Pooled 3 groups (sponsor's analysis)	4.0 (n = 6)	2.7 (n = 33)	P=0.003
Raxibacumab (pooled 20 and 40)	4.0 (n = 6)	2.7 (n = 17)	P = 0.002
Raxibacumab 40 mg	3.5 (n = 3)	2.5 (n = 8)	P = 0.032
Raxibacumab 20 mg	5.8 (n = 3)	2.7 (n = 9)	P = 0.027
Placebo	n/a (n = 0)	2.7 (n = 16)	n/a

* m = moderate, M = marked inflammation
(Tables from Dr Lan Zeng's review)

HGS initially stated that the animals with brain lesions lived longer, however the table below shows that there is no significant difference in survival among the arms, and the range of time to death is quite wide.

Table 3: NHP Time to death for animals that died

	Median	Range	Mean
Raxibacumab 40 mg (n = 5)	3.7	[2.3, 5.2]	3.7
Raxibacumab 20 mg (n = 7)	3.9	[2.3, 5.5]	3.9
Placebo (n = 12)	3.3	[1.9, 10.8]	4.3
Kruskal-Wallis, p-value=0.855			

Table 4: NZW rabbit Time to death for animals that died

	Median	Range	Mean
Raxibacumab 40 mg (n = 11)	2.9	[1.5, 4.1]	2.7
Raxibacumab 20 mg (n = 12)	3.0	[1.4, 6.9]	3.4
Placebo (n = 16)	2.7	[1.4, 4.9]	3.0
Kruskal-Wallis, p-value=0.655			

Comment: In his consult, Dr. Walton suggests one hypothesis that anthrax may be a rapidly progressive disease and significant progression of CNS involvement may be seen within a few hours, terminally. To that end, it is intriguing that the median duration of survival is the shortest in the placebo arm (3.3 days, 2.7 days) where animals had on average less severe lesions, and longest in the 20 mg/kg arm (3.9 days and 3.0 days) where animals had on average most severe lesions, and intermediate in the 40 mg/kg group (3.7 day and 2.9 days) also consistent with the findings. On the other hand the mean values do not show a similar patter. Given all these are post-hoc analyses, they should be viewed with caution.

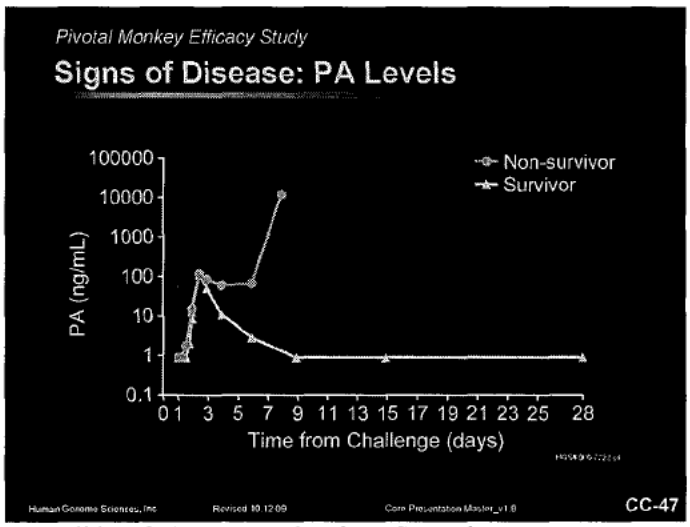
(2) <u>LT causes Immunosuppression</u>

The other hypothesis was offered by Dr. Frucht based on his research and is related to the immunosuppressive activity of LT (LT inhibits proliferation and function of T cells, B cells and PMLs), and effective treatment may play a part in reversing this inhibition (e.g., monoclonal, antimicrobials).

Also noteworthy, however, is that LT is considered to cause circulatory shock by direct effects on endothelial cells, vascular "leakiness," and cause cardiac toxicity (by endothelial necrosis, mitochondrial and myofilament degeneration). This may be relevant to the mechanism of death in the placebo animals which had more systemic anthrax present and less severe anthrax lesions in the brain.

3.8.4 PA and disease

PA levels detectable in animal correlated with survival or death. Surviving animals had an early rise followed by decline over 7-9 days peak and then drop, while animals that died had a rise-plateau-rise as PA levels continued to escalate as show in the figure below.



HGS slide CC-47 presented at the AC meeting

(3) Toxicity of Raxibacumab

The question was raised whether the CNS findings represent toxicity due to raxibacumab. This is not supported by the preclinical and human safety data in uninfected subjects. Raxibacumab did not bind or show toxicity in brain tissue. No CNS events in humans were noted -- the incidence of headaches was similar between raxibacumab and placebo arms, the one patient with schizophrenia had a pre-existing diagnosis. The likely explanation would seem to be related to a drug/disease interaction, but further data are needed.

(4) Other Mechanisms

The role of cytokines, chemokines, antigen/antibody complexes may be considered, although no data to confirm or refute this is available. CSF analysis or immunohistochemical staining of brain tissue for raxibacumab was not performed.

(5) Consult from DNP

DNP met with DSPTP for an informative discussion of the studies and commented that the precise mechanism of the brain lesions is not clear, but monoclonals are considered unable to penetrate the CNS.[8] Whether there is some drug disease interaction that changes the blood brain barrier characteristics and allows more bacteria and raxibacumab to penetrate, and somehow influences the severe inflammation and edema, is not known. As noted in the DNP consult, in the absence of information, it is not possible to determine whether the brain findings are relevant. DNP recommended that animal studies be repeated, all animals examined to see whether survivors had similar lesions, and histochemical staining of brain tissue be requested to evaluate distribution of raxibacumab.

(6) Consult from Dr Walton

[8] See consult reviews by Barbara Wilcox and Eric Bastings, and Louis Freed 8/18/09

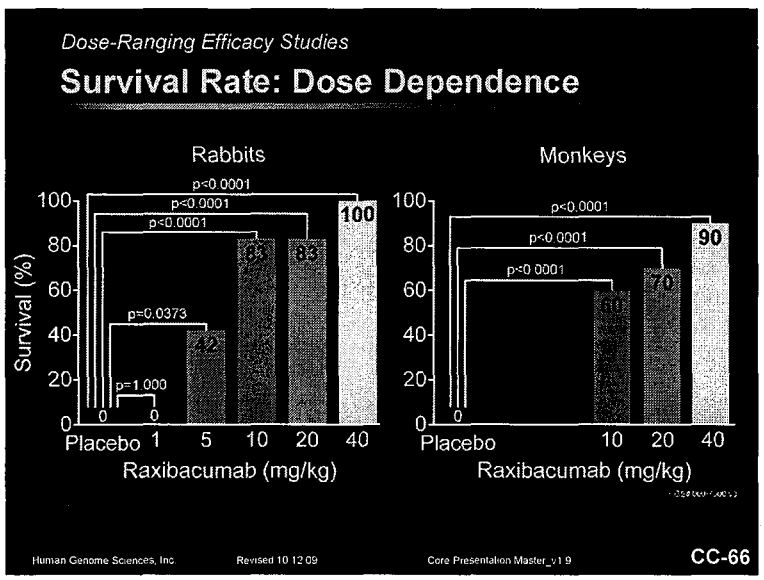
Dr. Walton reviewed the information from preclinical, animal efficacy, and human studies and noted that a direct toxicity due to the antibody was unlikely given the brain cross-reactivity studies were negative, suggested that the meninges in such studies be examined if this had not already been done. Absence of cross-reactivity makes direct toxicity very unlikely. The most likely drug disease interaction is insufficient treatment effect due to raxibacumab access to the brain, and did note that the survival benefit should not be dismissed because of the CNS findings.

Comment: Although the mechanism of brain findings in treated animals is not elucidated, CNS lesions were not seen in rabbits that survive treatment with antimicrobial and raxibacumab, or antimicrobial alone. Although there was concern articulated by some reviewers that surviving animals may have brain lesions, this is probably unlikely but not proven; survivors who received only raxibacumab have not been sacrificed and brain examined histologically.

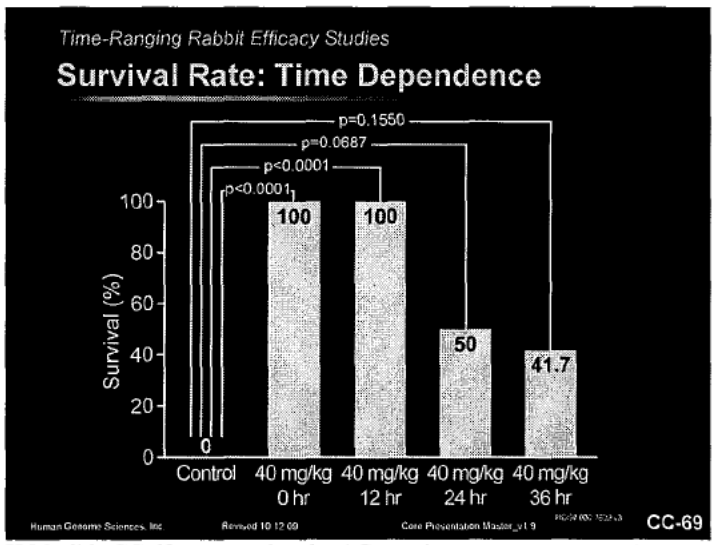
3.9 Raxibacumab Treatment Doses and Timing

Although survival was significantly higher with 40 mg/kg raxibacumab compared to placebo in rabbits (35% vs 0%) and in monkeys (69% vs. 0%), the question came up whether survival could be increased if ever higher doses of raxibacumab were tested. One reason for this suggestion was because the lesions seen in the brains were more frequent and severe in the lower dose of 20 mg/kg than the higher dose of 40 mg/kg. This pattern suggests a dose response seen, and raises the question whether a dose of 60 mg/kg or 80 mg/kg may be even more effective. The animal toxicology monkey study found a dose of 120 mg/kg safe for further testing; however, doses above 40 mg/kg have not been tested in humans and therefore would be need to be tested. Whether these higher doses would be safe in humans is not known, however, given that this is a fully humanized antibody infused intravenously, it is possible that toxicity would be similar to what was seen at 40 mg/kg (e.g., rash), although it is also possible that infusion time would need to be prolonged beyond the 2 hours 15 minutes used in human volunteer studies to minimize any infusion related adverse events.

Raxibacumab was also evaluated in post exposure prophylaxis studies which showed that there was a dose response (40 mg/kg most effective compared to lower doses) and a timing effect (raxibacumab 40 mg/kg given pre exposure or within 12 hours of exposure resulted in 100% survival) whereas delay of raxibacumab in rabbits of 24 and 36 hours resulted in about 45% survival.



HGS slide CC-66 presented at the AC meeting



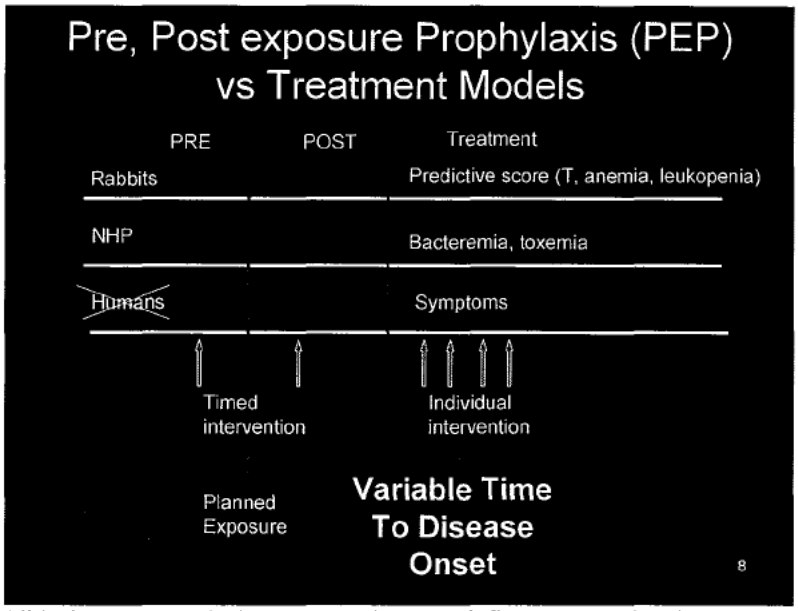
HGS slide CC-69 presented at the AC meeting

Interestingly, the delay also correlated with the appearance of bacteremia and temperature elevations, which are considered evidence that the animal is infected (i.e., the spores present in lungs have germinated and become vegetative cells which have gained access to blood stream and are capable of producing and releasing PA, EF, LF).

Time (hrs)	Rabbits	Monkeys
PA detected	24.0	36.0
Bacteremia (culture)	24.0	36.0
Temperature increase	26.7	47.5
First positive CRP	34.0	42.0
Death	70.5	96.3

Slide CC-35 from HGS AC presentation, adapted

The slide below summarizes the time sequence of *Bacillus* exposure and disease and shows that there is variability in the timing of onset and course of disease, which is relevant to the conduct of studies and is likely to impact outcome – the earlier treatment is initiation on this timeline, the better the outcome.



Slide from Dr. Yasinskaya's Regulatory Briefing presentation (October 2008)

3.10 Clinical Safety

The safety of raxibacumab 40 mg/kg IV was evaluated in 326 normal volunteers, 80 others received placebo. Also, 20 volunteers received two doses 4 months apart to evaluate immunogenicity and 23 patients received two doses 2 weeks apart to evaluate repeat dosing.

Study	Design	Population	Number of Subjects
C1064	Combination with ciprofloxacin	Raxibacumab Raxibacumab + ciprofloxacin	28 60
C1069	Repeat dose after 4 months	Raxibacumab	20
C1063	Placebo-controlled, single dose and repeat dose 2 weeks later	Placebo Raxibacumab, one dose Raxibacumab, two doses	80 217 23
		Raxibacumab Total	**326**

Serious events were death following a motor vehicle accident in a placebo patient, and a number of medical conditions, some probably pre-existing. (See MOR for details). Adverse event rates were similar between raxibacumab and placebo, except for rashes. No immunogenicity was shown after 14 day or 4 month single dose [Immunogenicity is generally expected to be lower for IV administered products than those given by other routes of administration].

Rash was seen in 6/25 (24%) patients without diphenhydramine pre-treatment and in 2/61 (3%) patients after institution of diphenhydramine pretreatment as shown in the table below.

Adverse Events and Drug Discontinuations Related to Rash

	Total number of rashes	Rashes attributed to raxibacumab	Discontinuation of infusion due to urticaria
All Subjects (N=86)	9/86 (10.4%)	7/86 (8.2%)	3/86 (3.5%)
Subjects w/o premedication (N=25)	6/25 (24%)	5/25 (20%)	2/25 (8%)
Subjects with premedication (N=61)	3/61 (4.9%)	2/61 (3.3%)	1/61 (1.6%)

Table from Dr Archdeacon's review

Other reactions were infusion site pain and headache. There were no thyroid abnormalities (there had been one case of auto-immune thyroiditis and one goiter in a previous study involving the M10 product). Cytokine levels were slightly elevated after infusion but not associated with any clinical findings.

3.12 Immunity

TNA titers indicative of immunity developed. Only in the pre-exposure prophylaxis monkey model were animals that had received raxibacumab before spore exposure challenged 11 months after the experiment and all the rechallenged monkeys survived, indicative that in this model, immunity developed.

4 ADVISORY COMMITTEE MEETING

The AC meeting was held October 27, 2009. Information on human safety and animal safety and efficacy was presented, but PK data was not presented or discussed because of issues with the bioanalytical assay. (See section 3.4 Clinical Pharmacology.)

During the meeting there was extensive discussion regarding the studies, concern about not being able to discuss PK data, and even unfortunate assumptions that the doses of antimicrobials were excessive, although they were humanized.

There were recommendations that more studies were needed, and benefit over antimicrobials should be shown, however, because approval was not an option on the table, comments were not specifically elicited whether the benefit studies should be pre or post approval. A few AC members volunteered that the studies should be done after approval, in rabbits and by perhaps someone other than HGS.

The committee voting was summarized in the Quick Minutes by Minh Doan, Pharm.D. presented below:

> Based on the information regarding the natural history of inhalational anthrax disease in man, in rabbits, and monkeys; the mechanism of action of raxibacumab; and the efficacy of raxibacumab 40 mg/kg IV in the animal studies, does the evidence from the animal models evaluating raxibacumab at 40 mg/kg IV predict response for treatment of humans with inhalational anthrax disease? If not, what additional studies should be conducted?
>
> *Vote: Yes 16 No 7 Abstain 1*
>
> *The majority of the Committee Members felt that the criteria for the Animal Rule were met and agreed that that the data from the animals studies could be used to extrapolate to humans. Concerns remain about dosing, and*

the timing of treatment after exposure. Further studies should include special populations, including the pediatric population.

Members of the Committee that voted 'no' thought that all four elements of the animal rule were not met and extrapolation to humans was not possible, and recommended studies be conducted that more accurately depict the course of disease in humans. Additional studies were requested to determine the benefit of raxibacumab in relation to timing post exposure.

The member of the Committee that abstained from voting did so because of a personal perception of conflict of interest.

In animal models of inhalational anthrax disease (both rabbit and monkey), a single dose of raxibacumab 40 mg/kg IV given in combination with antimicrobial therapy (levofloxacin in rabbits, ciprofloxacin in monkeys) resulted in efficacy similar to antimicrobial alone. Does the evidence provided in these studies support the conclusion that raxibacumab will not diminish the anticipated efficacy of antimicrobials in inhalational anthrax? If not, what additional studies should be conducted?

Vote: Yes 10 No 11 Abstain 3

Please note that one member changed their vote from 'yes' to 'no' during the discussion of the question.

Members of the Committee that voted 'yes' agreed that raxibacumab did not appear to diminish the effects of antimicrobials in treatment of inhalational anthrax.

Members of the Committee that voted 'no' thought that it was difficult to determine the effects of raxibacumab with antibiotics because there was no study with the antibody as the control arm. It could not be seen whether effects were due to the combination therapy or possibly antimicrobial therapy alone. It was noted that high doses of antimicrobial could have masked any detrimental effects of raxibacumab. Studies related to timing of administration, proper dosing of antimicrobials, and use of different antimicrobials were suggested.

Members of the Committee that abstained from voting did so because the need for more data and because of a personal perception of conflict of interest.

Given the high efficacy of the antimicrobial arms in the rabbit (95%) and monkey (100%) studies, the added benefit of raxibacumab to antimicrobial could not be determined. Should evidence be requested that raxibacumab makes a contribution to the efficacy over the antimicrobial alone (in rabbit and monkey animal models)? If yes, what types of additional studies should be requested and conducted?

Vote: Yes 17 No 5 Abstain 1 No Voting 1

Members of the Committee that voted 'yes' to the question thought that more evidence was needed to conclude that raxibacumab contributed to efficacy over antimicrobial therapy alone. Recommendations included studies where standard or suboptimal doses of antibiotic therapy in humans are used to see contributing effect of raxibacumab, use of timing to mimic course of infection in human, and the use of rabbits instead of primates. It was noted that additional studies should not necessarily be requested from the Sponsor.

Members of the Committee that voted 'no' to the question thought that raxibacumab possibly contributed to efficacy over antimicrobial therapy, but needed more precise animal studies to determine exact benefit. Additional studies should address when to start treatment and the state of the animal prior to treatment with raxibacumab. It was noted that the animal rule does not require benefit over conventional therapy.

The member of the Committee that abstained from voting did so because of a personal perception of conflict of interest.

There was one member of the Committee that left the room during voting and did not register a vote.

Based on the presentation of the histopathological findings from the animal efficacy studies, please discuss the implications of the CNS findings in the animal efficacy studies for humans with inhalational anthrax disease treated with raxibacumab. Do you have any recommendations on how this might be further evaluated?

The Committee recommended that CNS effects of raxibacumab be evaluated using rabbit models and not primates. It was noted that CNS effects of rabbits would be similar those of primates. It was also recommended that a study determine if the antigen-antibody complex is the reason for increased pathology due to complement being activated. The current studies showed that there were no findings of CNS effects in the absence of infection. A study to determine if supraoptimal doses of antibiotic therapy contributed to the CNS findings was also mentioned.

The safety of raxibacumab 40 mg/kg infused IV was assessed in healthy normal volunteers, who also received diphenhydramine. Due to the recent inspection report, at this time, relying on the raxibacumab concentrations and exposures reported for the 40 mg/kg IV infusion is not possible. Are there additional comments or further recommendations for safety evaluation in humans? If yes, what are these recommendations?

The Committee discussed the need for additional studies to evaluate the safety of raxibacumab in humans. Recommendations included studies to determine safety in the pediatric and elderly population. Studies are also needed to distinguish the effects of the infection, toxins, and the immune response. Again, it was noted that the issue of timing should be addressed in these studies.

5. DISCUSSION:

5.1 Clinical Considerations in Treatment

5.1.1 Infections where antimicrobial and antitoxin therapy is used

Given that the mechanism of action of antimicrobial and antitoxin is different, the likelihood they would interfere with each other is unlikely. Small molecule antimicrobials and antitoxin are used is in the treatment of a number of diseases:

- **Tetanus** (*Clostridium tetani*): antibiotics (e.g., metronidazole, penicillin), tetanus immunoglobulin (TIG) and tetanus toxoid
- **Botulinum** (*Clostridium botulinum*): botulinum antitoxin, antibiotics (e.g, penicillin, metronidazole)
- **Diphtheria** (*Corynebacterium diphtheriae)*: antibiotics (e.g., erythromycin, penicillin), dipththeria antitoxin

The effectiveness of these agents is stated to be greatest if administered early in the course of disease.[9] [10] Supportive measures are also needed.

5.1.2 Supportive measures in anthrax treatment

In animal studies, no supportive treatment was given in addition to treatment with raxibacumab and antimicrobials. In humans, however, patients were admitted to intensive care unit and supported as necessary and received multiple simultaneous interventions including BP and respiratory support, fluid drainage, dialysis, and other measures.

In the publication by Jernigan,[11] patients had pleural effusion, often requiring chest tube placement for removal of fluid. In the absence of such measures, it is possible that death could occur early due

[9] http://www.medicinenet.com/diphtheria/page3.htm

[10] Rob Roy MacGregor. Corynebacterium diphtheriae (Chapter 193) in Principles and Practice of Infectious Diseases. Mandell, Douglas and Bennett, Churchill Livingston 2000, fifth edition.

to for example due to the direct toxin mediated damage to the host, before the bacteria could be killed and toxin neutralized. In contrast, in humans, with supportive care in addition to antimicrobials, patients survived following hospitalizations ranging up to 3 weeks (Case 2).

5.1.3 Animal Efficacy Studies

Superiority of raxibacumab over placebo has been demonstrated. The rates were 35% in rabbits and 69% in monkeys. Evaluation of higher doses should be undertaken to see whether survival rate may increase.

Combination studies were conducted but the success rates of antimicrobials were 95-100%, exceeding the 55% seen in patients during the events of 2001. Additional model development is needed before repeat combination studies. Work at the NIAID to date has led to preliminary models with survival rate in the 10-20% based on antimicrobial administration in rabbits at 96 hours, and rates around 25% in monkeys based on administration of partially protective ciprofloxacin doses. The practical considerations that impact the design and conduct of these studies, and need to be kept in mind when designing these studies, are:

- BL4, BL3 laboratory
- Working with biologic agent
- Animal use, care, & handling issues
- Extensive real time diagnostics needed to guide initiation of therapy for **Treatment studies**
- Individualized intervention (24/7 monitoring)
- Duration of intervention and follow-up
- Animal availability (NHP but in rabbits sensitive to veterinary source, animal age, etc)
- Number of animals that can be studied in a cohort
  - 12 monkeys per group
  - 26 rabbits per group

Dr. Higgins calculated the sample size needed to show significance depending on anticipated benefit of drug over control regimen, and further provided the following information in the event that antibiotic efficacy is 50% or greater, and the combination has efficacy ranging from 60-90%. She notes:

> "The following sample size calculations are for a powered study to compare the antimicrobial to the combination of antimicrobial plus raxibacumab using a standard two-sided 0.05 test. Raxibacumab would need to provide fairly large additional benefit over the antimicrobial alone (20 – 30%) in order to have sample sizes of less than 100 per arm.

As seen below, the size of studies needed to show a significant difference in efficacy of the combination versus antimicrobial alone is likely to be quite large if the added benefit is small (e.g., 10%), but could be about 30 animals per arm if the benefit is about 30%.

[11] Jernigan DB, Raghunathan PL, Bell BP, et. al. Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. Emerg Infect Dis. Oct 2002;8(10):1019-28.

Sample sizes

Antibiotic Efficacy	Antibiotic + Raxibacumab Efficacy	Power 70% Sample size per arm	Power 80% Sample size per arm
.5	.6	303	385
.5	.7	71	91
.5	.8	29	36
.6	.7	278	354
.6	.8	62	79
.6	.9	23	29
.7	.8	229	291
.7	.9	47	59

6. RECOMMENDATIONS:

In summary, a **complete response** letter should be issued outlining the following deficiencies:

- DSI/Clinical Pharmacology: resolve the bioanalytical assay and PK issues
- Compliance/Manufacturing Inspections: resolve the listed deficiency on endotoxin measurement
- Field Study: A protocol to be used in the event of an exposure for study and data collection on raxibacumab use and patient course and outcome should be submitted

In addition, HGS should be requested to design protocols, and commit to initiate the following studies:

- Animal Study: A protocol to study the combination of raxibacumab and antimicrobial (e.g., fluoroquinolone) to show an added benefit of raxibacumab over antimicrobial. If a raxibacumab-alone arm is included in this study, all animals at the end of the study should be sacrificed and examined histologically, with special attention to the brain.
- Conduct a cross-reactivity to study raxibacumab bindings between human meninges, and rabbit and monkey meninges.
- Conduct, if feasible, immunohistochemical staining of brain and meningeal tissue for the presence of raxibacumab, and measure raxibacumab levels in animals with inhalational anthrax.
- Design and conduct an efficacy study exploring higher doses of raxibacumab (e.g., 60 mg/kg or 80 mg/kg)
- Address the remaining CMC microbiology issues related to methods validations and endotoxin measurements.

Please see Office Director Review
of BLA 125349 raxibacumab

11/13/09
Renata Albrecht, M.D.

11/14/09
Ed Cox, M.D. MPH

CLINICAL REVIEW - Addendum

Application Type	BLA
Application Number(s)	125,349
Priority or Standard	P
Submit Date(s)	May 13, 2009
Received Date(s)	May 14, 2009
PDUFA Goal Date	November 14, 2009
Division / Office	DSPTP/OAP
Reviewer Name	Sue Lim
Addendum Completion Date	November 12, 2009
Established Name	Raxibacumab
Therapeutic Class	Human monoclonal antibody
Applicant	Human Genome Sciences
Formulation(s)	Sterile liquid
Dosing Regimen	Single 40 mg/kg IV dose
Indication(s)	(b) (4) inhalational anthrax
Intended Population(s)	Subjects with a known or suspected exposure to *Bacillus anthracis*

Template Version: March 6, 2009

Executive Summary

On the basis of recommendations from the FDA's Division of Scientific Investigations' audit, the Division of Special Pathogen and Transplant Products will take a Complete Response action on BLA 125,349 (raxibacumab) on November 13, 2009.

The primary basis for this decision is the result of deficiencies in the analytical procedures used to collect pharmacokinetic data for raxibacumab and ciprofloxacin. A critical element of the Animal Rule which must be addressed for product approval is that "data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans". The inability to substantiate the reliability of the pharmacokinetic data generated for raxibacumab and ciprofloxacin in humans to provide the necessary link between the animal models and humans by the action date resulted in the Division's decision to take a Complete Response action.

A Complete Response (CR) action will be taken by FDA on the goal date of November 13, 2009 for BLA 125,349 (raxibacumab) as a result of analytical deficiencies identified by the FDA Division of Scientific Investigations' audit of the clinical investigation sites for this application.

This addendum to the full Clinical review of raxibacumab summarizes the key clinical points and recommendations from the expert panel discussion at the Raxibacumab Anti-infective Drug Committee Advisory Committee meeting, and from the Division of Scientific Investigations' audit, the latter of which resulted in the CR action.

Please refer to the Divisional Director's review and the issued CR letter for additional details. Please refer to the OC/DMPQ review for details regarding the compliance/manufacturing deficiencies.

1. Advisory Committee Recommendations - Clinical

At the Anti-infective Drug Committee Advisory Committee convened on October 27, 2009 to discuss the data on raxibacumab's efficacy in animal models and its safety in animals and human volunteers, the panel recommended additional information be obtained to evaluate added benefit or possible antagonism of raxibacumab on antimicrobials in animal models. It was agreed that the unanticipated high efficacy of antibiotics alone in the combination studies limited the determination of any added benefit of raxibacumab in studies submitted in the BLA. It was further discussed that such information was necessary because of anticipated conditions of use in the setting of a bioterrorism event, namely that antimicrobials are likely to be administered in the first-line treatment of inhalational anthrax unless contraindicated and therefore the effects of

combination use should be evaluated. Given that an animal model of approximately 50% decreased antibiotic efficacy to replicate the antibiotic efficacy observed in humans in the 2001 US attacks has not been developed, and questions remain regarding feasibility of a study demonstrating statistically significant added benefit of antitoxin treatment to a 50% efficacy antibiotic alone model (number of animals per arm ranging from 40 up to >300 depending on the effect of raxibacumab), details of the design and conduct of such study will require additional discussions. The Applicant should be required to submit a protocol of the added benefit study pending the establishment of an appropriate animal model for evaluation of added benefit.

The Applicant will also be required to conduct two additional clinical studies. The first study will be to investigate the effect of raxibacumab on the central nervous system. Similar to the added benefit study, details of the design and conduct of this study will require additional discussion between FDA and the Applicant. Second, to fulfill a requirement of the Animal Rule, the Applicant will also be asked to submit the field study protocol for review. While a study synopsis was submitted to FDA on November 5, 2009, a protocol for such a study will be requested as a Complete Response issue.

2. Division of Scientific Investigations' audit

In October 2009, the Division of Special Pathogen and Transplant Products (DSPTP) was made aware of the results of the Division of Scientific Investigations (DSI) audit of the clinical and analytical portions of studies HGS1021-C1063 and HGS1021-C1064. Upon inspection of the bioanalytical sites responsible for analysis of serum raxibacumab and plasma ciprofloxacin concentrations in humans, DSI reported several deficiencies in the analytical procedures used for both drugs. These findings raised concern about the reliability of the pharmacokinetic data generated for raxibacumab and ciprofloxacin in humans.

Upon review of these findings, the Clinical Pharmacology team has recommended the following:

1. Adequate revision of the analytical procedures to address these inspection findings.
2. Re-assay of the pharmacokinetic samples from human studies HGS1021-C1063, HGS1021-C1064, and HGS1021-C1069 using the revised procedures.
3. Re-calculation of the pharmacokinetic parameters.

Similarly, the plasma pharmacokinetic samples obtained from HGS1021-C1064 and the cynomolgus monkey study 789-G923702 for determining ciprofloxacin concentrations should be re-assayed using a revised analytical method that

adequately addresses the DSI findings. The pharmacokinetic parameters for ciprofloxacin should be re-calculated.

Lastly, as the pharmacokinetic data generated from the animal efficacy studies in New Zealand White rabbits and cynomolgus macaque monkeys used the same bioanalytical procedures as that for the human studies, FDA has also recommended repeat assay of the pharmacokinetic samples from animal studies 682-G005758 and 724-G005829 using the revised procedures followed by re-calculation of the pharmacokinetic parameters for raxibacumab.

Sue Lim 11-13-09

Sue Lim, M.D.
Medical Officer/Division of Special Pathogen and Transplant Products
November 13, 2009

11.13.09

Yuliya Yasinskaya, M.D.
Acting Medical Team Leader/Division of Special Pathogen and Transplant Products
November 13, 2009

Cross-Discipline/Medical Team Leader Review

Date	October 21, 2009
From	Yuliya Yasinskaya, M.D. Acting, Medical Team Leader Division of Special Pathogen and Transplant Products
Subject	Cross-Discipline Team/Medical Leader Review
NDA/BLA # **Supplement#**	125349
Applicant	Human Genome Sciences, Inc.
Date of Submission	May 4, 2009
PDUFA Goal Date	November 14, 2009
Proprietary Name / Established (USAN) names	Raxibacumab
Dosage forms / Strength	1700 mg vials
Proposed Indication(s)	Treatment of inhalational anthrax due to *B. anthracis*
Recommended:	*Complete Response*

Table of Contents

1. Introduction 2
2. Background 4
3. CMC/Device 6
4. Nonclinical Pharmacology/Toxicology 7
5. Clinical Pharmacology/Biopharmaceutics 8
6. Clinical Microbiology 11
7. Clinical/Statistical-Efficacy 12
7.1 Raxibacumab versus Placebo 13
7.1.1 Cynomolgus Macaque 13
7.1.2 New Zealand White Rabbit 17
7.2 Efficacy of Raxibacumab and Antimicrobial in Animal Models of Inhalational Anthrax 20
7.2.1 Cynomolgus Macaque 21
7.2.2 New Zealand White Rabbit 24
8. Safety 26
8.1 Histopathology findings from animal efficacy studies 26
8.2 Human Safety Findings 29
9. Advisory Committee Meeting 30
10. Pediatrics 31
11. Other Relevant Regulatory Issues 31
12. Labeling 31
13. Recommendations/Risk Benefit Assessment 31

1. Introduction

Inhalational anthrax due to *Bacillus anthracis* is a disease characterized by bacillary replication in mediastinal lymph nodes leading to septicemia with accumulation and dissemination of bacterial toxins to various organs. In the absence of adequate treatment, it is a highly fatal disease. Several drugs in the penicillin and tetracycline class are approved for the treatment of anthrax due to *B. anthracis* and include penicillin G procaine, penicillin G potassium, and penicillin G sodium as well as doxycycline, doxycycline calcium, and doxycycline hyclate. Tetracycline, demeclocycline hydrochloride and minocycline hydrochloride are recommended in situations when penicillin is contraindicated.

Antimicrobial treatment is directed at eradicating the bacterium. Antimicrobials, however, have no direct activity against the toxins of *B. anthracis:* lethal toxin (LT) and edema toxin (ET), the key pathogenic factors in anthrax disease responsible for the signs, symptoms, and high rates of mortality in infected individuals. These toxins are not preformed, but rather are assembled on the cell membrane of the host target cells after *B. anthracis* elaborates individual components of these toxins: protective antigen (PA), the binding moiety ([1]), lethal factor (LF), and edema factor (EF). The PA moiety of anthrax toxins binds the anthrax toxin receptors (ATR or CMG2) on host cells to initiate a cascade of molecular events that manifest as clinical disease. There are currently no approved therapies against anthrax toxins.

Raxibacumab is a first in class recombinant humanized ($IgG_1\lambda$) monoclonal antibody to *Bacillus anthracis* protective antigen (PA). Raxibacumab blocks the binding of PA to cell surfaces and prevents the anthrax toxins from entering host target cells and inducing their cytotoxic effects.

The proposed indication is treatment of inhalational anthrax, and the proposed treatment regimen for raxibacumab is 40 mg/kg administered as a single 2 hour 15 minutes IV infusion. Because of infusion reactions seen during drug development, pretreatment with diphenhydramine is recommended.

Early in the development program the Applicant requested Fast Track designation for raxibacumab, which was granted on August 15, 2003 for the investigation of raxibacumab for "*post-exposure prophylaxis and adjunctive therapy of inhalation anthrax disease, and for lessening the severity of inhalational anthrax disease, including disease caused by antibiotic resistant organisms*". Although the study reports were being submitted to the IND prior to BLA filing, no formal rolling BLA submission was pursued by the Applicant.

The Applicant also requested Orphan Drug designation for raxibacumab; the request was granted on November 12, 2003 for "*the treatment of anthrax*".

As inhalational anthrax is an extremely rare naturally-occurring disease in humans and intentional research exposure would be unethical, efficacy data on raxibacumab was obtained from animal models of anthrax infection, with the approval sought under Subpart H of the

[1] Inglesby TV, O'Toole T, Henderseon DA et al. Anthrax as a biological weapon, 2002 : Updated recomendations for management. JAMA 2002 ;287(17) :2236-52.

regulations 21 CFR 601 ("Approval of biological products when human efficacy studies are not ethical or feasible"). The Animal Rule, published in 2002 by the FDA ("New Drug and Biological Drug Products: Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible") provides a regulatory mechanism to accept efficacy from animal models when adequate and well-controlled studies in humans cannot be ethically conducted and field efficacy studies are not feasible. To obtain further guidance on whether approval should be based on the demonstration of superiority of monoclonal antitoxin over placebo, or whether animal efficacy studies should also show the contribution of monoclonal to antimicrobial efficacy in survival, the issue was presented at a Regulatory Briefing meeting in October 2006. The group recommended that statistical superiority of the monoclonal over placebo must be demonstrated, and studies evaluating the monoclonal and antimicrobials should be requested. However, recognizing the challenge in conducting these studies, the group did not recommend withholding approval if an additive effect of raxibacumab to antimicrobial efficacy could not be demonstrated. Furthermore, it was noted that the two products, i.e. antimicrobials and raxibacumab have different mechanisms of actions.

The Applicant's development program included studies showing that raxibacumab binds PA of *Bacillus anthracis* (Ames, Sterne, and Vollum strains tested) with high affinity and inhibits PA binding to the anthrax toxin receptor (ATR), on host cells; thereby, protecting the cells from anthrax toxin-mediated injury. Proof-of-concept studies with raxibacumab demonstrated a survival advantage in the rat lethal toxin infusion model, and in several pre-exposure and post-exposure prophylaxis animal model studies. The raxibacumab development program also included identification and characterization of the natural history of anthrax disease in animal models of infection (New Zealand White rabbits, cynomologus macaques), and these animal models were used to evaluate the efficacy of raxibacumab in the treatment of inhalational anthrax. Four key animal studies were conducted. These included 2 studies of raxibacumab monotherapy compared to placebo in the treatment of inhalational anthrax (rabbits, monkeys) and 2 studies of raxibacumab plus antimicrobials (levofloxacin in rabbits, ciprofloxacin in monkeys) in the treatment of inhalational anthrax.

Efficacy studies were conducted in animal models of anthrax infection because naturally-occurring inhalational anthrax infection in humans is rare to nonexistent and intentional exposure of healthy human volunteers to aerosolized *B. anthracis* spores for research purposes is unethical. In pivotal animal studies of raxibacumab monotherapy, raxibacumab conferred a statistically significant survival benefit over placebo in both monkey and rabbit animal models of inhalational anthrax. When raxibacumab was studied in combination with either levofloxacin or ciprofloxacin and compared to antimicrobial alone, there was no statistically significant difference between active treatment groups. These latter studies were not powered to detect added benefit of raxibacumab over antimicrobials alone. In addition, the animal models themselves were limited by the high (>90%) efficacy of antimicrobial monotherapy.

Raxibacumab safety was evaluated in a panel of preclinical studies and in 326 normal volunteers that received proposed 40mg/kg therapeutic dose of raxibacumab.
Upon histological examination of tissues from the non-survivor animals in pivotal animal studies it was noted that, although, all animals had evidence of anthrax disease in organs examined, the pattern of histopathologic changes was different between placebo and raxibacumab treated animals. While in placebo animals moderate to marked bacteria,

hemorrhage, inflammation, and necrosis were more frequently observed in the visceral organs including lungs, mediastinum, spleen and liver, in raxibacumab treated non-survivors the most prominent pathology findings were primarily localized to the brain. Such difference in the frequency and the severity of the pathologic findings in the brain between placebo and raxibacumab treated non-survivors across both animal species could not be fully explained by the duration of survival or duration of bacteremia prior to death. Direct CNS toxicity of raxibacumab appears to be unlikely in lieu of negative tissue cross-reactivity studies and favorable safety profile of raxibacumab in animal toxicology studies and in healthy human volunteers. While drug-disease interaction could not be excluded, in the context of survival benefit offered by raxibacumab and the knowledge that hemorrhagic meningitis is a pathognomonic feature of systemic anthrax disease reported in up to 66% of historical clinical cases, 50-60% in NHP and in 30% of rabbit animal models described in the scientific literature the clinical significance of this finding remains unclear.

Initial human safety studies showed that raxibacumab was associated with the development of infusion reactions (primarily rash); therefore, subsequent studies in human subjects and monkey efficacy studies included diphenhydramine pretreatment, and premedication with diphenhydramine is recommended in the proposed labeling. Immunogenicity was evaluated for this intravenous product and the results suggest a low risk for immunogenicity.

2. Background

Because inhalational anthrax is a rare naturally occurring disease (occupational exposure has been eradicated, intentional exposure is not ethical), efficacy of new products for the treatment of inhalational anthrax need to be derived from animal model of infection studies. In January 2009, the Agency published a draft *Guidance for Industry: Animal Models – Essential Elements to Address Efficacy Under the Animal Rule*,[2] which provides information on the development of animal models to study efficacy, including the critical characteristics of an animal model that need to be addressed under the Animal Rule. For example, these elements include the characteristics of the agent that causes the disease (in this case *Bacillus anthracis*), the host susceptibility and response to the agent, the natural history of the disease in humans and comparability in animal models, the trigger and timing of intervention, the characteristics of the medical intervention, and study design considerations.

All animal studies subject to this rule must be conducted in accordance with preexisting requirements under the good laboratory practices.

Safety evaluation of products is not addressed in the Animal Rule. Biological products (e.g., monoclonals) evaluated for effectiveness under subpart H of part 601 for are expected to be evaluated for safety under preexisting requirements for establishing the safety of new biological products. The agency believes that the safety of most of these products can be studied in human volunteers similar to the people who would be exposed to the product. Information on the safety of the product, immunogenicity, as well as potential drug interactions (e.g., raxibacumab and antimicrobial) would be expected. FDA recognizes that some safety data, such as data on possible adverse interactions between the toxic substance itself and the new product, may not be available.

[2] http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065014.htm

The Animal Rule[3] states that a biologic (e.g, raxibacumab) can be approved on the basis of adequate and well-controlled animal studies when the results of those animal studies establish that the biological product is reasonably likely to produce clinical benefit in humans. In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency. FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

- There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;
- The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;
- The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and
- The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

Therefore, data from appropriate studies to address each of the above bullet points have to be provided to support the conclusion that the product is effective.

In addition, approval under this subpart is subject to three requirements:

1. Postmarketing studies. The applicant must conduct postmarketing studies, such as field studies, to verify and describe the biological product's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical. A commitment to conduct such studies is typically included in the letter to the applicant that their biological licensing application (BLA) is "approved." Such postmarketing studies would not be feasible until an exigency arises. When such studies are feasible, the applicant must conduct such studies with due diligence. Applicants must include as part of their application a plan or approach to postmarketing study commitments in the event such studies become ethical and feasible.
2. Approval with restrictions to ensure safe use. If FDA concludes that a biological product shown to be effective under this subpart can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to ensure safe use of the biological product, commensurate with the specific safety concerns presented by the biological product, such as:
    - Distribution restricted to certain facilities or health care practitioners with special training or experience;
    - Distribution conditioned on the performance of specified medical procedures, including medical follow-up; and

[3] Request For Comments: 62 FR 40996 (July 31, 1997); Proposed Rule: 64 FR 53960 (Oct 5, 1999); Final Rule: 67 FR 37988 (May 31, 2002); Regulations: 21 CFR § 314.600-650 (New Drugs), § 601.90-95 (Biologics)

- Distribution conditioned on specified recordkeeping requirements.

3. Information to be provided to patient recipients. For biological products or specific indications approved under this subpart, applicants must prepare, as part of their proposed labeling, labeling to be provided to patient recipients. The patient labeling must explain that, for ethical or feasibility reasons, the biological product's approval was based on efficacy studies conducted in animals alone and must give the biological product's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, adverse reactions, anticipated benefits, drug interactions, and any other relevant information required by FDA at the time of approval. The patient labeling must be available with the product to be provided to patients prior to administration or dispensing of the biological product for the use approved under this subpart, if possible.

FDA may withdraw approval if:

- A postmarketing clinical study fails to verify clinical benefit;
- The applicant fails to perform the postmarketing study with due diligence;
- Use after marketing demonstrates that postmarketing restrictions are inadequate to ensure safe use of the drug product;
- The applicant fails to adhere to the postmarketing restrictions applied at the time of approval under this subpart;
- The promotional materials are false or misleading; or
- Other evidence demonstrates that the drug product is not shown to be safe or effective under its conditions of use.

3. CMC/Device

Product Quality review for this BLA was conducted by David Frucht, M.D., Team Leader in the Division of Monoclonal antibodies. Upon review of the data submitted in the application Dr. Frucht concludes that the manufacture of raxibacumab is well controlled, and leads to a product that is pure and potent. The product is free from endogenous or adventitious infectious agents sufficient to meet the parameters recommended by FDA. The conditions used in manufacturing have been sufficiently validated, and a consistent product is produced from the multiple production runs presented.

Long term stability testing (24 months) of raxibacumab bulk drug substance (BDS) and final drug product (FDP) demonstrated negligible degradation when stored at recommended storage conditions. Raxibacumab however is nearly completely degraded by (b) (4) of storage at room temperature with light exposure (b) (4)).

The quality of raxibacumab was not affected by dilution with normal saline or intravenous delivery at normal or slow rates of infusion (15 mL/hour for the first 20 minutes followed by approximately 125 mL/hour until completion) with and without an in-line filter.
Dr. Frucht indicated that HGS' proposal to extend expiry for the final drug product to (b) (4) (b) (4) based on data from two full-scale GMP lots and two (b) (4) developmental lots (otherwise manufactured identically) is acceptable, assuming (b) (4) data from these lots meets pre-determined specifications.

Early proof-of concept studies, including toxin infusion, pre- and post-exposure prophylaxis in animal models of inhalational anthrax, as well as the first in human dose ranging study of raxibacumab in healthy volunteers were conducted using raxibacumab product manufactured by the processes different from the one being proposed for licensure. Comparability analyses of raxibacumab product manufactured by pre (M10) and post (M11, proposed for licensure) scale up processes were preformed. (b) (4) Therefore, the Applicant was advised to and conducted their pivotal animal efficacy and all subsequent clinical studies with raxibacumab product manufactured by M11 (to-be-licensed) process. Clinical safety review also focused on the safety data from the studies tested to-be-licensed raxibacumab product (M11 process). It is also important to note that animal raxibacumab monotherapy and raxibacumab antibiotic combination efficacy studies in both animal models were conducted with M11 raxibacumab product.
Facilities inspections were performed by Office of Compliance/DMPQ revealed raxibacumab drug product facility, (b) (4) was classified as acceptable. A two-item FDA form 483 was issued to HGS drug substance facility citing deficiencies in the production and materials management system. Although CGMP compliance was acceptable to the inspectors for the approval, inspection was classified as VAI. It was recommended that the Applicant should complete a study (b) (4) and conduct a validation study supporting the proposed hold times for in-process materials with re-assessment (b) (4) of endotoxin and bioburden limits for in-process materials to reflect the current drug substance manufacturing process. These recommendations were to be included in the action letter as postmarketing commitments and had been communicated and agreed to by the Applicant. ***However, as a Complete Response Action is recommended for this application the postmarketing commitment*** (b) (4) ***endotoxin limits for in-process materials for raxibacumab drug substance was classified as a deficiency by OC/DMPQ team as discussed during the wrap-up meeting on 11/08/2009 and will need to be addressed by the Applicant prior to the approval of this BLA.***

4. Nonclinical Pharmacology/Toxicology

Drs. Terry Miller, Tracy McGill, and Ying Mu reviewed the nonclinical toxicity studies in this application. The following are their findings:

There were no significant toxicologic observations in either of the submitted toxicity studies of raxibacumab in healthy animals. The NOAEL values obtained in both animal studies were the high doses tested; i.e., 40 mg/kg in healthy cynomolgus macaques and 120 mg/kg (3 times the clinical dose) in an embryo-fetal development study in pregnant New Zealand White rabbits. Based on comparisons of AUC in monkey and human, there is an approximately 2-fold safety margin; based on comparisons of C_{max}, there is a less than 2-fold safety margin. Based on comparisons of C_{max} in rabbits and humans (AUCs were not generated in the rabbits), there is a 2- to 4-fold safety margin.

Tissue cross-reactivity studies with raxibacumab show significant cytoplasmic cross-reactive binding only to the thyroid of both non-human primates and human donor tissues; however the significance of these findings remains unknown.

NZW rabbits and cynomolgus macaques that succumbed to inhalation anthrax disease after treatment with raxibacumab alone or in combination with antibiotics showed enhanced CNS bacterial infiltration, inflammation, and meningitis compared to placebo. In the absence of any similar findings in healthy animals or in human volunteers, the clinical significance of these findings and their responsible mechanism(s) are unknown.

Results of the dose-ranging placebo controlled animal studies appear to confirm a "superior" treatment benefit of intravenous raxibacumab over placebo alone in preventing anthrax-induced lethality. Although the CNS findings are concerning and should be further investigated, the benefits of raxibacumab treatment in combination with antibiotics most likely will exceed any risk of potential adverse CNS effects that may develop in the clinic.

Due to the DSI identified deficiencies in the bioanalytical assay measuring raxibacumab concentrations at HGS serum samples from the monkey and embryo-fetal toxicology study need to be re-analyzed after revising the bioanalytical procedures to correct the deficiencies identified.

5. Clinical Pharmacology/Biopharmaceutics

Kimberly Bergman, Pharm. D. performed Clinical Pharmacology review of this application. Analyses of raxibacumab pharmacokinetic data allowed for the selection of the therapeutic raxibacumab dose in humans based on the comparable PK parameters (Cmax, AUC) between the efficacious 40mg/kg single raxibacumab dose in animals and the 40mg/kg single raxibacumab dose tested in human volunteers.

Ms. Bergman documents the following in her review of the data submitted by the Applicant:

The clinical pharmacology characteristics of raxibacumab have been defined in rabbits, monkeys and humans. Raxibacumab dose selection is supported by a cross-species comparison of exposure and exposure-response (E/R) relationships described in *in vivo* animal models of inhalation anthrax infection. These studies show raxibacumab demonstrates the following clinical pharmacology characteristics:

- Following single intravenous administration of raxibacumab 40 mg/kg in healthy, male and female human subjects, mean raxibacumab steady-state volume of distribution was greater than plasma volume, suggesting some tissue distribution. Clearance values were much smaller than the glomerular filtration rate indicating that there is virtually no renal clearance of raxibacumab. These findings were similar in monkeys and rabbits.
- Humans achieve similar to or greater exposure to raxibacumab following a single 40 mg/kg IV dose compared to rabbits and monkeys receiving the same dose. Mean raxibacumab Cmax in humans following a single 40 mg/kg IV dose was similar to or greater than mean Cmax values in monkeys and rabbits. Mean raxibacumab AUCinf

in humans following a single 40 mg/kg IV dose was 2.4 and 4.6-fold that of the mean AUCinf values in monkeys and rabbits, respectively. Mean total clearance of raxibacumab was substantially slower in humans as compared to rabbits by a factor of approximately 5 and in monkeys by a factor of approximately 3. Thus, half-life of raxibacumab was longer in humans compared to mean half-lives observed in monkeys and rabbits (20.6 ± 6.5 days versus 10.1 ± 2.4 days and 4.1 ± 0.85 days, respectively). Variability in Cmax was similar across species; %CV values ranged between approximately 13 to 17%. Variability in AUCinf was wider, ranging between 13 and 32% across species.

- Serum raxibacumab concentration-time profiles were similar following administration of raxibacumab alone and with both PO and IV ciprofloxacin. Overall, exposure to ciprofloxacin appears to have no consistent or meaningful impact on raxibacumab PK. Likewise, ciprofloxacin exposure is equivalent when ciprofloxacin is administered alone and with raxibacumab. Thus, there is no significant interaction between raxibacumab and ciprofloxacin.
- The exposure-response data suggest a relationship between raxibacumab dose, concentration, and survival. The dose and dosing regimen selected by the applicant, raxibacumab 40 mg/kg IV single dose, is consistent with the known relationship between dose-concentration-response.
- Dose and concentration response for the endpoint of survival indicates potential to achieve higher response rates at higher doses. If future trials are conducted, higher doses (e.g. 60 or 80 mg/kg) should be explored along with 40 mg/kg.
- Based on *in vitro* binding kinetics studies, the proposed dose of 40 mg/kg raxibacumab in humans would be expected to maintain levels required for virtually complete binding (99.9%) of PA for 7 days and those required for 99% binding for up to 42 days. Despite the theoretical importance of targeting virtually complete binding of PA, the duration of time raxibacumab concentrations remain above the threshold of 202 μg/mL (99.9% PA binding) does not appear to impact efficacy in inhalational anthrax models.
- Based on predicted human PK profiles, more than 95% of humans administered a 40 mg/kg IV raxibacumab dose can be expected to achieve serum raxibacumab concentrations that are equimolar to or in excess of the highest observed serum/plasma PA concentration in any animal that died in the therapeutic intervention studies (for 48 and 28 days relative to rabbits and monkeys, respectively). In addition, modeling and simulation indicated that a single 40 mg/kg IV raxibacumab dose in humans is adequate to bind at least 99.7% of serum PA for up to 28 days after administration, in at least 95% of patients who receive raxibacumab.
- In the pivotal animal studies, animals that were treated with raxibacumab and died exhibited a higher incidence and severity of histopathologic findings in the brain versus placebo treated animals. No clear relationship between exposure and CNS pathology can be discerned based on the limited amount of data in animals that died. Overall, the raxibacumab 20 mg/kg dose group had a higher incidence and severity of CNS findings than the 40 mg/kg group in both rabbits and monkeys. Due to the small numbers of animals that exhibited specific pathology findings, no definitive conclusions about exposure-response for bacteria, hemorrhage, inflammation, or necrosis could be made.

- Raxibacumab was non-immunogenic in humans and there were no subjects with a positive anti-raxibacumab antibody response in the human studies following single or repeat (every 14 days or following ≥ 4 months) doses.

Also, Ms. Bergman finds that ciprofloxacin administered at the labeled oral dose of 500 mg in humans is expected to achieve higher exposures (Cmax) than those observed in combination ciprofloxacin/raxibacumab study conducted in monkeys with inhalational anthrax. Levofloxacin administered at the labeled oral dose of 500 mg in humans is expected to achieve slightly higher exposures (Cmax) than those observed in this study conducted in rabbits with inhalational anthrax.

Clinical pharmacology conclusions described above hinge on the assurance that pharmacological data is reliable, i.e. collected and processed according to GLP standards. ***However, the reliability of clinical pharmacokinetic data came under question as a result of the issues identified during DSI inspections of the bioanalytical sites at HGS and*** (b) (4) (b) (4) ***The inspectional issues identified included incomplete data and documentation, questions regarding some of the quality control samples, questions regarding several SOPs, among others. The assays under question measured raxibacumab and ciprofloxacin concentrations in clinical study C-1064. Raxibacumab pharmacokinetic data from this study is crucial and was used for the above human raxibacumab exposure analyses for the determination of the appropriate human therapeutic dose when compared to the exposures at the 40mg/kg efficacious dose from the animal models of anthrax. Ciprofloxacin PK data was necessary to document the absence of PK/PD drug interaction between raxibacumab and ciprofloxacin. Thus, the following deficiencies are to be included in the Complete Response Action letter and to be addressed by the Applicant prior to approval of this application:***

a. ***Following adequate revision of the analytical procedures to address these inspection findings, the pharmacokinetic samples from human studies HGS1021-C1063, HGS1021-C1064, and HGS1021-C1069 should be re-assayed using the revised procedures. Pharmacokinetic parameters for raxibacumab from these re-analyses should be re-calculated in humans following re-assay of the samples. Depending on the outcome of these re-analyses, additional pharmacokinetic studies may be required.***

b. ***The plasma pharmacokinetic samples obtained from the human study HGS1021-C1064 and the plasma samples obtained from the cynomolgus monkey study 789-G923702 should be re-assayed for ciprofloxacin concentrations using a revised analytical method that adequately addresses the DSI findings for the ciprofloxacin assay. Pharmacokinetic parameters for ciprofloxacin from these re-analyses should be re-calculated in both humans and monkeys following re-assay of the samples. Depending on the outcome of these re-analyses, additional pharmacokinetic studies may be required.***

c. ***Because of the outstanding issues with the assay of raxibacumab in human serum, we have now become concerned about the reliability of the pharmacokinetic data that was generated from the animal efficacy studies in New Zealand White rabbits and cynomolgus monkeys, which used similar bioanalytical procedures as that for***

the human studies. Therefore, the pharmacokinetic samples from animal studies 682-G005758 and 724-G005829 should also be re-assayed using the revised procedures. Pharmacokinetic parameters for raxibacumab from these re-analyses should be re-calculated in rabbits and monkeys following re-assay of the samples. Depending on the outcome of these re-analyses, additional pharmacokinetic studies may be required.

6. Clinical Microbiology

Drs. Maureen Davidson and Lynnette Berkeley reviewed the natural history studies of inhalational anthrax in cynomolgus macaques and NZW rabbits, *in vitro* PA binding studies, performance of PA detection and Toxin Neutralization assays as well as the provided an insight on the animal efficacy studies.

These reviewers concluded that *in vitro* studies show that raxibacumab can bind PA in culture supernatants from Ames, Sterne and Vollum strains of *B. anthracis*. Raxibacumab binds free but not cell surface bound PA.

Studies in rabbits and monkeys show that raxibacumab is effective in improving survival. The survival rate decreases as the time to treatment is increased.

In animal efficacy studies in rabbits and monkeys, detection of PA was used as a trigger for intervention. Due to the limitation of the detection of PA by ECL assay, the cure rates should be based on presence or absence of bacteremia alone. Results show that 40 mg/kg raxibacumab is effective in improving survival in monkeys. Efficacy in rabbits showed a similar trend like the monkeys, however, the difference between the treated and untreated animals was less.

Raxibacumab appears to decrease the numbers of bacteria present in tissues other than the brain. Greater proportion of lesions (necrosis, hemorrhages, suppurative inflammation, and meningitis) was reported in the brain of raxibacumab treated animals compared to placebo. It is unclear whether an increase in lesions in the brain in animals treated with raxibacumab is due to longer survival time in treated animals compared to placebo, longer duration of bacteremia prior to therapeutic intervention, activation of inflammatory responses to treatment with raxibacumab or due the presence of bacteria, toxins or raxibacumab in the brain. Necropsies of animals that survived were not done in the studies except rabbits treated with levofloxacin or levofloxacin + raxibacumab.

The reviewers were reassured that no serious safety concerns were observed in the nonclinical pharmacology/toxicology studies. The data from healthy subjects in the clinical safety studies showed that adverse reactions frequently observed included headaches, cough, arthralgia, and paresthesias. Blood levels of IL-2, IL-4, IL-5, IL-8, IL-10, IL-12, IL-13, and histamine were slightly higher on average after the raxibacumab infusion in blood samples from first 25 subjects dosed with raxibacumab from to-be-licensed raxibacumab (M11process), but did not correlate with any clinically significant outcomes. However, the presence of infection such as that with *B. anthracis* can potentially damage the blood-brain barrier, activate immune cells,

and induce innate and acquired immune responses. Additionally, the possibility of raxibacumab or toxins crossing the blood brain barrier and inducing inflammatory responses in the brain, either alone or in combination with *B. anthracis*, cannot be ruled out.

The recommendation was made in the future animal studies to perform necropsy of all surviving animals in addition to non-survivors in order to understand the differences in inflammatory responses between the animals that survive and those that die. Additionally, immunohistochemical staining of brain tissue sections to evaluate whether the cause of CNS lesions might be due to the presence of raxibacumab, bacteria, or both, could be helpful.

7. Clinical/Statistical-Efficacy

Efficacy of raxibacumab as treatment of inhalational anthrax was evaluated in 2 animal models of inhalational anthrax: NZW rabbits and cynomolgus macaques, selected based on the results of their respective natural history studies. Four treatment studies with raxibacumab were conducted. The initial two efficacy studies tested raxibacumab versus placebo; one study was conducted in rabbits and the other one in monkeys. Both of these studies demonstrated that the 40 mg/kg IV raxibacumab dose was superior to placebo.

The subsequent two studies evaluated the efficacy of raxibacumab plus antimicrobial (a fluoroquinolone) versus antimicrobial alone in the treatment of anthrax; a placebo arm was included in these studies as well. As designed the main purpose of these studies was to see whether there may be any antagonism when the two components are used together. However, it was also of interest whether raxibacumab made a contribution to the efficacy of the combination (raxibacumab and antimicrobial) and whether the efficacy of the combination was higher than the efficacy of antimicrobial alone. In these studies, unexpectedly, the efficacy of the antimicrobial (levofloxacin in rabbit and ciprofloxacin in monkey) was 95-100%, and similarly, high rates of efficacy were also seen in the raxibacumab plus antimicrobial combination arm. Given the high efficacy of the antimicrobial in these studies, it was not possible to demonstrate a contribution of raxibacumab to the efficacy of the regimen, raising the question about the need for an animal model that more closely approximates the 55% survival seen in 2001 in the patients with inhalational anthrax.

Analyses populations:
In The FDA reviews the analysis populations for the four efficacy studies were defined as follows:

Intention-to-treat (ITT): all animals that were randomized and challenged with *B. anthracis*. The ITT analysis was to be based on the treatment group planned at randomization rather than the actual treatment the animals received. Animals that were spore-challenged, but died before receiving placebo or active treatment, were to be included in this population as treatment failures.

FDA Primary: all animals that were bacteremic (defined as a positive blood culture) at the time of therapeutic intervention, and according to the actual treatment administered. FDA used this as its primary analysis population because both PA assays quantitative ELISA and ECL trigger for intervention exhibited high intra and inter animal variability as well as a

significant operator-dependency. The FDA primary analysis using only animals that were bacteremic at the time of treatment was specified in order to clearly differentiate the animals that received raxibacumab for treatment as opposed to post-exposure prophylaxis.

As treated: all treated animals according to the actual treatment administered.

Toxemic at treatment: the ITT population according to the detection of toxemia at treatment, defined as the detection of PA by quantitative ECL.

Excluding non-anthrax death: the above analysis populations, excluding animal(s) that died from non-anthrax causes.

7.1 Raxibacumab versus Placebo

The efficacy of raxibacumab monotherapy was compared to placebo in the treatment of inhalational anthrax in two pivotal animal studies (Cynomolgus macaque and New Zealand White rabbit).

7.1.1 Cynomolgus Macaque

Study 724-G005829: "Evaluation of Raxibacumab Efficacy as Therapeutic Treatment Against Inhalation Anthrax in the Cynomolgus Macaque"

Objectives

- To evaluate the efficacy of raxibacumab when administered as a therapeutic treatment against lethality upon appearance of clinical symptoms due to inhalation exposure to *B. anthracis* in cynomolgus macaques.
- Although not pre-specified as primary or secondary study objectives in the protocol, additional objectives of this study were to determine the pharmacokinetics (PK) of raxibacumab following a single IV raxibacumab dose and the kinetics of *B. anthracis* PA in monkeys with inhalation anthrax.

Study Design
This was a randomized, double-blind, placebo-controlled efficacy study designed to evaluate single IV doses of raxibacumab vs. placebo as therapeutic treatment against lethality in cynomolgus macaques with symptomatic inhalation anthrax. This study also evaluated the kinetics of *B. anthracis* PA and the PK of IV raxibacumab in symptomatic monkeys.

Methods
Forty naïve cynomolgus macaques were randomized into two separate groups of 14 monkeys and one group of 12 monkeys (each group 50% male, 50% female) for treatment as delineated in the table 7.1.1-1 below:

Table 7.1.1-1 Treatment Groups

Group	Number of NHPs	Dose	Route/Frequency	Treatment Point
Group 1	14	40 mg/kg	IV/single dose	Individual

		raxibacumab (1 mL/kg)		treatment times were based on serum PA levels as detected by ECL
Group 2	14	20 mg/kg raxibacumab (1 mL/kg)	IV/single dose	
Group 3 (placebo)	12	Raxibacumab buffer (1 mL/kg)	IV/single dose	

(Adapted from HGS' BLA submission, p.21)

The study was conducted at Battelle Biomedical Research Center (BBRC), West Jefferson, OH. Monkeys were randomized to 1 of 3 aerosol challenge days and a challenge order per day. On Study Day 0, monkeys were challenged with a targeted 200 x lethal dose (LD50) (1.24 x 107 spores) dose of *B. anthracis* spores (Ames strain) via a nebulizer in a head-only inhalation exposure chamber.

After aerosolization the animals were observed twice daily throughout the study, with observations performed every six hours from 24 through 192 hours post challenge. Body temperature was monitored hourly throughout the course of the study. Beginning at 24 hours through up to 54 hours post spore challenge, blood samples for culture and PA were taken every six hours up to the time of treatment.

When an animal was found positive for PA in serum via the ECL screening assay or at 54 hours post challenge, a single bolus IV injection of either 40 or 20 mg/kg raxibacumab, or 1 mL/kg raxibacumab buffer (dependent upon that animal's treatment assignment) was administered. Just prior to treatment intervention (± 5 minutes), monkeys were administered a single 1 mg/kg dose of diphenhydramine (or equivalent) intramuscularly (IM) to parallel the treatment paradigm for humans in Phase 2/3 studies. Staff members administering the study agent were blinded to study arm assignments and study drug preparation.

Death or euthanasia was recorded at the time observed and a complete necropsy was performed. Blood samples were collected during the study for hematology and CRP, as well as for bacteremia by culture and PCR, serum PA levels, anti-PA and TNA levels, and serum raxibacumab levels. Blood was collected 3 days prior to spore challenge, just prior to spore challenge, and 5 minutes, 12 and 24 hours, and 3, 5, 8, 14, and 28 days following treatment intervention. A terminal blood sample was also collected if possible.

Demographics and Anthrax Exposure

The treatment groups were comparable with respect to sex, weight, and age at randomization. The anthrax spore challenge by treatment group is shown in the table 7.1.1-2 below:

Table 7.1.1-2 Spore Challenge Exposure

	Placebo N=12	Raxibacumab 20 mg/kg N=14	Raxibacumab 40 mg/kg N=14	All Groups N=40	P-value[1]
Anthrax exposure (x LD_{50})					0.0255
N	12	14	14	40	
Mean ± SD	197.6 ± 43.5	199.0 ± 52.4	157.2 ± 32.4	184.0 ± 46.8	
Median	207.9	208.5	161.6	175.0	

Range	121.0-277.0	130.0-301.0	106.9-218.0	106.9-301.0	

[1] P-value based on 1-way ANOVA for comparison among all treatment groups
(Adapted from HGS' BLA submission, p.45)

The spore challenge dose was lower on average in the 40 mg/kg raxibacumab group than in the other two treatment groups. There were statistically significant differences in mean spore exposure levels among the three treatment groups (F test, p=0.0255). The difference in inhaled spores between the 40 mg/kg raxibacumab group and the placebo group was statistically significant even after the conservative Bonferroni multiplicity adjustment. However, there did not appear to be a relationship between challenge day, challenge dose, and survival.

The time to treatment for animals that were bacteremic at the time of treatment was no significantly different between the groups.

Statistical Methods
According to the HGS protocol, the primary efficacy analysis was to be subject to multiple comparison adjustment using a step-down sequential testing procedure.

The primary efficacy analysis was performed using a 2-sided likelihood test and was to be subject to multiple comparison adjustment using a step-down sequential testing procedure. First the 40 mg/kg raxibacumab group was compared with placebo. If this was statistically significant then the 20 mg/kg raxibacumab group was compared with placebo for statistical significance. Sensitivity analyses were to be performed for the as-treated population, HGS' modified ITT population, the FDA primary population, and the group of all animals that were randomized including any that were randomized but not challenged with spores.

Efficacy Results

Primary Endpoint
The primary efficacy endpoint was survival at Day 28. The efficacy described in Table 7.1.1-3 is based on the HGS ITT statistical analysis and the FDA primary statistical analysis of those animals that were bacteremic (as defined by positive blood culture) at the time of treatment.

Table 7.1.1-3 Primary Efficacy Results in Cynomolgus Macaques (Day 28)

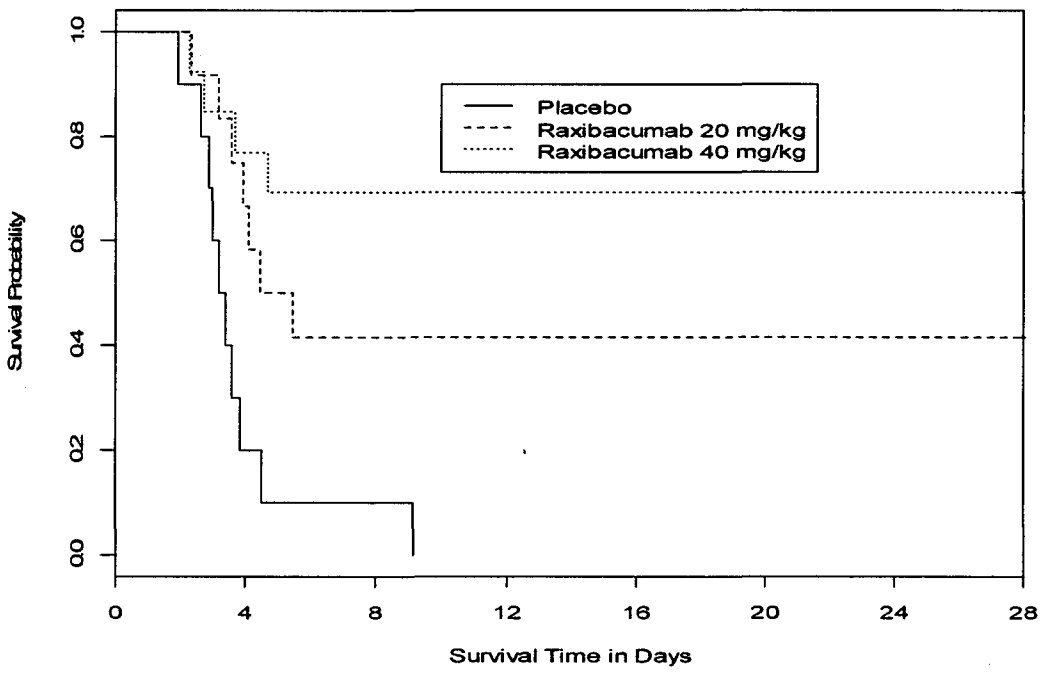
Population	Treatment	N	No. of Survivors (%)	P-value*	95% CI of Raxi-placebo**	97.5% CI of Raxi-placebo**
HGS ITT	Placebo	12	0 (0.0%)			
	20 mg/kg raxibacumab	14	7 (50.0)	0.0064	(19.3, 73.7)	(13.2, 77.0)
	40 mg/kg raxibacumab	14	9 (64.3)	0.0007	(31.6, 84.7)	(27.0, 87.4)
FDA Primary	Placebo	10	0 (0.0%)			
	20 mg/kg raxibacumab	12	5 (41.7%)	0.0396	(7.2, 68.7)	(1.5, 72.4)
	40 mg/kg raxibacumab	13	9 (69.2%)	0.0016	(31.1, 88.9)	(25.7, 90.9)

* P-values are based on two-sided Fisher's exact test for comparisons between the raxibacumab group and placebo.
** CIs are exact confidence intervals.

HGS concluded that both the 20 mg/kg and the 40 mg/kg doses achieved a statistically significant mortality difference compared to placebo. For the FDA primary analysis, two animals in the placebo group, two animals in the raxibacumab 20 mg/kg group, and one animal in the raxibacumab 40 mg/kg group were excluded because they were not bacteremic at the time of treatment. After exclusion of these animals, both the 20 mg/kg and the 40 mg/kg doses achieved a statistically significant mortality difference compared to placebo for the FDA primary analysis.

Secondary Endpoint
The secondary efficacy endpoint was survival time, defined as the time from spore challenge to death during the 28 day study period. The figure below (Figure 7.1.1-1) is a graph of the Kaplan-Meier survival curves for the three treatment groups of the monkeys in the FDA primary analysis group. The median survival times were 3.3 days in the placebo group, 5.0 days in the raxibacumab 20 mg/kg group and beyond 28 days in the raxibacumab 40 mg/kg group because 69.2% of the animals survived at the end of the study. There was a statistically significant difference in terms of survival time among the three groups (log rank test, p=0.0006). Pairwise comparisons also show statistically significant differences between each raxibacumab group and the placebo ($p = 0.0098 < 0.025$ for 20 mg/kg group versus placebo, and $p = 0.0005 < 0.025$ for 40 mg/kg group versus placebo) after the Bonferroni adjustment for multiple comparisons (0.025=0.05/2).

Figure 7.1.1-1 Survival Probability of Monkeys in FDA Primary Analysis Population



Necropsy and Histopathology
Necropsies were performed on all animals that died. Discussion of histopathology findings from all four animal efficacy studies as well as a detailed discussion of the pathologic CNS

findings in the context of time-to-event and exposure-response analyses could be found in Section 8 (Safety) of this document.

7.1.2 New Zealand White Rabbit

Title of Study: 682-G005758: Evaluation of Raxibacumab Efficacy as Therapeutic Treatment Against Inhalation Anthrax in the Rabbit Model

Objectives

- To evaluate the efficacy of raxibacumab when administered as a therapeutic treatment against lethality upon appearance of clinical symptoms due to inhalation exposure to *B. anthracis* in New Zealand White (NZW) rabbits.
- To determine the PK of raxibacumab following a single IV raxibacumab dose and the kinetics of *B. anthracis* PA in rabbits with inhalation anthrax.

Study Design, and Methods

This study was designed as an open-label, single center, randomized, placebo controlled dose-ranging study of raxibacumab given as a single IV bolus at either 20 or 40mg/kg dose or placebo to NZW rabbits to evaluate the therapeutic efficacy of a single IV dose of raxibacumab in anthrax spore inhalation challenged rabbits experiencing the symptoms of inhalation anthrax. The study was conducted at BBRC. The primary objective was to evaluate raxibacumab efficacy as therapeutic treatment against inhalation anthrax induced lethality in a NZW rabbit model of established inhalational anthrax. Survival in the bacteremic population was the primary analytic endpoint in the FDA analysis, as had been discussed with HGS, whose protocol prespecified primary analysis in the ITT population. Assuming raxibacumab efficacy of 50%, with three cohorts of 18 animals per group, the study provides 80% power at overall significance level of 0.05 to detect an absolute difference of 45% or more in 14 day survival in one of the raxibacumab treatment groups (20 or 40 mg/kg). Animals received the following interventions when disease was identified (trigger) by bacteremia, or either PA toxemia and temperature elevation of ≥2°F. The latter criteria correlate with bacteremia development in the natural history study in NZW rabbits.

Table 7.1.2-1 illustrates the treatment groups. Raxibacumab was administered as a bolus infusion. The animal's disposition is illustrated in Figure 7.1.2-1.

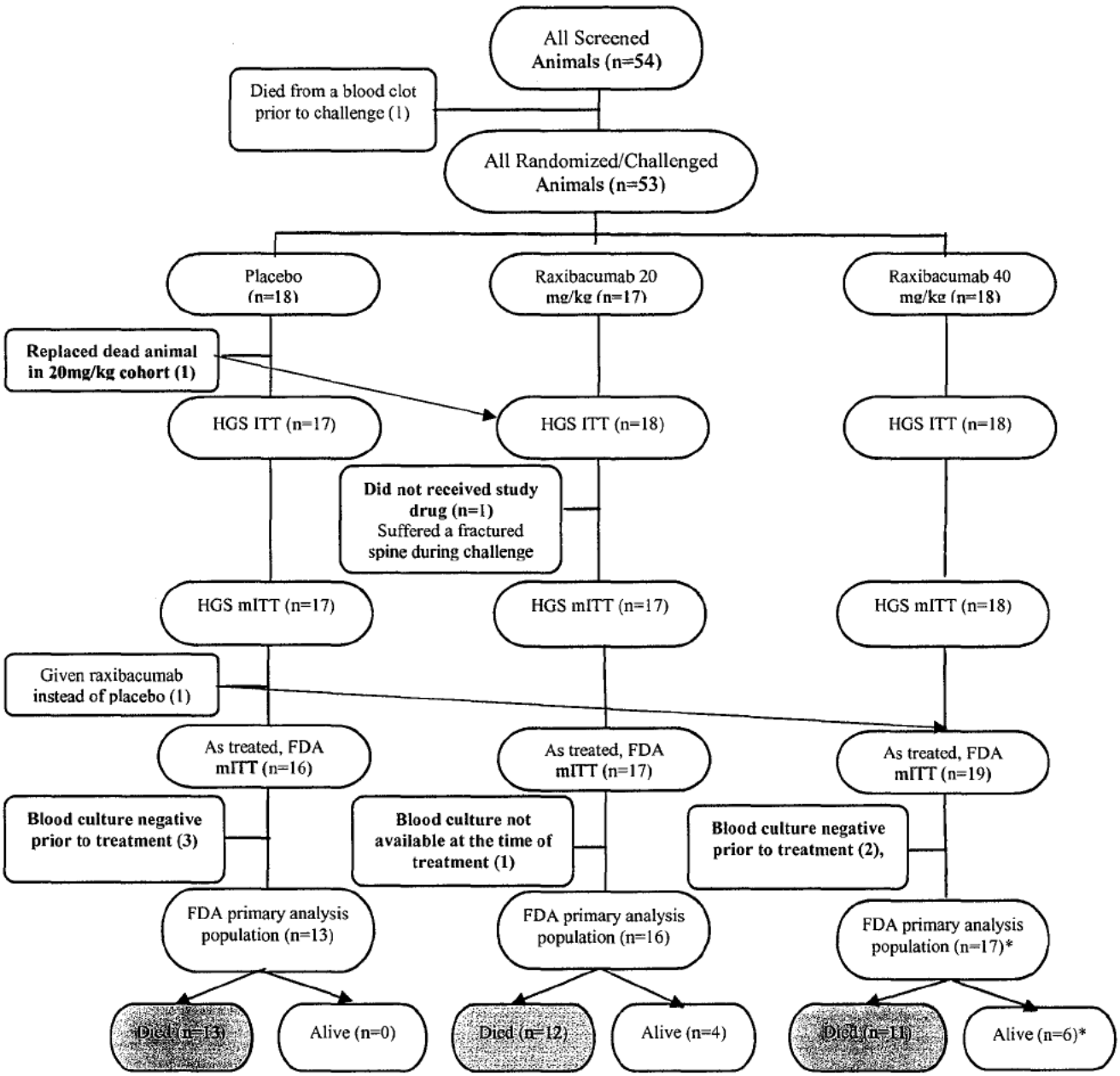
Table 7.1.2-1 Treatment Groups

		Raxibacumab Treatment		
Group	**# of Animals**	**Dose (mg/kg)**	**Dose Schedule and Route**	**Treatment Point Post-Challenge**
1 (Control Group)	18	Raxibacumab buffer [a]	Single IV	Individual times will be based on serum PA levels or body temperature [b]
2	18	20 Raxibacumab	Single IV	Individual times will be based on serum PA levels or body temperature [b]
3	18	40 Raxibacumab	Single IV	Individual times will be based on serum PA levels or body temperature [b]

a Dose equivalent in ml/kg volume to Group 3.
b Each individual rabbit in groups 1- 3 will receive assigned treatment immediately upon either its first positive PA result, or its first two consecutive time points of a body temperature 2 or more degrees F higher than the baseline average (whichever occurs first).

Study animals were randomized by dose group and gender, and aerosol challenged with the *B. anthracis* Ames strain at 200 x LD50 at Day 0. Temperature was measured every hour from 12h to 72h post-challenge. Blood samples were collected for detection of PA (ECL and ELISA), culture, PCR for *B. anthracis*, and CBC/CRP every 4 hours from 12h to 72h post-challenge. Intervention was to occur based on the trigger for intervention: positive PA toxemia by ECL or a 2° F sustained temperature increase over baseline. Clinical observations were recorded every 12h and animals were euthanized at day 14 or if found moribund. Necropsy and histopathology were conducted on all euthanized for moribundity and all dead animals.

Figure 7.1.2-1 Animal's Disposition- NZW Rabbits



**Animal L08147 was bacteremic by culture 1.4hours prior to treatment with raxibacumab 40mg/kg; however, sample drawn immediately prior to raxibacumab administration did not grow bacteria. Blood cultures remained*

negative for the remainder of the study period. Animal was febrile for 5 hours prior to treatment and remained febrile for additional 17 hours after treatment. Animal's PA levels by ELISA at the time of treatment were at lower limit of detection. This animal was considered to be bacteremic prior to treatment administration by FDA

Efficacy Results

The efficacy described below in Table 7.1.2-2 is based on the FDA statistical analyses of the population of animals that were identified by clinical/microbiology reviewers as having inhalational anthrax by the presence of bacteremia prior to treatment. The results indicate that only the 40 mg/kg raxibacumab dose achieved a statistically significant mortality difference compared to placebo.

Table 7.1.2-2 Primary Efficacy Results in NZW Rabbits (Survival at Day 14)

Population	Treatment	N	No. of Survivors (%)	P-value*	95% CI** of Raxibacumab-placebo (%)	97.5% CI** of Raxibacumab – placebo (%)
HGS ITT	Placebo	17	0 (0.0%)			
	20 mg/kg Raxibacumab	18	5 (27.8%)	0.0455	(6.6, 52.5)	(1.4, 54.5)
	40 mg/kg Raxibacumab	18	8 (44.4%)	0.0029	(21.3, 66.7)	(16.1, 69.6)
FDA primary	Placebo	13	(0.0%)			
	20 mg/kg Raxibacumab	16	4 (25.0%)	0.1067	(-2.2, 50.9)	(-7.8, 53.4)
	40 mg/kg Raxibacumab	17	6 (35.3%)	0.0237	(7.3, 59.6)	(2.2, 62.3)

*two-sided Fisher's exact test for comparisons between the treatment and placebo
** CIs are exact confidence intervals.

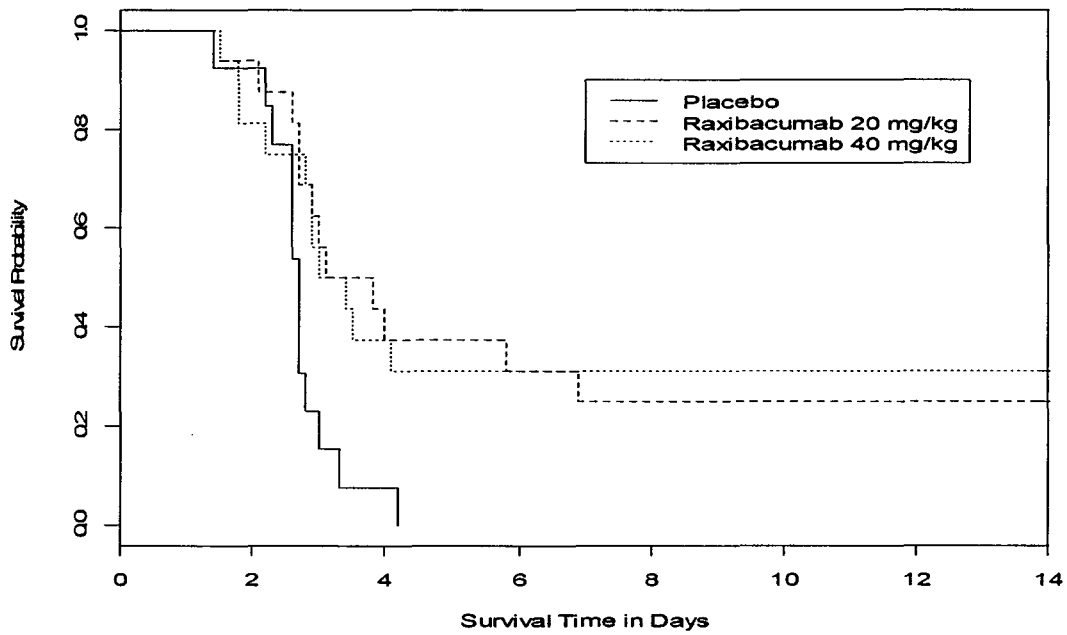
The FDA exploratory analyses showed a similar trend in survival with the 40mg/kg dose when disease was defined as presence of toxemia by the screening and quantitative toxin assays.

An issue of weak statistical significance (p value of 0.0237) between 40mg/kg raxibacumab arm and placebo was raised by statistical reviewer Dr. Hongling Zhou.
Specifically, it was pointed out that the result of statistically significant difference between raxibacumab at 40mg/kg arm and placebo is predicated on inclusion of a single animal (L08147) in the FDA primary analysis population (see animal disposition Figure 7.1.2-1 above). However, this medical reviewer argues to retain the animal in the FDA primary analysis population (animals bacteremic prior to study treatment administration) to be consistent with current standards of clinical practice (even a single positive blood culture for B. anthracis along with the elevated body temperature is reflective of bacteremia, i.e. a documented systemic bacterial illness). In addition, this finding of statistical significance is seen in the most conservative approach in selecting the primary analysis population for efficacy as a population of animals bacteremic by culture prior to study treatment and assigning the study group to an animal made according to the actual treatment received. In addition, as stated above, the finding of statistically significant difference for 40mg/kg raxibacumab holds true in the sensitivity analyses of the ITT, mITT population (both FDA and Sponsor's) as well as in the analyses of efficacy in toxemic animals.

Secondary Efficacy Endpoint

The secondary efficacy endpoint was survival time defined as the time from the beginning of spore challenge to death during the 14-day study period. The graph of the Kaplan-Meier survival curves for the three treatment groups of rabbits is presented in Figure 7.1.2-2 below for the FDA primary analysis population. The median survival times were 2.7 days in the placebo group, 3.5 days in the raxibacumab 20 mg/kg group and 3.2 days in the raxibacumab 40 mg/kg group. There were statistically significant differences in terms of survival time among the three groups (log rank test, p=0.0125).

Figure 7.1.2-2 Survival Probability of Rabbits in FDA Primary Analysis Population



Necropsy and Histology
The animals that succumbed to disease or were sacrificed due to moribundity were necropsized. The internal organs were examined for gross pathology and then sections were examined microscopically. The necropsy and histopathology readings were initially performed by the pathologist at Battelle in a blinded fashion. Upon Division's request, the organs and histopathology slides were re-examined and read by an independent peer review pathology panel (EPL). Discussion of histopathology findings from all four animal efficacy studies as well as a detailed discussion of the pathologic CNS findings in the context of time-to-event and exposure-response analyses could be found in Section 8 (Safety) of this document.

7.2 Efficacy of Raxibacumab and Antimicrobial in Animal Models of Inhalational Anthrax

In addition to conducting studies of raxibacumab versus placebo in monkeys and rabbits, HGS also conducted studies to test the efficacy of raxibacumab in combination with antimicrobial therapy in monkeys and in rabbits. These studies were performed to simulate anticipated conditions of use in clinical practice, when antimicrobial treatment will be administered to anthrax patients along with raxibacumab.

The analysis populations for these studies were defined in the same way as for the raxibacumab versus placebo studies. The study design and outcome for each study are summarized below.

7.2.1 Cynomolgus Macaque

Study 789-G923702: "Evaluation of the Efficacy of Raxibacumab in Combination with Ciprofloxacin for Therapeutic Treatment in the Cynomolgus Macaque Inhalation Anthrax Model"

Objectives

- To evaluate the efficacy of raxibacumab when administered as a therapeutic agent in combination with ciprofloxacin against lethality due to inhalation exposure of *B. anthracis* in cynomolgus macaques.
- To determine the PK of intragastric (IG) ciprofloxacin doses and of an IV raxibacumab dose when co-administered, as well as the kinetics of *B. anthracis* protective antigen (PA), in monkeys with inhalation anthrax.

Study Design

This was a parallel-group, double-blind, randomized, placebo-controlled study to evaluate the efficacy of raxibacumab in combination with ciprofloxacin as a therapeutic treatment in the cynomolgus macaque inhalation anthrax model. PA kinetics and raxibacumab and ciprofloxacin PK were also examined.

Methods

Forty (50% male, 50% female) juvenile (< 5 years of age) cynomolgus macaques (*Macaca fascicularis*) weighing between 2.0 to 6.0 kg at randomization were randomized into 3 treatment groups (see table 7.2.1-1 below), and randomly assigned to 1 of 3 aerosol challenge days and a challenge order.

Table 7.2.1-1 Treatment Groups

Group	N	Ciprofloxacin/Control	Raxibacumab/Control	Treatment Point
Group 1 (Control)	12	Sterile water IG	Raxibacumab buffer IV (1mL/kg)	Individual treatment times were based on serum PA levels as detected by ECL
Group 2 (Ciprofloxacin)	14	75 mg IG every 12h for 6 doses	Raxibacumab buffer IV (1mL/kg)	
Group 3 (Ciprofloxacin/ raxibacumab)	12	75 mg IG every 12h for 6 doses	Raxibacumab IV 40mg/kg after the first ciprofloxacin dose	

(Adapted from HGS' BLA submission, p.29)

The study was conducted at BBRC. On Day 0, monkeys were aerosol challenged with a targeted 200 x LD50 [1.24 x 107 spores] dose of aerosolized *B. anthracis* spores (Ames strain).

Monkeys were observed every 6 hours beginning 24 hours through 192 hours (8 days) post median challenge time for clinical signs including, but not limited to, anorexia, lethargy, respiratory distress, moribundity, activity (recumbent, weak, or unresponsive), seizures, and

other abnormal clinical observations, afterwards they were observed twice daily, or more often at any sign of distress.

Treatment with study agent was triggered by detection of serum PA via the ECL screening assay or at 54 hours post-challenge. Ciprofloxacin (75 mg) or control material (sterile water for injection, WFI) was administered IG for 6 doses: initially once the criterion for treatment was met and then every (Q) 12 hours (± 1 hour) for 5 doses. In addition, a single IV bolus dose of raxibacumab (40 mg/kg) or raxibacumab buffer (1 mL/kg) was administered immediately after administration of the first ciprofloxacin dose. Within five minutes prior to treatment each monkey received a single 1 mg/kg intramuscular (IM) dose of diphenhydramine to parallel the treatment paradigm for humans in Phase 2/3 studies. Staff members administering study agents were blinded to study agent preparation to preclude introduction of bias into the study following treatment.

Monkeys surviving the post-challenge could be returned to HGS (if required) or donated following observation for an additional 60 days under BL-2 laboratory conditions for recrudescence. Gross necropsy and histopathology were performed on all monkeys that were found dead or were euthanized. Blood samples were collected for hematology and CRP, as well as to measure bacteremia by culture, serum PA levels, anti-PA and TNA levels, serum raxibacumab levels and ciprofloxacin levels.

Statistical Analysis
All statistical tests were to be 2-sided and performed at a significance level of 0.05.

Demographics and Anthrax Exposure
The treatment groups were comparable with respect to sex, weight, age at randomization, and anthrax spore challenge.

Efficacy Results

Primary Endpoint
The primary efficacy endpoint was survival at Day 28, defined as the percent of animals alive at Day 28. The efficacy described below in Table 7.2.1-3 is based on the HGS ITT statistical analysis and the FDA primary analysis of those animals that were bacteremic (as defined by positive blood culture) at the time of treatment.

Table 7.2.1-3 Primary Efficacy Analysis in Monkeys

Population	Treatment	N	No. of Survivors (%)*	95% CI** of Cipro/Raxi – Cipro (%)
Sponsor's ITT	Placebo	12	0 (0.0%)	
	Ciprofloxacin	14	14 (100%)	
	Cipro/Raxi	14	12 (85.7%)	(-42.8, 11.9)
FDA primary	Placebo	10	0 (0.0%)	
	Ciprofloxacin	13	13 (100%)	
	Cipro/Raxi	13	11 (84.6%)	(-45.5, 11.4)

*There is significant difference between each active group and placebo (all p-values <0.0001).
**CIs are exact confidence intervals for comparison between cipro/raxi and ciprofloxacin.

None of the placebo treated animals survived. One (C24791) of the two non-survivors in the ciprofloxacin/raxibacumab group bacteremic and toxemic at 36 h post challenge and at the time of the treatment administration died from pneumonia related to a potential gavage error. PA levels in this animal were declining from the time of treatment administration to the time of death; blood culture was negative at 24 h post treatment and at the time of death.

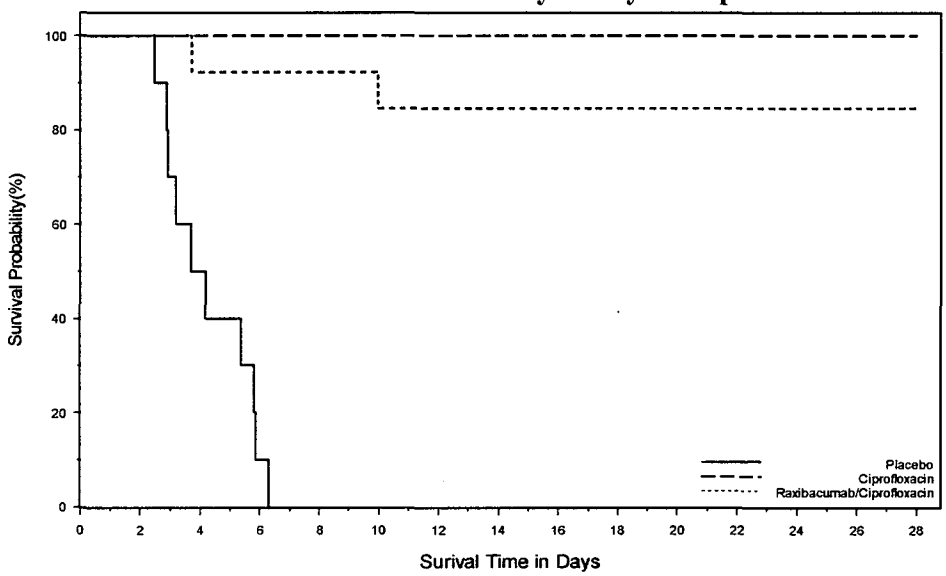
The other non-survivor in the ciprofloxacin/raxibacumab group tested negative for bacteremia at death but had evidence of meningitis and CNS hemorrhage. One animal in the ciprofloxacin group died after study end (D36 postchallenge) due to left leg arterial thrombosis with inflammation and fibrosis. No anthrax bacteria were found in any tissue in this animal. All survivors in the study tested negative for TNA at baseline and positive for both anti-PA antibodies and TNA on Day 28.

For the FDA primary analysis the 28-day survival rates were 0%, 100%, and 84.6% in the placebo, ciprofloxacin, and ciprofloxacin/raxibacumab combination groups, respectively. There was no difference (p=0.222) in survival rates between the ciprofloxacin and ciprofloxacin/raxibacumab combination groups (-15.4%, 95% CI [-45.5%, 11.4%].

Sensitivity analyses with respect to the primary efficacy endpoint were performed on several populations. Sensitivity analyses as well as analysis excluding the non-anthrax death did not alter the conclusions of the primary efficacy analyses. There was no statistical difference in survival rate at Day 28 between the ciprofloxacin and ciprofloxacin/raxibacumab, both of which maintained statistically significant survival benefit as compared to placebo.

Secondary Endpoint
The secondary efficacy endpoint was survival time from spore challenge to death during the study period. The median survival time in the placebo group was 4.2 days based on HGS' ITT and 3.9 days based on the FDA primary analysis (Figure 7.2.1-1). Median survival time could not be determined for the ciprofloxacin and ciprofloxacin/raxibacumab groups because the medians extended beyond 28 days.

Figure 7.2.1-1 Study 789-G923702: Survival Probability of Monkeys in FDA Primary Analysis Population



7.2.2 New Zealand White Rabbit

Study 781-G923701: "Evaluating the efficacy of raxibacumab in combination with levofloxacin for treatment in the New Zealand White Rabbit inhalational anthrax model"

Objectives

• To evaluate the efficacy of raxibacumab when administered in combination with levofloxacin as a therapeutic treatment against lethality due to inhalation exposure to *B. anthracis* in NZW rabbits.

• To determine the PK of intragastric (IG) levofloxacin doses and of an IV raxibacumab dose when co-administered, as well as the kinetics of *B. anthracis* protective antigen (PA), in rabbits with inhalation anthrax.

Study Design

This was a parallel-group, randomized, double-blind, placebo-controlled GLP study to evaluate the efficacy of raxibacumab in combination with levofloxacin for therapeutic treatment in the NZW rabbit inhalational anthrax model. PA kinetics and raxibacumab and levofloxacin PK were also examined.

Methods

Fifty-two rabbits New Zealand White rabbits (*Oryctolagus cuniculus*) (b) (4) weighing 2.97 to 4.02 kg at randomization, were randomized into one group of 12 rabbits and two groups of 20 rabbits (each group approximately 50% male, 50% female) and assigned to 1 of 3 aerosol challenge days and a challenge order. The eight remaining rabbits were to serve as replacements.

Rabbits were to be aerosol challenged on Day 0 with a targeted 200x the median lethal dose (LD50) (1.24 x 107 spores) of *B. anthracis* (Ames strain) spores. Treatment was administered based on a positive plasma PA result via ECL screening assay or two consecutive timepoints of a body temperature 2 or more °F higher than the baseline average temperature, whichever was noted first. Beyond 36 hours postchallenge until 72 hours postchallenge, only temperature was used as a trigger for treatment. If an animal had not been treated by 72 hours postchallenge (negative for PA and did not meet the temperature criteria), the animal was to be treated after its last hourly temperature assessment.

The following three treatment groups were evaluated:

- Group 1 (X): Individual rabbits received sterile WFI (2 mL/kg) IG upon trigger symptom detection, immediately followed by a single IV dose of raxibacumab buffer (1 mL/kg), and then one dose of sterile WFI (2 mL/kg) at 24 and 48 hours after trigger symptom detection.

- Group 2 (Y): Individual rabbits received levofloxacin (50 mg/kg) IG upon trigger symptom detection, immediately followed by a single IV dose of raxibacumab buffer (1 mL/kg), and then one dose of levofloxacin (50 mg/kg) at 24 and 48 hours after trigger symptom detection.

- Group 3 (Z): Individual rabbits received levofloxacin (50 mg/kg) IG upon trigger symptom detection, immediately followed by a single IV dose of raxibacumab (40 mg/kg), and then one dose of levofloxacin (50 mg/kg) at 24 and 48 hours after trigger symptom detection.

Demographics and Anthrax Exposure
The treatment groups were comparable with regard to sex, weight, and age at randomization. The extent of anthrax exposure was similar between groups, with no statistically significant difference in mean spore dose among the three treatment groups. The mean inhaled spore dose exceeded the targeted dose of 200x LD50 in all groups.

Efficacy Results

Primary Endpoint

The primary efficacy endpoint was survival at Day 28, defined as the percent of animals alive at Day 28. In the FDA primary analysis, the 28-day survival rates were 0%, 95.0%, and 94.1% in the placebo, levofloxacin, and levofloxacin/raxibacumab combination groups, respectively (Table 5.2.2-1). There was no difference (p=0.947) in survival rates between the levofloxacin and levofloxacin/raxibacumab combination groups (-0.88%, 95% CI [-23.9%, 19.6%]).

None of the 12 rabbits in the placebo group survived. There was one death in the levofloxacin group and one death in the levofloxacin/raxibacumab group. The one non-survivor in the levofloxacin group was discovered moribund and was euthanized 11 days post challenge. The one non-survivor in the levofloxacin/raxibacumab group (K99246) was bacteremic and toxemic at 20h post challenge and at the time of treatment administration; blood cultures were negative at the time of death and histopathology findings were consistent with aspiration pneumonia. There were no meningeal findings on histopathology of this animal. This animal was considered to have died as the result of a dosing accident (inadvertent intratracheal gavage of levofloxacin).

Table 7.2.2-1: Survival at Day 28 in the ITT and FDA Primary Populations for NZW Rabbits

Population	Treatment	N	No. of Survivors (%)*	95% CI** of Levo/Raxi – Levo (%)
Sponsor's ITT	Placebo	12	0 (0.0%)	
	Levofloxacin	20	19 (95.0%)	
	Levo/Raxi	20	19 (95.0%)	(-20.4, 20.4)
FDA primary	Placebo	10	0 (0.0%)	
	Levofloxacin	20	19 (95.0%)	
	Levo/Raxi	17	16 (94.1%)	(-23.9, 19.6)

*There is significant difference between each active group and placebo (all p-values <0.0001).
**CIs are exact confidence intervals for comparison between levo/Raxi and levofloxacin.

Sensitivity analyses as well as analysis excluding the non-anthrax death did not alter the conclusions of the primary efficacy analyses. There was no statistical difference in survival rate at Day 28 between the levofloxacin and levofloxacin/raxibacumab, both of which maintained statistically significant survival benefit as compared to placebo.

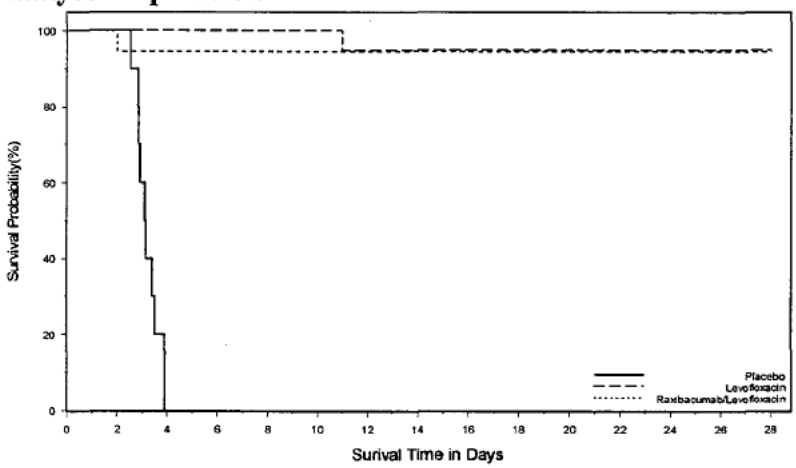
Table 7.2.2-2: Survival at Day 28 in FDA Primary Excluding Non-anthrax Death

Study population	Placebo	Levofloxacin	Levofloxacin/Raxibacumab
FDA Primary excluding non-anthrax death			
	N=10	**N=20**	**N=16**
Survival n (%); (95% CI)	0	19 (95); (65.9, 99.9)	16 (100); (69.2, 100)
p-value*	-	<0.0001	<0.0001
95% CI** of Levo/raxi – Levo (%)	-	-	-15.2, 25.6

Secondary Endpoint

The secondary efficacy endpoint was survival time, defined as the time from the beginning of spore challenge to death during the 28-day postchallenge period. As shown in the Figure 5.2.2-1 below, survival time was significantly longer in the levofloxacin group ($p < 0.0001$) and the levofloxacin/raxibacumab group ($p < 0.0001$) relative to the placebo group. The difference in survival times between the 2 active treatment groups was not statistically significant ($p = 0.9855$ as determined by log-rank test). The median survival time in the placebo group was 78.5 hours, median survival time could not be determined for the levofloxacin and levofloxacin/raxibacumab groups because the medians extended beyond 28 days.

Figure 5.2.2-1 Study 781-G923701: Survival Probability of Rabbits in FDA Primary Analysis Population



8. Safety

8.1 Histopathology findings from animal efficacy studies

Upon histological examination of tissues from the non-survivor animals in pivotal animal studies it was noted that, although, all animals had evidence of anthrax disease in organs examined, the pattern of histopathologic changes was different between placebo and raxibacumab treated animals. There was greater incidence and/or severity of CNS lesions in raxibacumab-treated non-survivors as compared to placebo non-survivors. Histopathologic examination revealed that more bacteria (intra-, peri-vascular areas, subarachnoid space, choroid plexus), fibrinoneutrophilic inflammation, hemorrhage, fibrinoid or neuropil necrosis were present in the brains of raxibacumab-treated non-survivors.

A consistent method was applied to compare histopathology findings between the study groups and between the studies with focus on "high grade" lesions defined as presence of either bacteria hemorrhage, or inflammation of >2 or any evidence of necrosis.

Table 8.1-1 Raxibacumab Monotherapy Studies: High Grade pathology in Non-Survivors

Organ/animal	Placebo (%)	Raxibacumab 20 mg/kg (%)	Raxibacumab 40 mg/kg (%)	P-value (20 vs. p)*	p-value (40 vs. p)*
Cynomolgus Macaque (As treated)					
Mortality	**12/12 (100)**	**7/14 (50)**	**5/14 (35.7)**	**0.006**	**0.001**
CNS	**1/12 (8)**	**6/7 (86)**	**3/5 (60)**	**0.002**	**0.053**
Liver	3/12 (25)	1/7 (14)	0/5 (0)	1.0	0.515
Spleen	11/12 (92)	5/7 (71)	4/5 (80)	0.523	0.515
Mediastenitis	10/11 (91)	4/7 (57)	4/5 (80)	0.245	1.0
Pneumonia	5/12 (42)	0/7 (0)	1/5 (20)	0.106	0.6
Nephritis	2/12 (17)	0/7 (0)	0/5 (0)	0.509	1.0
NZW Rabbit (As treated)					
Mortality	**16/16 (100)**	**12/17 (70.5)**	**11/19 (57.9)**	**0.044**	**0.004**
CNS	**2/16 (12)**	**9/12 (75)**	**6/11 (55)**	**0.001**	**0.033**
Liver	6/16 (38)	3/12 (25)	3/11 (27)	0.687	0.692
Spleen	11/16 (69)	2/12 (17)	2/11 (18)	0.009	0.018
Mediastenitis	15/16 (94)	7/12 (58)	8/11 (73)	0.057	0.273
Pneumonia	10/16 (63)	0/12 (0)	0/11 (0)	0.001	0.001
Nephritis	4/16 (25)	1/12 (8)	2/11 (18)	0.355	1.0
Cardiac	0/16 (0)	0/12 (0)	2/11 (18)	ns	0.157

* two-sided Fisher's exact test

While in placebo animals moderate to marked bacteria, hemorrhage, inflammation, and necrosis were more frequently observed in the visceral organs including lungs, mediastinum, spleen and liver, in raxibacumab treated non-survivors the most prominent pathology findings were primarily localized to the brain.

The trend in longer (1-2 days) median duration of survival or bacteremia prior to death in the non-survivor animals that had moderate/marked inflammatory changes (well correlated with high level of pathology by FDA algorithm) in the CNS was observed in all treatment groups. However, the analyses are limited by the number of non-survivor animals in the active treatment arms. These additional analyses were performed by the primary medical and statistical reviewers. The tables below prepared by the statistical reviewer Dr. Hongling Zhou illustrate this finding.

Table 8.1-2: NHP Median time to death by brain inflammation status

	M/M brain inflammation	Not M/M brain inflammation	Wilcoxon, p-value
Pooled 3 groups (Applicant's analysis)	4.5 (n = 9)	3.2 (n = 15)	P = 0.017
Raxibacumab (pooled 20 and 40)	4.3 (n = 8)	2.5 (n = 4)	P = 0.022
Raxibacumab 40 mg	4.9 (n = 2)	2.7 (n = 3)	P = 0.149
Raxibacumab 20 mg	4.0 (n = 6)	2.3 (n = 1)	P = 0.211
Placebo	4.5 (n = 1)	3.3 (n = 11)	P = 0.385

M/M –moderate/marked

Table 8.1-3: NZW rabbits Median time to death by brain inflammation status

	M/M brain inflammation	Not M/M brain inflammation	Wilcoxon, p-value
Pooled 3 groups (Applicant's analysis)	4.0 (n = 6)	2.7 (n = 33)	P=0.003
Raxibacumab (pooled 20 and 40)	4.0 (n = 6)	2.7 (n = 17)	P = 0.002
Raxibacumab 40 mg	3.5 (n = 3)	2.5 (n = 8)	P = 0.032
Raxibacumab 20 mg	5.8 (n = 3)	2.7 (n = 9)	P = 0.027
Placebo	n/a (n = 0)	2.7 (n = 16)	n/a

M/M –moderate/marked

The difference in the frequency and the severity of the pathologic findings in the brain between placebo and raxibacumab treated non-survivors across both animal species could not be fully explained by the duration of survival from challenge or duration of bacteremia prior to death due to limited number of non-survivor animals in the active treatment arms and also due to a considerable overlap in the time to death or duration of bacteremia prior to death between placebo animals with low level CNS pathology and raxibacumab treated non-survivors with high level CNS pathology.

Additional hypotheses on the mechanism of the CNS findings in the raxibacumab-treated non-survivors should be considered further:

- Increased permeability of blood-brain barrier in a setting of severe systemic disease that allows raxibacumab penetration into CNS and potential binding to the toxin damaged brain tissue with ensuing inflammatory response. This could potentially be evaluated by systematic collection and examination of histopathology data from survivors and non-survivors and immunohistochemistry of the brain/meninges.
- Inhibition of inflammatory response by lethal toxin in a setting of acute systemic anthrax disease does not allow for recruitment and activation of inflammatory cells into CNS. In the presence of raxibacumab action of lethal toxin will be blocked and host inflammatory response is restored. *Ex vivo* studies of neutrophil activation from raxibacumab treated and untreated exposed animals could potentially be performed.
- Additional drug-disease interaction hypotheses included: immune-complex disease (unlikely as kidney damage was more pronounced in the placebo animals), and cytokine, chemokine storm, complement activation in the presence of raxibacumab during ongoing anthrax infection.

Direct CNS toxicity of raxibacumab appears to be unlikely in lieu of negative tissue cross-reactivity studies and favorable safety profile of raxibacumab in animal toxicology studies and in studies of healthy human volunteers. While drug-disease interaction could not be excluded, in the context of survival benefit offered by raxibacumab and the knowledge that hemorrhagic meningitis is a pathognomonic feature of systemic anthrax disease reported in up to 66% of historical clinical cases, 50-60% in NHP and in 30% of rabbit animal models described in the scientific literature the clinical significance of this finding remains unclear.

Consults on the significance and potential mechanisms of the above histopathology findings were requested from the DNP and Marc Walton, M.D. former Director of ODE

VI. The findings and conclusions were similar to that of the primary reviewers of this BLA.

Additional studies were recommended to investigate the relationship of raxibacumab to the CNS findings in treated non-survivors and the significance of such findings for the survivors and include the following:

a. ***Evaluate neurological function/behavior in survivor and non-survivor animals in further efficacy studies recommended***
b. ***Conduct necropsy on all survivor and non-survivor animals and histopathology examination of tissues, particularly the brain and meninges***
c. ***Report the findings on raxibacumab binding to the meninges from previously conducted tissue cross reactivity studies, if available, or conduct a new cross reactivity study on rabbit, monkey, and human meningeal tissue.***
d. ***Perform an immunohistochemical staining of the brains of raxibacumab treated animals to evaluate whether raxibacumab is present in CSF and brain, if feasible.***

8.2 Human Safety Findings

The safety database consisted of 326 healthy subjects treated with the recommended dose of raxibacumab of 40 mg/kg manufactured by the M11 process (the version intended for licensure), with 303 subjects receiving a single dose (studies HGS1021-C1064 and HGS1021-C1063), 23 subjects receiving a 2nd dose 2 weeks after their first dose (study HGS1021-1063), and 20 subjects from study HGS1021-C1064 who received a 2nd dose more than 4 months after their initial dose (study HGS1021-C1069). In Study HGS1021-C1064, 48 subjects who received raxibacumab also received ciprofloxacin as part of this drug interaction study.

The M11 formulation of raxibacumab is safe for use in the adult population at the intended single IV dose of 40 mg/kg when administered after diphenhydramine pre-medication. There was only 1 death in a subject who received placebo from injuries sustained in a motor vehicle accident. In addition to this death, there were 2 additional SAEs reported, 1 case of cholecystitis and 1 case of schizophrenia, both of which were likely related to underlying conditions. There were 4 subjects (1.2%) who discontinued raxibacumab during infusion because of AEs. Two of these were cases of urticaria in subjects who did not receive premedication with diphenhydramine. AEs of severe intensity were infrequently reported (1.9%). The most frequently reported AE was headache; other commonly reported AEs included upper respiratory tract infection and rash.

As the safety database consisted of healthy volunteers, a study agent/disease interaction could not be directly assessed. While CNS AEs were reviewed, it appears from the animal models that systemic anthrax disease must be present for CNS pathology to be observed. A review for CNS safety in humans was therefore limited. Other limitations with respect to safety include the exclusion of pediatric subjects and pregnant patients from the safety database. While subjects greater than 65 years of age were enrolled in the study, they represented only 6.4% (21 subjects) of the population which is insufficient to make any conclusions as to whether these subjects respond differently to raxibacumab compared to subjects less than 65 years of age. Similarly, non-white subjects represented 28% of the

safety database. Therefore, it is unclear how generalizable the results of raxibacumab safety assessments are to the above populations.

9. Advisory Committee Meeting

Advisory Committee meeting was held on October 27, 2009. The following questions were posed to the committee:

(1) Does the evidence from the animal models evaluating raxibacumab at 40 mg/kg IV predict response for treatment of humans with inhalational anthrax disease?

16 members voted Yes, 7 voted No, 1 abstained

Minutes excerpt: *The majority of the Committee Members felt that the criteria for the Animal Rule were met and agreed that that the data from the animal studies could be used to extrapolate to humans. Concerns remain about dosing and the timing of treatment after exposure. Further studies should include special populations, including the pediatric population.*

Members of the Committee that voted 'no' thought that all four elements of the animal rule were not met and extrapolation to humans was not possible, and recommended studies be conducted that more accurately depict the course of disease in humans. Additional studies were requested to determine the benefit of raxibacumab in relation to timing post exposure.

The member of the Committee that abstained from voting did so because of a personal perception of conflict of interest.

(2) Does the evidence provided in these studies support the conclusion that raxibacumab will not diminish the anticipated efficacy of antimicrobials in inhalational anthrax?

10 members voted Yes, 11 voted No, 3 abstained

Minutes excerpt: *Members of the Committee that voted 'yes' agreed that raxibacumab did not appear to diminish the effects of antimicrobials in treatment of inhalational anthrax.*

Members of the Committee that voted 'no' thought that it was difficult to determine the effects of raxibacumab with antibiotics because there was no study with the antibody as the control arm. It could not be seen whether effects were due to the combination therapy or possibly antimicrobial therapy alone. It was noted that high doses of antimicrobial could have masked any detrimental effects of raxibacumab. Studies related to timing of administration, proper dosing of antimicrobials, and use of different antimicrobials were suggested.

Members of the Committee that abstained from voting did so because the need for more data and because of a personal perception of conflict of interest.

(3) Given the high efficacy of the antimicrobial arms in the rabbit (95%) and monkey (100%) studies, the added benefit of raxibacumab to antimicrobial could not be determined. Should evidence be requested that raxibacumab makes a contribution to the efficacy over the antimicrobial alone (in rabbit and monkey animal models)?

17 members voted Yes, 5 voted No, 1 abstained, 1 not voted

Minutes excerpt: *Members of the Committee that voted 'yes' to the question thought that more evidence was needed to conclude that raxibacumab contributed to efficacy over antimicrobial therapy alone. Recommendations included studies where standard or suboptimal doses of antibiotic therapy in humans are used to see contributing effect of raxibacumab, use of timing to mimic course of infection in human, and the use of rabbits instead of primates. It was noted that additional studies should not necessarily be requested from the Sponsor.*

Members of the Committee that voted 'no' to the question thought that raxibacumab possibly contributed to efficacy over antimicrobial therapy, but needed more precise animal studies to determine exact benefit. Additional studies should address when to start treatment and the state of the animal prior to treatment with raxibacumab. It was noted that the animal rule does not require benefit over conventional therapy.

10. Pediatrics

Raxibacumab has not been studied in pediatric patients or healthy pediatric subjects. Raxibacumab has been granted Orphan Designation for "the treatment of anthrax". It is exempt from PREA obligations.

11. Other Relevant Regulatory Issues

None

12. Labeling

Not applicable at this time

13. Recommendations/Risk Benefit Assessment

- **Recommended Regulatory Action:**
  Complete response pending resolution of bioanalytical assays' deficiencies. Under the Animal Rule 21CFR 601.90 the animal efficacy studies did establish raxibacumab efficacy (prevention of anthrax induced lethality) in two animal models of inhalational anthrax disease (NZW rabbits and cynomolgus macaques) in the FDA primary population of animals bacteremic prior to raxibacumab administration. The reviewer concludes that requirements 1-3 of the Animal Rule were met in this application:
  1. There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;

Mechanism of action of anthrax toxin is well understood and proximal targets for anthrax toxin toxicity are well established in humans and animals. Raxibacumab binds PA and prevents formation of anthrax toxin and its downstream cytotoxic effects. This is documented in the in vitro binding studies of recombinant PA as well as the PA from antibiotic susceptible *B. anthracis* strains: Ames, Sterne, and Vollum. In proof of concept/mechanism of action rat lethal toxin infusion study administration of raxibacumab improved survival of the animals as compared to placebo.

2. The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;

Statistically significant improvement in survival with raxibacumab administered as a 40mg/kg IV single dose as compared to placebo was observed in two animal models of inhalational anthrax disease (NZW rabbits and cynomolgus macaques) in the FDA primary analysis population of animals bacteremic prior to the study treatment intervention. The selected animal models were sufficiently similar to humans with respect to etiology, pathogenesis, route of exposure, and host susceptibility factors. ***The course of the disease was shorter in the animal models as compared to historical data on human disease, but the spore challenge dose of approximately 200xLD_{50} in the animals was highly lethal and represented the worse case scenario (100% mortality in the placebo group). However, the FDA efficacy analyses focused on the animals that were bacteremic prior to study treatment, ensuring that the animals, in fact, had systemic anthrax disease and would reflect the human patient population diagnosed with anthrax disease by culture.***

There are additional limitations of the efficacy data with respect to predicting response in humans. First, the time of intervention could be different between animals and humans. In the controlled research environment setting, animals were closely monitored for either an elevation in temperature (rabbits) or a positive serum PA (rabbits and cynomolgus macaques), and once detected, a therapeutic intervention was delivered. Humans will likely to have variable time to presentation to the health care setting or be given alternative diagnoses prior to the definitive diagnosis of anthrax being made. ***Although animal efficacy data might not be applicable to the whole spectrum of human inhalational anthrax disease at the time of the health care setting presentation, it likely to be applicable to a subset of patients with positive blood cultures at the time of presentation possibly in the prodromal phase of the disease.***

Second, the combination efficacy studies demonstrated nearly 100% efficacy of levofloxacin in NZW rabbits and ciprofloxacin in cynomolgus macaques, respectively. In contrast, during the 2001 anthrax attacks, 5 of the 11 people who developed inhalational anthrax died despite best available treatment. Both of these factors may affect the degree of certainty in extrapolation of efficacy

from the animal models to humans. ***Although helpful for the healthcare provider to be aware of the window of a successful therapeutic intervention to be characterized in an animal model of delayed treatment (antibiotic efficacy of only 55%) the studies evaluating added benefit of raxibacumab to antimicrobial under these conditions have not been conducted to date and in the preamble to the Final Animal Rule is discussed as not required preapproval.***[4]

Third, ***in the animal studies therapeutic interventions were limited only to study drugs while in clinical care setting multiple supportive measures will likely be instituted including but not limited to cardio-respiratory support, fluid resuscitation, dialysis, pleural fluid drainage, etc.***

3. The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity;

   Survival was the primary endpoint measured at 14 days post challenge in rabbit and 28 days post challenge in NHP animal models.

The results of the safety evaluation of raxibacumab as a 40mg/kg IV single dose in healthy human volunteers were unremarkable, except for mild self limiting infusion related rashes prevented with diphenhydramine premedication. However, this BLA application cannot be approved as PK/PD link (4th requirement under the Animal Rule) between the efficacious dose in animals and proposed efficacious and safe dose in humans cannot be reliably established at this time due to remaining concerns from DSI inspections about the raxibacumab assays from human PK/safety studies C-1064 and C-1063.

The following deficiencies should be included in the Complete Response letter and must be addressed by the Applicant prior to approval of this BLA:

a. ***Following adequate revision of the analytical procedures to address these inspection findings, the pharmacokinetic samples from human studies HGS1021-C1063, HGS1021-C1064, and HGS1021-C1069 should be re-assayed using the revised procedures. Pharmacokinetic parameters for raxibacumab from these re-analyses should be re-calculated in humans following re-assay of the samples. Depending on the outcome of these re-analyses, additional pharmacokinetic studies may be required.***

b. ***The plasma pharmacokinetic samples obtained from the human study HGS1021-C1064 and the plasma samples obtained from the cynomolgus monkey study 789-G923702 should be re-assayed for ciprofloxacin concentrations using a revised analytical method that adequately addresses the DSI findings for the ciprofloxacin assay. Pharmacokinetic parameters for ciprofloxacin from these re-analyses should be re-calculated in both humans and monkeys following re-assay of the samples. Depending on the outcome of these re-analyses, additional pharmacokinetic studies may be required.***

[4] Final Rule 21 CFR Parts 314 and 601 (p. 39790-1)

c. ***Because of the outstanding issues with the assay of raxibacumab in human serum, we have now become concerned about the reliability of the pharmacokinetic data that was generated from the animal efficacy studies in New Zealand White rabbits and cynomolgus monkeys, which used similar bioanalytical procedures as that for the human studies. Therefore, the pharmacokinetic samples from animal studies 682-G005758 and 724-G005829 should also be re-assayed using the revised procedures. Pharmacokinetic parameters for raxibacumab from these re-analyses should be re-calculated in rabbits and monkeys following re-assay of the samples. Depending on the outcome of these re-analyses, additional pharmacokinetic studies may be required.***

d. ***Re-assess and*** (b) (4) ***the bacterial endotoxin specification for the raxibacumab final drug product. The re-assessment should consider the endotoxin contribution from the saline solution used to dilute raxibacumab drug product prior to administration. The revised specification should provide a safety factor (e.g., 2-4 fold) when the endotoxin limits from both the saline and the drug product are considered.***

- **Risk Benefit Assessment**

Raxibacumab is a recombinant humanized monoclonal antibody that binds the protective antigen (PA) of *B. anthracis* and prevents the binding of PA with its receptors. As the PA-receptor interaction is required for disease progression, its inhibition represents a direct antitoxin therapy to complement very limited armamentarium of approved anti-anthrax treatments, i.e. antibiotics ([5],[6],[7]).

Current approved treatments of anthrax include antibiotics: penicillin G and tetracyclines[1]. Antibiotics however have several limitations: the absence of direct activity against anthrax toxin and anthrax spores[8], resistance to engineered antibiotic-resistant strains[9], and poor patient adherence with the recommended 60 day treatment duration[1]. Furthermore, the effectiveness of antimicrobial therapy is less than ideal; following the dissemination of anthrax spores through the US Postal Service system in September 2001 where 11 people developed inhalational anthrax, the survival rate was 55% (6/11) despite early intervention with antimicrobial therapy and advanced supportive care[10]. Benefit of raxibacumab would also be expected in individuals for whom antibiotics are contraindicated or who are non-adherent. Although could not be

[5] Little SF, Leppla SH, Friedlander AM. Production and characterization of monoclonal antibodies against the lethal factor component of *Bacillus anthracis* lethal toxin. Infect Immun 1990 ;58(6) :1606-13.
[6] Maynard JA, Maassen CB, Leppla SH et al. Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. Nat Biotechnol 2002 ;20(6) :597-601.
[7] Kobiler D, Gozes Y, Rosenberg H et al. Efficiency of protection of guinea pigs against infection with *Bacillus anthracis* spores by passive immunization. Infect Immun 2002 ;70(2) :544-60.
[8] Friedlander AM, Wellkos SL, Pitt ML et al. Postexposure prophylaxis against experimental inhalational anthrax. J Infect Dis 1993 ;167(5) :1239-43.
[9] Athamna A, Athamna M, Abu-Rashed N et al. Selection of Bacillus anthracis isolates resistant to antibiotics. J Antimicrob Chemother 2004 ;54(2) :424-8.
[10] Inglesby TV et al. Anthrax as a biological weapon, 2002: Updated recommendations for management. JAMA 2002:May 1;287(17):2236-52.

tested against PA from multi-drug resistant *B. anthracis,* mechanism of action of raxibacumab is different from those of antibiotics and it is potentially could serve as an antitoxin therapy for those patients in whom disease is due to antibiotic-resistant anthrax.

In a proof-of-concept rat lethal toxin infusion model study conducted by the Applicant, raxibacumab at a concentration of ten-fold the concentration of recombinant PA (rPA) resulted in 100% survival for 24 hours, while isotype antibody treated control rats died within 90 minutes of LT administration. These results support the current thought that secreted *B. anthracis* toxins are responsible for the morbidity and mortality characteristic of anthrax infection despite appropriate antimicrobial therapy.

In animal studies (rabbit and monkey models of inhalational anthrax), raxibacumab conferred a statistically significant survival benefit over placebo when administered to the animals confirmed to be bacteremic with *B. anthracis*. When raxibacumab was studied in the same animal models (rabbit and monkey) of inhalational anthrax in combination with either levofloxacin or ciprofloxacin and the efficacy of combination was compared to antibiotics alone, there was no statistically significant difference in survival between both active treatment groups. While these supportive studies were not powered to detect added benefit or antagonism between raxibacumab and antibiotics and the animal models themselves are limited by the high efficacy of antibiotics alone, the absence of numerical difference (a single anthrax death in both models: on combination arm in the NHP study and on levofloxacin alone arm in the rabbit study) and the absence of a pharmacokinetic drug-drug interaction are reassuring and the additional benefit conferred with concurrent use of raxibacumab in humans remains a viable possibility and might need to be further explored in animal studies when a suitable animal model of delayed intervention in inhalational anthrax becomes available.

From a safety perspective, the M11 formulation of raxibacumab is safe for use in the adult population at the intended single IV dose of 40 mg/kg when administered after diphenhydramine pre-medication based on the analysis of a database composed of 326 subjects. There were no deaths in raxibacumab-treated subjects, and only 2 SAEs reported (0.6%) that were likely related to the subjects' underlying conditions. There were 4 subjects (1.2%) who discontinued raxibacumab during infusion because of infusion-related AEs. Two of these were cases of rash in subjects who did not receive premedication with diphenhydramine. AEs of severe intensity were infrequently reported (1.9%). The most frequently reported AE was headache; most cases were mild in severity, did not lead to study agent discontinuation and resolved spontaneously during the study period.

Risks

While the anthrax animal models have been carefully and extensively tested and characterized, it is still unknown as to how well they predict efficacy in humans for several reasons. First, the time of intervention is likely to be different between animals under study conditions and humans in real life clinical care settings. Animals evaluated in efficacy studies have received therapeutic intervention at the time of trigger positivity (early in the course of the disease) compared to humans who may

present to the health care setting with variable time of symptom onset, including delayed presentation, or have the appropriate treatment delayed due to alternative diagnoses considered prior to the establishment of definitive diagnosis due non-specific nature of symptoms in the prodromal phase of inhalational anthrax disease. However, there would be patients with inhalational anthrax disease who will present and will be diagnosed with inhalational anthrax and receive treatment in the prodromal phase when blood cultures are positive for *B. anthracis*, similar to the conditions of treatment in the animal studies conducted.
In addition, antibiotic efficacy observed in the two combination efficacy studies was close to 100%, which is far higher than the rates observed historically during the 2001 anthrax attacks (55% efficacy). ***The additional benefit that raxibacumab may offer could not be determined in animal models studied and, therefore, it is difficult to predict the extent of survival improvement what might occur in humans with the addition of raxibacumab. The delayed treatment models in inhalational anthrax are under development. It is unclear when such model will become available. Multiple factors will need to be well characterized and considered in designing added benefit delayed model study, including but not limited to: desired efficacy of antimicrobials to be replicated, humanized dose and regimen of antimicrobial (relevant human exposures for an approved duration achieved in an animal), therapeutic window post challenge or trigger detection for each animal model, statistical power to detect superiority of combination treatment versus antimicrobial alone, ethical issues of allowing large numbers (hundreds) of animals to die prior to treatment initiation in order to mimic 55% human survival with antibiotics in 2001, and feasibility of conducting such a study under BL-3 laboratory conditions. Although, this study will provide additional information on the potential synergy/added benefit of a combination therapy, the Reviewer concludes that it is not required for product approval in accordance with the defined goal of a therapeutic product development in the Final Rule 21 CFR Parts 314 and 601 (p. 39790-1), specifically for a bioterrorism target, where the requirement for products to demonstrate meaningful therapeutic benefit over existing treatments was eliminated, in order to allow for a wide range of products to be available for treatment indications studied under the Animal Rule, i.e. inhalational anthrax where current therapeutic options are limited to only 2 classes of antibiotics. However, a study evaluating added benefit of antitoxin to antimicrobial will be requested as a postmarketing commitment upon application approval, contingent on the development of a delayed treatment (50% antimicrobial efficacy) animal model of treatment of inhalational anthrax.***

While animal models represent the best studied efficacy evidence to date, the true efficacy of raxibacumab can only be determined under field study conditions.

Next, the risk of central nervous system (CNS) toxicity in humans associated with raxibacumab exposure remains unclear. In the pivotal rabbit and NHP efficacy studies, animals treated with raxibacumab and died of anthrax had more CNS pathology compared with placebo animals that died. Survivors were not sacrificed and, therefore, it could not be determined if the same pathophysiologic process occurred in survivors. In the rabbit combination antibiotic study, all animals (survivors and non-survivors), were sacrificed and had their tissues examined microscopically. While all placebo animals had both systemic and CNS findings

consistent with anthrax, only 1 animal in an active treatment group died of anthrax, but did not have CNS findings. None of the rabbits surviving to study termination had lesions attributable to anthrax on histopathologic examination. Similarly, in the combination NHP study, only 1 animal in the ciprofloxacin/raxibacumab group died of anthrax. While this animal did have evidence of hemorrhagic meningitis, the single death limits the degree of analysis that can be performed. However, negative brain tissue cross reactivity and animal toxicology studies as well as unremarkable safety of raxibacumab in healthy volunteers argue against the direct CNS toxicity of raxibacumab. Although drug-disease interaction cannot be ruled out, its' clinical significance has to be assessed in lieu of survival benefit provided by raxibacumab monotherapy and will not likely be easily discernable even with the conduct of the studies recommended by the reviewers and consultants. The studies recommended to be conducted to evaluate potential mechanisms of CNS findings in raxibacumab treated animals include the following:

- ***Evaluate neurological function/behavior in survivor and non-survivor animals in further efficacy studies recommended***
- ***Conduct necropsy on all survivor and non-survivor animals and histopathology examination of tissues, particularly the brain and meninges in further efficacy studies recommended***
- ***Report the findings on raxibacumab binding to the meninges from previously conducted tissue cross reactivity studies, if available, or conduct a new cross reactivity study on rabbit, monkey, and human meningeal tissue.***
- ***Perform an immunohistochemical staining of the brains of raxibacumab treated animals to evaluate whether raxibacumab is present in CSF and brain, if feasible.***

There are several limitations of the clinical safety data. First, a safety database of 326 subjects cannot exclude a serious adverse event (SAE) or fatal event occurring at less than 1% frequency. In addition, as the safety database consisted only of healthy volunteers, any possible study agent/disease interaction could not be assessed. Lastly, the exclusion of pediatric subjects and other special populations (pregnant patients, and patients with renal and hepatic dysfunction), and insufficient numbers of subjects aged 65 years and older limit the generalizability of safety results to these populations.

- **Recommendation for Postmarketing Risk Management Activities**

None

- **Recommendation for other Postmarketing Study Commitments**

To be determined

BLA 125349 (Raxibacumab)

Signatory Page

Medical/ Cross-Discipline Team Leader (CDTL): Yuliya Yasinskaya, M.D.

Date: October 21, 2009

Division Director: Renata Albrecht, M.D.

Yuliya Yasinskaya, M.D. — 11.15.2009 Date

Renata Albrecht, M.D. — 11-13-09 Date

CLINICAL REVIEW

Application Type	BLA
Application Number(s)	125,349
Priority or Standard	P
Submit Date(s)	May 13, 2009
Received Date(s)	May 14, 2009
PDUFA Goal Date	November 14, 2009
Division / Office	DSPTP/OAP
Reviewer Name(s)	Sue Lim
Review Completion Date	October 19, 2009
Established Name	Raxibacumab
(Proposed) Trade Name	
Therapeutic Class	Human monoclonal antibody
Applicant	Human Genome Sciences
Formulation(s)	Sterile liquid
Dosing Regimen	Single 40 mg/kg IV dose
Indication(s)	[redacted] (b) (4) inhalational anthrax
Intended Population(s)	Subjects with a known or suspected exposure to *Bacillus anthracis*

Table of Contents

1 RECOMMENDATIONS/RISK BENEFIT ASSESSMENT 5

1.1 Recommendation on Regulatory Action 5
1.2 Risk Benefit Assessment 5
1.3 Recommendations for Postmarketing Risk Evaluation and Mitigation Strategies 8
1.4 Recommendations for Postmarketing Requirements and Commitments 8

2 INTRODUCTION AND REGULATORY BACKGROUND 8

2.1 Product Information 8
2.2 Tables of Currently Available Treatments for Proposed Indications 11
2.3 Availability of Proposed Active Ingredient in the United States 12
2.4 Important Safety Issues With Consideration to Related Drugs 12
2.5 Summary of Presubmission Regulatory Activity Related to Submission 12
2.6 Other Relevant Background Information 13

3 ETHICS AND GOOD CLINICAL PRACTICES 14

3.1 Submission Quality and Integrity 14
3.2 Compliance with Good Clinical Practices 15
3.3 Financial Disclosures 15

4 SIGNIFICANT EFFICACY/SAFETY ISSUES RELATED TO OTHER REVIEW DISCIPLINES 15

4.1 Chemistry Manufacturing and Controls 15
4.2 Clinical Microbiology 15
4.3 Preclinical Pharmacology/Toxicology 19
4.4 Clinical Pharmacology 20
4.4.1 Mechanism of Action 21

5 SOURCES OF CLINICAL DATA 21

5.1 Tables of Studies/Clinical Trials 21
* Study was reviewed but not included in the integrated database for safety analysis 24
5.2 Review Strategy 24
5.3 Discussion of Individual Studies/Clinical Trials 25

6 REVIEW OF EFFICACY 25

Efficacy Summary – Study 781-G923701 25
6.1 Indication 27
6.1.1 Methods 28
6.1.2 Demographics 35
6.1.3 Subject Disposition 36
6.1.4 Analysis of Primary Endpoint(s) 38

6.1.5 Analysis of Secondary Endpoints(s) 45
6.1.7 Subpopulations 64
6.1.8 Analysis of Clinical Information Relevant to Dosing Recommendations 64
6.1.9 Discussion of Persistence of Efficacy and/or Tolerance Effects 64
6.1.10 Additional Efficacy Issues/Analyses 65

7 REVIEW OF SAFETY 66

Safety Summary 66
7.1 Methods 67
7.1.1 Studies/Clinical Trials Used to Evaluate Safety 67
7.1.2 Categorization of Adverse Events 69
7.1.3 Pooling of Data Across Studies/Clinical Trials to Estimate and Compare Incidence 70
7.2 Adequacy of Safety Assessments 70
7.2.1 Overall Exposure at Appropriate Doses/Durations and Demographics of Target Populations 70
7.2.2 Explorations for Dose Response 71
7.2.3 Special Animal and/or In Vitro Testing 72
7.2.4 Routine Clinical Testing 73
7.2.5 Metabolic, Clearance, and Interaction Workup 73
7.2.6 Evaluation for Potential Adverse Events for Similar Drugs in Drug Class 74
7.3 Major Safety Results 74
7.3.1 Deaths 74
7.3.2 Nonfatal Serious Adverse Events 74
7.3.3 Dropouts and/or Discontinuations 76
7.3.4 Significant Adverse Events 77
7.3.5 Submission Specific Primary Safety Concerns 79
7.4 Supportive Safety Results 81
7.4.1 Common Adverse Events 81
Laboratory Findings 86
7.4.3 Vital Signs 89
7.4.4 Electrocardiograms (ECGs) 90
7.4.5 Special Safety Studies/Clinical Trials 90
7.4.6 Immunogenicity 90
7.5 Other Safety Explorations 91
7.5.1 Dose Dependency for Adverse Events 92
7.5.2 Time Dependency for Adverse Events 92
7.5.3 Drug-Demographic Interactions 92
7.5.4 Drug-Disease Interactions 97
7.5.5 Drug-Drug Interactions 98
7.6 Additional Safety Evaluations 98
7.6.1 Human Carcinogenicity 98
7.6.2 Human Reproduction and Pregnancy Data 98
7.6.3 Pediatrics and Assessment of Effects on Growth 99

7.6.4 Overdose, Drug Abuse Potential, Withdrawal and Rebound 99
7.7 Additional Submissions / Safety Issues.....99

8 POSTMARKET EXPERIENCE 100

9 APPENDICES 101

9.1 Literature Review/References.....101
9.2 Labeling Recommendations.....101
9.3 Advisory Committee Meeting 101
9.4 Individual Study Report HGS1021-C1063.....102
9.5 Individual Study Report HGS1021-C1069.....122
9.6 Individual Study Report 789-G923702.....135
9.7 Individual Study Report 724-G005829.....167
9.8 Individual Study Report 682-G005758.....226

1 Recommendations/Risk Benefit Assessment

1.1 Recommendation on Regulatory Action

Raxibacumab, a monoclonal antibody against protective antigen (PA) of *Bacillus anthracis*, manufactured by the process proposed for licensure (M11) administered as a single intravenous dose of 40 mg/kg dose is safe and effective (b) (4) A premedication regimen of diphenhydramine administered within 1 hour prior to the raxibacumab dose is recommended.

The efficacy recommendation is based on 2 pivotal efficacy studies, studies 682-G005758 (New Zealand White (NZW) rabbit) and 724-G005829 (nonhuman primate, NHP) and 2 supportive efficacy studies of raxibacumab used in combination with antibiotics, studies 781-G923701 (NZW rabbit) and 789-G923702 (NHP).

The safety recommendation is based on the pooled safety analyses of 326 subjects treated with the recommended dose of raxibacumab of 40 mg/kg manufactured by the M11 process. The database analysis consisted of 303 subjects receiving a single dose (studies HGS1021-C1064 and HGS1021-C1063), 23 subjects receiving a 2nd dose 2 weeks after their first dose (study HGS1021-1063), and 20 subjects from study HGS1021-C1064 who received a 2nd dose more than 4 months after their initial dose (study HGS1021-C1069).

1.2 Risk Benefit Assessment

Raxibacumab is a human monoclonal antibody which binds the PA of *B. anthracis* and prevents the binding of PA to its receptors. As this PA-receptor interaction is required for disease progression, its inhibition represents a novel and viable clinical strategy to complement the bactericidal activity of antibiotics (1,2,3).

The only therapies currently approved for the treatment of anthrax due to *B. anthracis* are tetracycline and penicillin antibiotics:

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1 Little SF, Leppla SH, Friedlander AM. Production and characterization of monoclonal antibodies against the lethal factor component of *Bacillus anthracis* lethal toxin. Infect Immun 1990 ;58(6) :1606-13.
2 Maynard JA, Maassen CB, Leppla SH et al. Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. Nat Biotechnol 2002 ;20(6) :597-601.
3 Kobiler D, Gozes Y, Rosenberg H et al. Efficiency of protection of guinea pigs against infection with *Bacillus anthracis* spores by passive immunization. Infect Immun 2002 ;70(2) :544-60.

Tetracyclines: doxycycline, doxycycline calcium, doxycycline hyclate Tetracycline, demeclocycline hydrochloride and minocycline hydrochloride are recommended in situations when penicillin is contraindicated

Penicillins: penicillin G procaine, penicillin G potassium, and penicillin G sodium.

These agents are approved on the basis on *in vitro* activity against *B. anthracis*.

Limitations of antibiotics include the absence of activity against anthrax toxin and anthrax spores (4), resistance of engineered strains (5), and poor patient adherence. Furthermore, the effectiveness of antimicrobial therapy is less than ideal; following the dissemination of anthrax spores through the US Postal Service system in September 2001 where 11 people developed inhalational anthrax, the survival rate was 55% (6/11) despite early intervention with antimicrobial therapy and supportive care (6). Benefit would also be expected for individuals for whom antibiotics are contraindicated or who are non-adherent.

In a proof-of-concept toxin rat model study conducted by the Applicant, raxibacumab at a concentration of ten-fold the concentration of recombinant PA (rPA) was administered up to three weeks prior to challenge with recombinant lethal toxin, rLT (composed of recombinant lethal factor, rLF + rPA) in F344 rats and resulted in 100% survival for 24 hours. In contrast, isotype antibody treated control rats died within 90 minutes of LT administration. These results demonstrate that secreted *B. anthracis* toxin alone in the absence of bacteremia is lethal. These results, in addition to the results of the pivotal efficacy studies of anthrax bacterial disease in cynomolgus monkeys and New Zealand White (NZW) rabbits demonstrate that raxibacumab prevents death in both toxin infusion and bacterial disease models.

In animal studies, raxibacumab conferred a statistically significant survival benefit over placebo. When raxibacumab was studied in combination with either levofloxacin or ciprofloxacin and compared to antibiotics alone, there was no statistically significant difference between either active treatment group. While these latter studies were not powered to detect added benefit of raxibacumab to antibiotics alone and the animal models themselves are limited by the high efficacy of antibiotics monotherapy, the absence of interaction is reassuring. However, potential additional benefit conferred with concurrent use of raxibacumab and antibiotics in humans remains to be evaluated.

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4 Friedlander AM, Wellkos SL, Pitt ML et al. Postexposure prophylaxis against experimental inhalational anthrax. J Infect Dis 1993 ;167(5) :1239-43.
5 Athamna A, Athamna M, Abu-Rashed N et al. Selection of Bacillus anthracis isolates resistant to antibiotics. J Antimicrob Chemother 2004 ;54(2) :424-8.
6 Inglesby TV et al. Anthrax as a biological weapon, 2002: Updated recommendations for management. JAMA 2002:May 1;287(17):2236-52.

From a safety perspective, the M11 formulation of raxibacumab is safe for use in the adult population at the intended single IV dose of 40 mg/kg when administered after diphenhydramine pre-medication. This finding is based on the analysis of a database composed of 326 subjects. There were no deaths in raxibacumab-treated subjects, and only 2 SAEs reported (0.6%) which were likely related to the subjects' underlying conditions. There were 4 subjects (1.2%) who discontinued raxibacumab during infusion because of infusion-related AEs. Two of these were cases of rash in subjects who did not receive premedication with diphenhydramine. AEs of severe intensity were infrequently reported (1.9%). The most frequently reported AE was headache; most cases were mild in severity, did not lead to study agent discontinuation and resolved during the study period.

Risks

Limitations of animal efficacy findings
While the anthrax animal models have been carefully and extensively tested and characterized, it is unknown as to how well they predict efficacy in humans for several reasons. First, the time of intervention is likely to differ between animals and humans. In the clinical trial setting, animals were closely monitored for either an elevation in temperature (rabbits) or a positive serum PA (rabbits and cynomolgus monkeys), and once detected, a therapeutic intervention was delivered. Animals enrolled in clinical studies are therefore likely to have earlier therapeutic intervention compared to humans who may either have delayed presentation to the health care setting, or have alternative diagnoses made prior to the definitive diagnosis of anthrax given the non-specific signs and symptoms in early inhalational anthrax disease. In addition, antibiotic efficacy observed in the two combination efficacy studies was close to 100%, which is far higher than rates seen historically with the 2001 anthrax attacks (55% efficacy). Both of these factors may affect the degree of extrapolation of efficacy from the animal models to humans. Lastly, additional potential benefit of raxibacumab could not be determined with animal models, and therefore it is unclear what will occur in humans. The efficacy of delayed administration of raxibacumab after the onset of clinical symptoms has not been reported in animal models and therefore the therapeutic window for effective raxibacumab delivery is unknown. While animal models still represent the best studied efficacy evidence to date, the true efficacy of raxibacumab can only be determined under field conditions.

Limitations of animal safety (central nervous system) findings
Next, the risk of central nervous system (CNS) toxicity in humans associated with raxibacumab exposure remains unclear. In the pivotal rabbit and NHP efficacy studies, animals treated with raxibacumab and died of anthrax had more CNS pathology compared with placebo animals that died. Survivors were not sacrificed, and therefore it could not be determined if the same pathophysiologic process occurred in survivors. In the rabbit antibiotic combination study, all animals (survivors and non-survivors), were sacrificed and had their tissues examined microscopically. While all placebo

animals had both systemic and CNS findings consistent with anthrax, only 1 animal in an active treatment group died of anthrax but did not have CNS findings (rabbit K99203 in the levofloxacin group; note rabbit K99246 in the levofloxacin/raxibacumab group died of a gavage accident). None of the rabbits surviving to study termination had lesions attributable to anthrax at sacrifice. Similarly in the combination NHP study, only 1 animal in the ciprofloxacin/raxibacumab group died of anthrax. While this animal did have evidence of hemorrhagic meningitis, the single death limits the degree of analysis that can be performed.

Limitations of clinical safety findings
There are several risks with respect to safety as determined in human subjects. First, a safety database of 326 subjects cannot exclude a serious adverse event (SAE) or fatal event occurring at less than 1% frequency. In addition, as the safety database consisted only of healthy volunteers, any possible study agent/disease interaction could not be directly assessed. Lastly, the exclusion of pediatric subjects and other special populations (pregnant patients, and patients with renal and hepatic dysfunction), and insufficient numbers of subjects aged 65 years and older limit the generalizability of safety results to these populations.

1.3 Recommendations for Postmarketing Risk Evaluation and Mitigation Strategies

Recommendations for postmarketing risk evaluation and mitigation strategies are pending the discussion at the pending Anti-Infectives Advisory Committee Meeting on October 27, 2009.

1.4 Recommendations for Postmarketing Requirements and Commitments

Recommendations for postmarketing risk evaluation and mitigation strategies are pending the discussion at the pending Anti-Infectives Advisory Committee Meeting on October 27, 2009.

2 Introduction and Regulatory Background

2.1 Product Information

Anthrax, caused by *Bacillus anthracis*, is a disease characterized by bacterial replication and septicemia with accumulation of bacterial toxins in the blood. In the absence of

adequate treatment, this is a highly fatal disease. While *B. anthracis* replication can be affected by antibiotics, it is the secreted anthrax toxin (AT) that is primarily responsible for the symptoms, signs and high rates of mortality in infected individuals.

AT is a protein complex consisting of lethal factor (LF), edema factor (EF) with protective antigen (PA) constituting the binding moiety (7). The PA moiety of AT binds the AT receptors (ATR or CMG2) on host cells to initiate a cascade of molecular events that manifest as clinical disease.

Raxibacumab is a recombinant, fully human immunoglobulin ($IgG_1\lambda$) monoclonal antibody to *Bacillus anthracis* protective antigen (PA). Raxibacumab blocks the binding of PA to cell surfaces and prevents the anthrax toxins from entering and killing cells.

The indication sought for raxibacumab is the treatment of subjects with inhalational anthrax due to *B. anthracis*.

As inhalational anthrax is an extremely rare naturally-occurring disease in humans, and intentional exposure would be unethical, efficacy data on raxibacumab was obtained from animal models of infection, with approval sought under Subpart H of the regulations 21 CFR 601 ("Approval of biological products when human efficacy studies are not ethical or feasible"). The Animal Rule, published in 2002 by the FDA ("New Drug and Biological Drug Products: Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible") provides a regulatory mechanism to accept efficacy from animal models when adequate and well-controlled studies in humans cannot be ethically conducted and field efficacy studies are not feasible. To obtain guidance on whether approval should be based on the demonstration of superiority of monoclonal antibody over placebo, or whether animal efficacy studies should also show the contribution of monoclonal and antimicrobial to survive, this issue was presented at a Regulatory Briefing meeting in October 2006. The group recommended that statistical superiority must be shown of the monoclonal over placebo, and studies evaluating the monoclonal and antimicrobials should be requested. However, recognizing the challenge to conducting these studies, the group did not recommend that approval be withheld if an additive effect of each component could not be demonstrated. Furthermore, it was noted that the two products have different mechanisms of actions.

The development program for raxibacumab was designed to meet the criteria described in the draft Guidance for Industry: Animal Models – Essential Elements to Address Efficacy Under the Animal Rule to support the licensure of raxibacumab for treatment of

7 Inglesby TV, O'Toole T, Henderseon DA et al. Anthrax as a biological weapon, 2002 : Updated recomendations for management. JAMA 2002 ;287(17) :2236-52.

inhalational anthrax. The criteria and HGS' response are summarized in the following table.

Table: Efficacy elements for raxibacumab under the Animal Rule

Animal rule criteria	HGS response
Reasonably well-understood pathophysiological mechanism for the toxicity and its amelioration or prevention by the product	Mechanism for anthrax toxin toxicity well understood - Nonclinical studies which demonstrated that raxibacumab binds PA and specifically interferes with the binding of PA to its receptor to prevent macrophage killing - Animal studies in the rat, rabbit and cynomolgus monkey showed raxibacumab provided protection from anthrax toxin effects and improved survival of infected animals
Demonstration of effectiveness in more than 1 animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans	Natural history studies (characterize clinical progression of inhalational anthrax disease): 615-N104504: New Zealand White (NZW) rabbits 685-G005762: Cynomolgus monkeys Pivotal efficacy studies: Study 682-G005758: NZW rabbits Study 724-G005829: Cynomolgus monkeys Efficacy of antibiotics administered concomitant with raxibacumab: 781-G923701: NZW rabbits 789-G923702: Cynomolgus monkeys
Animal study endpoint is clearly related to the desired benefit in humans	Primary endpoint used was survival rate Secondary endpoint was survival time (time to death during the study period)
Pharmacokinetics and pharmacodynamics of the product in animals and humans is sufficiently well understood to allow selection of an effective dose in humans	Raxibacumab PK has been evaluated in rabbits, cynomolgus monkeys and humans. The PK has been consistent and predictable with comparable peak exposures across the species; specifically, humans administered a 40 mg/kg IV raxibacumab dose have achieved serum levels that are comparable to the concentrations that provide a survival benefit in rabbits and monkeys, with immediate high exposure, sufficient concentrations for an adequately prolonged duration.
All studies subject to this rule must be conducted in accordance with preexisting requirements under the GLP regulations and the Animal Welfare Act	The pivotal and confirmatory efficacy studies and the raxibacumab/antibiotic combination efficacy studies were conducted in accordance with GLP and the Animal Welfare Act. Human clinical trials for safety data were performed in compliance with GCP and ICH guidelines.

Approval under the Animal Rule is also subject to three requirements:

1. <u>Postmarketing studies</u>

Postmarketing studies, such as field studies, must be conducted when such studies are feasible and ethical to verify and describe the biological product's clinical benefit and to assess its safety when used as indicated.

2. Approval with restrictions to ensure safe use
If the FDA concludes that a biological product shown to be effective under this subpart can be safely used only if distribution or use is restricted, postmarketing restrictions will be required

3. Information to be provided to patient recipients
Applicants must provide labeling for patient recipients which explains that the biological product's approval was based on efficacy studies conducted in animals only due to ethical or feasibility reasons. The biological product's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, anticipated benefits, adverse reactions, drug interactions, and any other relevant information required by FDA at the time of approval must also be provided.

2.2 Tables of Currently Available Treatments for Proposed Indications

Raxibacumab is a first-in-class agent, with no other approved antibody therapies available for the proposed indication of treatment of inhalational anthrax. However, antibiotics approved for the treatment of anthrax disease include the tetracyclines (doxycycline; tetracycline and minocycline are recommended in situations where penicillin is contraindicated) and penicillin G procaine. These were approved based on *in vitro* activity against *B. anthracis*.

The fluoroquinolones ciprofloxacin and levofloxacin have been approved for post-exposure prophylaxis of inhalational anthrax based on animal pharmacokinetic data that were likely to predict clinical benefit in humans. These approvals antedated the Animal Rule and were based on subpart H regulations (8).

Table: Approved drugs and doses for *B. anthracis* exposure in adults – Treatment and post-exposure prophylaxis

Drug	Dose	Duration	Notes
Treatment of anthrax			
Doxycycline (doxycycline calcium, doxycycline hyclate)	100 mg po q12h 100 mg IV q12h	60 days 60 days	Members of the tetracycline class are generally not indicated for the treatment of patients under 8 years of age (tooth discoloration, enamel hypoplasia, delays in bone development with

8 21 CFR 314.510 (Subpart H – Accelerated Approval of New Drugs for Life-Threatening Illnesses)

			prolonged use)
Penicillin G procaine	1.2 million units IV q12h	60 days	
Post-exposure prophylaxis of anthrax			
Ciprofloxacin	10 mg/kg IV (maximum 400 mg/dose) q12h 15 mg/kg po (maximum 500 mg/dose) q12 hours	60 days	Approved for post-exposure inhalational anthrax based on pharmacokinetic data (human exposures matched exposures that proved efficacious as post-exposure prophylaxis in rhesus monkeys exposed to inhalational anthrax)
Levofloxacin	500 mg po QD	60 days	Approved for post-exposure inhalational anthrax based on pharmacokinetic data (human exposures matched exposures that proved efficacious as post-exposure prophylaxis in rhesus monkeys exposed to inhalational anthrax)

2.3 Availability of Proposed Active Ingredient in the United States

Human Genome Sciences (HGS), located in Rockville, MD, USA is responsible for the manufacture of raxibacumab.

2.4 Important Safety Issues With Consideration to Related Drugs

N/A, as raxibacumab is a first-in-class agent. There are no class effects to consider.

2.5 Summary of Presubmission Regulatory Activity Related to Submission

The following table summarizes the key presubmission regulatory activities and actions.

Date	Type of correspondence	Action
28 August 2002	Meeting request and package	Pre-IND submitted for raxibacumab
22 May 2003	Applicant submission	IND filing
15 August 2003	Fast track designation letter	Fast track development program for the investigation of PA mAB for post exposure prophylaxis and adjunctive therapy of inhalational anthrax disease designated
12 November	Orphan drug	Request for orphan drug designation granted

2003	designation letter	
12 July 2006	Type B (pre-Phase 3) meeting minutes	Agreed on development plans for raxibacumab; agreed on therapeutic treatment indication
27 September 2006	Follow-up Type B meeting minutes	Confirmed immunogenicity study with wash-out period was required in humans
17 November 2006	FDA written correspondence	Defined scope of animal efficacy studies needed to support licensure (2 animal models required; demonstration that the monoclonal is superior to placebo; compare the efficacy of the monoclonal with antimicrobial to the antimicrobial alone
11 December 2006	Type A meeting minutes	Discussion of design for rabbit efficacy studies
6 February 2007	FDA written correspondence	Confirmed embryo-fetal toxicity study was required
3 December 2007	FDA written correspondence	Confirmed scope of studies required for licensure (Efficacy – require 2 models of monoclonal with antimicrobial; Safety – established that PAM-NH-01 study cannot be counted towards the 300 subjects safety database because its safety profile (M10) is different from the safety profile of the M11 product.
21 October 2008	Type B (pre-BLA) meeting minutes	Reviewed data to support submission of a BLA (FDA agreed that therapeutic efficacy studies reviewed to date were sufficient to file the BLA; discussed presentation of safety data, namely separate presentation of the M10 and M11 data, separate presentation of single dose (n=326) and double dose with and without washout also separately presented)
30 January 2009	FDA written correspondence	Confirmed raxibacumab efficacy, safety and product quality data provided by HGS were sufficient to support the use of a single 40 mg/kg IV dose for the treatment of inhalational anthrax under IND 11,069 ("safe to proceed" issued by FDA on Jan 23, 2009)

2.6 Other Relevant Background Information

Recommendation for Stockpiling

In October 2005, an End of Phase 1 meeting was held between the FDA and HGS to discuss the issues related to the licensure and use of raxibacumab under Emergency Use Authorization (EUA) for the post-exposure treatment of anthrax. During this meeting, the Agency indicated that unmet medical need exists for the treatment of anthrax rather than postexposure prophylaxis and expressed the need for studies in healthy volunteers and animal efficacy studies.

In December 2007, agreement was reached on the data necessary to support the Centers for Disease Control and Prevention (CDC) IND submission to use raxibacumab in the Strategic National Stockpile (SNS), and subsequent additional studies needed in the BLA submission to support licensure.

In January 2009, DSPTP determined that a single dose of raxibacumab 40 mg/kg IV was acceptable for use under HGS' and the CDC's IND for the treatment of inhalational anthrax. This decision was based on the review of various preclinical studies (including pivotal efficacy studies in animals as required under the Animal Rule), human safety and PK studies, acceptable product quality assessments, and the results of the Division of Scientific Investigation (DSI) and Biotechnology Manufacturing Team's inspections.

3 Ethics and Good Clinical Practices

3.1 Submission Quality and Integrity

The Division of Scientific Investigations (DSI) was involved in site inspections of Human Genome Sciences, as well as two sites of the clinical investigators who enrolled the majority of subjects in safety studies HGS1021-C1063 and HGS1021-C1064. The studies appeared to have been conducted adequately, and therefore the data generated appeared acceptable to support the proposed indication with the exception of three findings:

There were two subjects with adverse events reported to the sponsor which do not appear in the data listings submitted in the NDA.

- In Study HGS1021-C1063, Subject US002-000003 had the adverse event of eczematous dermatitis (start date 3/25/08 and end date of 4/7/08) documented in the case report form, and this AE was reported to the Applicant. However, this AE was not in the data listings submitted by the Applicant to the BLA.
- In Study HGS1021-C1064, Subject US 003-000056 had an adverse event of "regularly irregular rhythm, probably frequent premature beats or sinus arrhythmia" from 6/18/07 to 8/11/07 recorded on the CRF, but this adverse event was not included in the Applicant's data listings.

Lastly, a refrigerator containing all vials of raxibacumab dropped in temperature below the specified 2-8°C on November 24 - 25, 2007 with the lowest temperature recorded being 1.8°C. All vials were transferred to another refrigerator on November 25.

Please refer to the DSI consult in DFS for complete details.

3.2 Compliance with Good Clinical Practices

All animal pivotal and the raxibacumab/antibiotic combination efficacy studies were conducted in accordance with GLP and the Animal Welfare Act. Human clinical trials for safety data were performed in compliance with GCP and ICH guidelines.

3.3 Financial Disclosures

The Applicant obtained certification from each investigator and sub-investigator who enrolled subjects in the clinical (human) trials, as well as the pivotal nonclinical efficacy studies in rabbits and monkeys. No investigator or sub-investigator had any conflict of interest information to disclose.

4 Significant Efficacy/Safety Issues Related to Other Review Disciplines

4.1 Chemistry Manufacturing and Controls

Please refer to the final chemistry manufacturing and control review by Dr. David Frucht, M.D., PhD. for complete details.

4.2 Clinical Microbiology

Please refer to the final microbiology reviews by Drs. Maureen Davidson, PhD and Lynette Berkeley, PhD for complete details. A summary of their reviews follows:

Dr. Davidson's summary:
The applicant has provided *in vitro* studies that indicate the mechanism of action appears to be neutralization of free protective antigen (PA) and that raxibacumab binds PA in supernatants from Sterne, Ames and Vollum strains of *Bacillus anthracis.* Raxibacumab does not bind recombinant PA (rPA) after it has bound to mammalian cellular receptors. *In vitro* studies used rPA, recombinant lethal factor (rLF), and recombinant edema factor (rEF) to create complete lethal toxin (LT) and edema toxin (ET). These studies indicated that raxibacumab inhibited killing of murine macrophage (J774-A.1) cells when rPA was pre-incubated with raxibacumab prior to addition of rLF.

In an epithelial cell line (CHO-K1 cells), raxibacumab blocked cAMP induction by rET when PA was pre-incubated with raxibacumab prior to addition of rEF.

In proof of concept studies in rats given a slow infusion of the LD_{50} dose of LT over a 24 hour period, the timing and dose of raxibacumab was evaluated in seven day survival studies. The dose of raxibacumab was based on the concentration of rPA used to make LT. In one study, the rats were treated with ten times the molar concentration of PA at either three, six, nine or twelve hours after initiation of LT infusions. Treatment with raxibacumab up to six hours after LT infusion initiation significantly increased the number of rats surviving for seven days (p=0.001). For the rats receiving the placebo treatment, the overall survival rate (mean ± SE) on day 7 was 60 % ± 2 % and the median time to death was 16 hours (range 8 to 152 hours). In the experiment that examined the effect of different doses of raxibacumab on seven day survival, rats that received raxibacumab at either 10X, 1X, 0.5X or 0.05X the molar concentration of rPA at six hours after the beginning of the LT infusion had ≥95 % survival rates at seven days. However, the number of rats surviving in the placebo group (35/48; 73 %) was much higher than the expected 50% survival rate, so comparisons among raxibacumab treatment groups should be interpreted with caution.

In rabbits infected with the $100LD_{50}$ dose of *B. anthracis* Ames strain spores given by aerosol exposure, the activity of different doses of intravenous raxibacumab was examined different times post infection (PI). All rabbits (24/24) treated with raxibacumab within 12 hours PI survived for 42 days while 11/12 placebo treated rabbits died within four days PI. About half the rabbits treated with raxibacumab at 24 and 36 hours PI survived, suggesting that the treatment window for optimal treatment efficacy was less than 24 hours PI. In the next experiment, raxibacumab doses of 5, 10, 20, or 40 mg/kg, given intravenously at 24 hours PI, were examined. An additional treatment group was given 20 mg/kg raxibacumab at 36 hours PI. The primary efficacy endpoint was survival at Day 14 PI. The only significant improvement in survival was seen in the 20 mg/kg raxibacumab treatment group (5/12 (42 %; p= 0.04) treated at 24 hours PI as compared to the placebo treated rabbits (0/12 (0 %). All (12/12) rabbits treated with the 20 mg/kg dose of raxibacumab at 36 hours PI died. The reason for the lower survival rate in the 40 mg/kg raxibacumab treatment group in this experiment (4/12; 33 %) as compared to that seen with the same treatment regimen in the previous study (6/12; 50%) is unknown. Likewise, the reasons for a lower survival rate in the rabbits that received 40 mg/kg of raxibacumab (4/12; 33 %) vs. the survival rate in the 20 mg/kg raxibacumab treatment group (5/12; 42 %) are unknown, but may be related to the inherent variability in this experimental model.

Efficacy studies evaluating monotherapy with raxibacumab and studies in which treatment with raxibacumab was combined with an antimicrobial were performed in both rabbits and monkeys. In all of the applicant's studies, lack of consistency in methodology and variability in the models confounds interpretation of the data. The experimental assays used throughout the studies were found to be non-specific and/or

highly variable for the parameters of interest. Of particular interest was the ECL based screening assay for detection of PA in serum or plasma that was used as a trigger for treatment. These assays had major quality control problems that rendered them unsuitable for use as a trigger for treatment. Therefore, cure rates are based on culture positive findings and not PA positive results at the time treatment was initiated. In this review, efficacy analyses were evaluated using only those animals that had positive blood cultures at, or before, the time of treatment. The applicant's studies indicated that treatment with the recommended 40 mg/kg dose of raxibacumab, in the absence of concomitant antimicrobial treatment, resulted in 35% survival in rabbits and 69% in cynomolgus macaques. These survival rates were significantly better than the survival rates for the placebo treatment groups. However, statistical improvement in survival in the rabbit study was weak and depended on the assignment of one rabbit to a particular treatment group. Treatment of rabbits and monkeys with either levofloxacin or ciprofloxacin, for only three days and without supportive therapy, resulted in >95% survival in either species, which is much higher than the 45% survival rate in humans during the 2001 anthrax attacks in the USA. Thus, it is possible that these animal models overestimate the survival benefit of raxibacumab when administered after bacteremia is established.

The largest problems with the animal model studies were in the necropsy and pathology data. Systematic, detailed, comprehensive, necropsies and histopathological evaluation of all tissues were not done in any study. There was little detail in descriptions of gross and microscopic lesions. It is unclear, both within an individual study and between studies, if all tissues were evaluated on every animal. The protocols for some studies specifically state that tissues were examined for microscopic lesions at the discretion of the pathologist. At the request of the Agency, the applicant performed microscopic assessment of lesions from stored tissues in some studies, but because not all tissues were collected, results were not available for all tissues from every animal. The lack of detail in pathology assessments was of most concern in the brains because there were more lesions, and the lesions are more severe, in the brains of rabbits and monkeys that received monotherapy with raxibacumab than in placebo treated animals. Surviving monkeys were never necropsied and surviving rabbits were necropsied only in the antimicrobial + raxibacumab combination study. There was not a raxibacumab alone treatment arm in either of the antimicrobial treatment combination studies, which makes direct comparison among treatment groups difficult because comparison to the raxibacumab only treatment group must be done with historical controls from the other efficacy studies. In the rabbit combination study in which survivors were necropsied, there were few lesions of any kind, and none in brains, in the animals that were treated with levofloxacin or raxibacumab + levofloxacin. In this study, all placebo treated rabbits had microscopic lesions in the brain that were consistent with anthrax. The reason for higher incidence of microscopic lesions in the placebo treatment group in this study as compared to the placebo treated rabbits in the monotherapy efficacy study, and compared to the published data for rabbits is unknown. In the monkey combination treatment study, monkeys that survived inhalational anthrax were not necropsied so no

data regarding brain lesions in surviving animals were available. Finally, the region(s) of the brain affected, and the extent of the lesions within each region, cannot be determined. Taken together, the lack of information regarding the lesions seen in the brains of rabbits and monkeys that received raxibacumab without antimicrobial treatment makes interpretation of these studies impossible. The biological basis, and the clinical significance, of the brain lesions is unknown. Future studies must include all treatment arms in a balanced experimental design, animals that survive inhalational anthrax must be necropsied, and assessment of gross and microscopic lesions must be done in a detailed, comprehensive, systematic manner in all animals. In the brain, all regions must be carefully evaluated for gross and microscopic lesions. It will be useful to rule out whether lesions are associated with the presence of raxibacumab in the brain.

Dr. Berkeley's summary:
Screening and quantitative electrochemiluminescence (ECL) assays were designed for the purpose of detecting protective antigen (PA), a toxin produced by *Bacillus anthracis* in infected individuals and to use this as a trigger for intervention in rabbits (serum and plasma) and monkeys (serum). The principle and methodology are similar for both tests and are based on that of ELISA with rabbit generated anti-PA tagged either to biotin or a Sulfo luminescent compound. There are shortcomings in both tests. Common to both are the inadequate testing of specificity and variability. The quantitative assay had greater precision and sensitivity than the screening assay and is overall a more accurate test. Nevertheless, the presence of PA by either of these assays should not, as presently reported in this study, be used alone as a trigger for intervention in rabbits or monkeys. The efficacy results should be based on presence or absence of bacteremia alone.

While the applicant lowered the maximum acceptable level for precision for the screening test of PA in plasma samples of rabbits to 15% to improve the specificity for all concentrations of PA tested to ≤8.0%, the limit was not changed for the quantitative rabbit plasma assay. There was significant variation in the range of results. In addition, the effect of raxibacumab on PA differed between matrices of rabbit serum and plasma. In serum, the concentrations of PA increased beyond its nominal value whether raxibacumab concentration of 100 or 1000 µg/mL was added to dilutions of PA; whereas in plasma the presence of raxibacumab at the same concentrations caused a decrease in PA below its nominal value.

PCR assay shows high precision and can be used as a trigger for intervention in rabbits and monkeys infected with the Ames strain. For future studies, it is recommended that PCR assay be tested against *B. anthracis* strains other than Ames.

Toxin neutralization test, for the detection of lethal toxin or neutralizing antibodies appears to be a reasonable test. However, it is unclear whether the assay will

differentiate between raxibacumab and the anti-toxin antibodies induced *in vivo*. The neutralizing antibody titers were higher in rabbit plasma than in serum.

The ELISA for the detection of anti-PA antibodies in rabbit plasma and in monkey serum showed good precision and could be used to measure anti-PA antibody in rabbits and monkeys. However, studies evaluating the specificity were limited to testing for the presence of antibodies against CCR5.

4.3 Preclinical Pharmacology/Toxicology

Please refer to the final pharmacology/toxicology review by Dr. Terry Miller, PhD for complete details. A summary of his review follows:

The decision to support approval of raxibacumab for the treatment of *Bacillus anthracis* disease is based on the absence of any reported adverse findings in the nonclinical pharmacology/toxicology studies submitted by the sponsor for BLA 125,349 and IND 11,069. Two tissue cross-reactivity studies with raxibacumab (study nos. 1494-95 and 1M1634) showed no *ex vivo* positive staining of most donor tissues from human, monkey, and rabbit. Strong, punctuate, intracellular staining of the thyroid with raxibacumab was observed in human and monkey donor tissue in both studies, however evaluation of the findings from the repeat dose 120-day toxicity study in healthy monkeys (study no: 6962-140) showed no thyroid histopathology or abnormal levels of circulating thyroid hormones so the human implication of this finding remain unknown. Weak, inconsistent staining of several other tissues in observed in the earlier study were not observed in the later study in any species most likely due to a modified manufacturing process for antibody production (developmental process vs. M11).

Raxibacumab treatment up to 40 mg/kg on multiple days (3 doses, once every 12 days) by several routes of exposure (S.C., I.V., and I.M.) showed no test article related effects in healthy cynomolgus monkeys. (Study no: 6962-140). The data collected in the 120-day toxicity study in healthy cynomolgus monkeys support a NOAEL of 40 mg/kg, identical to the proposed clinical dose for raxibacumab. Slow intravenous injection of raxibacumab in cynomolgus monkeys resulted in a 2.5 fold greater Cmax value (< 1000 µg/mL) but only slightly greater $AUC_{(0-\infty)}$ exposure (1.2-fold) compared to an S.C. dose, with near equal $t_{1/2}$ of 12-14 days. Despite the long half life, optimal for scavenging free PA in anthrax disease, raxibacumab is not retained in the serum between dosing and induces no significant antibody neutralization with multiple dosing up to 12 days between doses. Dose-normalization of serum levels after repeat dosing showed nearly identical PK after a single dose indicating dose linearity of PA mAb serum concentration up to the high dose of 40 mg/kg. Serum concentration profiles of raxibacumab were similar in both sexes.

Similarly, intravenous treatment with PA mAb up to 120 mg/kg (3 times the clinical dose) on gestation days 7 and 14 is well tolerated by pregnant rabbits and appears to pose no significant risk for maternal health, abnormal pregnancy or drug-related teratology while used for the prevention or treatment of infection by *Bacillus anthracis.* With respect to the teratology portion of the main study, there were no deaths or significant treatment-related adverse effects observed in the fetuses from raxibacumab treated dams. The only potential treatment related effect in the teratology portion of the study was in the skeletal variation findings (unossified 5th sternebra and presence of 13th rudimentary ribs) present in the high dose group. However, these minor variations do not impact the conformity or well-being of the animal, and thus were not considered an adverse effect. Therefore, the data support the stated NOAEL of 120 mg/kg for both maternal and fetal effects. Toxicokinetic analysis showed proportionally higher concentrations for the 120 mg/kg relative to the 40 mg/kg group at all time points in both the main and TK studies. Additionally, dose normalized serum raxibacumab levels were the same across doses and groups showing linearity in response similar to findings in cynomolgus monkeys.

In both healthy cynomolgus monkeys and pregnant healthy rabbit, raxibacumab does not appear to cause a significant immunogenic response above background levels in either species. This evidence supports the PK findings of dose linearity in the absence of any significant dose neutralization in both animals in the pharmacology/toxicology studies and patients evaluated in the clinical trials (PAM-NH-01, HGS1021-C1064, HGS1021-C1069). However, in both animal studies, the assays used to determine immunogenicity appeared not to be very reliable or sensitive in the presence of high serum raxibacumab concentrations (> 150 μg/ml) and perhaps other interfering anti-PA-antibodies with high titer levels in normal, untreated animals. Based on these findings as well as highly variable individual animal data in both studies, the relative immunogenicity of raxibacumab in these animal models and the implications for the clinic remain unclear.

From a pharmacology/toxicology perspective, in the relative absence of any clear adverse findings in the submitted healthy animal studies, drug approval for raxibacumab to be used in the treatment of inhalation anthrax disease should be granted by the Agency.

4.4 Clinical Pharmacology

Please refer to the final clinical pharmacology review by Dr. Kimberly Bergman, PhD. for complete details. A summary of her review follows:

To allow selection of an effective dose in humans for therapeutic treatment of anthrax, the Applicant has submitted clinical pharmacology data for raxibacumab in humans and

in the two pivotal animal species, rabbits and cynomolgus monkeys. The pharmacokinetics (PK) and safety of raxibacumab administered as the product proposed for licensure has been evaluated in a Phase 1 dose-ranging study (PAM-NH-01), an antibiotic interaction study with ciprofloxacin (HGS1021-C1064), a repeat dose immunogenicity study (HGS1021-C1069) and a Phase 2/3 safety study (HGS1021-C1063). Based on a cross-species comparison of raxibacumab exposure and exposure-response information for raxibacumab dose/concentration, PA concentration and survival, the clinical pharmacology information provided by the Applicant is acceptable and supports the use of the 40 mg/kg dose of raxibacumab. The dose and concentration response relationship with the endpoint of survival indicates potential to achieve higher response rates at higher doses. If future trials are conducted, higher doses (e.g. 60 or 80 mg/kg) should be explored along with 40 mg/kg. In any future trials studying higher doses in animals, safety assessments should include an examination of CNS pathology.

4.4.1 Mechanism of Action

In vitro studies demonstrated that raxibacumab binds PA in supernatants from Sterne, Ames and Vollum strains of *B. anthracis*. The mechanism of action appears to be by neutralization of free PA. Studies show that PA binds the surface of human and murine macrophages, and the epitope on recombinant PA that binds the cell surface receptor appears to be the same as the one binding raxibacumab (9), thereby suggesting that some structural similarity between the antigen recognition site on raxibacumab and the cell surface receptors binding site for PA exists. Raxibacumab blocks cAMP induction by recombinant ET when PA is preincubated with raxibacumab prior to addition of EF. Similarly, raxibacumab inhibits killing of murine macrophage (J774-A.1) cells when PA is preincubated with raxibacumab prior to addition of LF.

5 Sources of Clinical Data

5.1 Tables of Studies/Clinical Trials

The following table summarizes the clinical studies reviewed to support the efficacy and safety of raxibacumab.

9 Xu L, Frucht DM. Bacillus anthracis: a multi-faceted role for anthrax lethal toxin in thwarting host immune defenses. Internat J. Biochem. Cell Biol 2007:39;20-4.

Table: Clinical studies reviewed in support of BLA 125,349

Study number	Title	Study design/ objective	Test product/ dosage/ route	Number (sex) of subjects per group	Healthy or exposed	Study duration
Efficacy Studies						
682-G005758	Evaluation of raxibacumab efficacy as therapeutic treatment against inhalational anthrax in the rabbit model	Randomized, placebo-controlled/ rabbit efficacy	Placebo or raxibacumab 20 and 40 mg/kg IV	9M, 9F	Anthrax challenged	14 days
724-G005829	Evaluation of raxibacumab efficacy as therapeutic treatment against inhalational anthrax in the cynomolgus macaque	Randomized, double-blind, placebo-controlled/ monkey efficacy	Placebo or raxibacumab 20 and 40 mg/kg IV	Placebo 12M, 12 F Treatment groups 14M, 14F	Anthrax challenged	28 days
781-G923701	Evaluating the efficacy of raxibacumab in combination with levofloxacin for post-exposure treatment in the New Zealand White Rabbit inhalational anthrax model	Randomized, double-blind, placebo-controlled/ rabbit therapeutic efficacy	Placebo (raxibacumab buffer) Levofloxacin 50 mg/mL po q12h x 6 Levofloxacin 50 mg/mL po q12h x 6 + raxibacumab 40 mg/kg	Placebo 12 M, 12 F Treatment groups 20M, 20 F	Anthrax challenged	28 days
789-G923702	Evaluation of the efficacy of raxibacumab in combination with ciprofloxacin for therapeutic treatment in the cynomolgus monkey inhalational anthrax model	Randomized, double-blind, placebo-controlled/ monkey therapeutic efficacy	Placebo (raxibacumab buffer) Ciprofloxacin 75 mg/mL po q12h x 6 Ciprofloxacin 75 mg/mL po q12h x 6 + raxibacumab 40 mg/kg	Placebo12 M, 12 F Treatment groups 14M, 14 F	Anthrax challenged	28 days
Safety Studies						
PAM-NH01*	A phase 1 single-blind, placebo-controlled, single-injection dose-escalation study to evaluate the safety and pharmacokinetics of PA mAb (human Monoclonal Antibody	Single-blind, dose-escalation, placebo-controlled study/ Phase 1 human study,	Raxibacumab or placebo single dose 0.3, 1 and 3 mg/kg IM 1 and 3 mg/kg IM	80 raxibacumab, 25 placebo	Healthy subjects	56 days

	to *B. anthracis* Protective Antigen) in healthy subjects	immunogenicity and raxibacumab PK	1, 3, 10, 20 and 40 mg/kg IV			
HGS1021-C1063	A randomized, single-blind, placebo-controlled study to evaluate the safety and tolerability of raxibacumab (human monoclonal antibody to B. anthracis protective antigen) in healthy subjects	Randomized, single-blind, placebo-controlled /human safety	Raxibacumab or placebo (raxibacumab buffer) single or double dose 40 mg/kg raxibacumab IV single dose on day 0 or double dose on days 0 and 14	165M, 164F 240 raxibacumab (217 single dose, 23 double dose) and 80 placebo (74 single dose, 6 double dose)	Healthy subjects	56 days after last dose
HGS1021-C1064	An Open-Label Study to Evaluate the Pharmacokinetics and Safety of Raxibacumab (Human Monoclonal Antibody to *B. anthracis* Protective Antigen) Administered in Combination with Ciprofloxacin in Healthy Subjects	Randomized (Groups 1 & 2), open-label/ safety and PK of raxibacumab in combination with ciprofloxacin	Raxibacumab single dose and/or ciprofloxacin for 7.5 days Group 1: Ciprofloxacin 500 mg po q12h x 15 doses on days 0-7 + raxibacumab 40 mg/kg IV on day 5 Group 2: Raxibacumab 40 mg/kg IV on day 0 Group 3: Ciprofloxacin 400 mg IV q12h x 2 doses + raxibacumab 40 mg/kg IV on day 0 and Ciprofloxacin 500 mg po q12h x 13 doses on days 1-7	43M, 45 F	Healthy subjects	56 days
HGS1021-C1069	An open-label study to evaluate the immunogenicity and safety of raxibacumab (human monoclonal antibody to B. anthracis protective antigen) administered in healthy subjects	Open-label/ safety of raxibacumab reinjection after washout	Raxibacumab single dose Raxibacumab 40 mg/kg IV (all subjects enrolled had received a single	12M, 8F	Healthy subjects	70 days

			40 mg/kg raxibacumab IV dose at least 4 months prior to receiving treatment in this study)			

* Study was reviewed but not included in the integrated database for safety analysis

5.2 Review Strategy

The approval to stockpile raxibacumab in the SNS under IND 11069 in January 2009 was based upon the review of two pivotal efficacy studies (NZW rabbit 682-G005758, NHP 724-G005829) and two safety studies (HGS-1021-C1064 and PAM-NH-01). Please refer to the following reviews in DFS for these studies.

Efficacy studies:
682-G005758 – Evaluation of raxibacumab efficacy as therapeutic treatment against inhalational anthrax in the rabbit model (Dr. Yuliya Yasinskaya)

724-G005829 – Evaluation of raxibacumab efficacy as therapeutic treatment against inhalational anthrax in the cynomolgus macaque (Dr. Susan McCune)

Safety studies:
PAM-NH-01 – Pharmacokinetic analysis of a phase 1 single-blind, placebo-controlled, single-injection dose-escalation study to evaluate the safety and pharmacokinetics of PA mAb (human Monoclonal Antibody to *B. anthracis* Protective Antigen) in healthy subjects (Dr. Eileen Navarro-Almario)

HGS1021-C1064 – An Open-Label Study to Evaluate the Pharmacokinetics and Safety of Raxibacumab (Human Monoclonal Antibody to *B. anthracis* Protective Antigen) Administered in Combination with Ciprofloxacin in Healthy Subjects (Dr. Patrick Archdeacon)

The following additional studies were reviewed for this BLA application:

Study number	Title	Efficacy or safety	Reviewer
781-G923701 – rabbit	Evaluating the efficacy of raxibacumab in combination with levofloxacin for post-exposure treatment in the New Zealand White Rabbit	Efficacy	Sue Lim

	inhalational anthrax model		
789-G923702 – NHP	Evaluation of the efficacy of raxibacumab in combination with ciprofloxacin for therapeutic treatment in the cynomolgus monkey inhalational anthrax model	Efficacy	Susan McCune
HGS1021-C1063	A randomized, single-blind, placebo-controlled study to evaluate the safety and tolerability of raxibacumab (human monoclonal antibody to *B. anthracis* protective antigen) in healthy subjects	Safety	Sue Lim
HGS1021-C1069	An open-label study to evaluate the immunogenicity and safety of raxibacumab (human monoclonal antibody to *B. anthracis* protective antigen) administered in healthy subjects	Safety	Sue Lim

The review of Study 789-G923702 by Dr. Susan McCune is attached in the Appendix of this review. A brief summary of her review can be found in Section 6.1.10 Additional Efficacy Issues/Analyses.

5.3 Discussion of Individual Studies/Clinical Trials

Individual study reports for safety Studies HGS1021-C1063 and HGS1021-C1069 can be found in the Appendix of this review.

6 Review of Efficacy

Efficacy Summary – Study 781-G923701

Study 781-G923701 was a double-blind, placebo-controlled GLP efficacy study which examined the efficacy of 50 mg/kg qd x 3 IG administration of levofloxacin as therapeutic treatment in rabbits with symptomatic inhalational anthrax when administered in combination with a single dose of 40 mg/kg IV raxibacumab.

The study consisted of three treatment arms: placebo, levofloxacin alone, and levofloxacin/raxibacumab. All 3 groups were comparable with regard to anthrax exposure, treatment trigger, and number of animals with detectable PA. Time to PA detection (screening and quantitative), time to bacteremia, and time to treatment were also comparable among survivors and non-survivors across groups. One survivor in the levofloxacin group (K99234) tested borderline (just above LLOQ) positive for anti-PA antibodies at baseline. All animals were toxin neutralization assay (TNA) negative at baseline.

The primary efficacy endpoint of survival at Day 28 relative to placebo was met, with statistically higher 28-day survival in the FDA Primary population for both the levofloxacin group (FDA Primary 19/20, 95.0%, p<0.0001) and levofloxacin/raxibacumab group (16/17, 94.1%, p<0.0001) relative to placebo (0%). Rabbit K99251 was randomized to the levofloxacin/raxibacumab group but was not dosed with raxibacumab, and was assigned to the levofloxacin group in the As treated population analysis, while the 1 non-survivor in the levofloxacin/raxibacumab group (K99246) died as the result of a gavage accident (non-anthrax death analysis). Sensitivity analyses of the "As treated" and "As treated excluding non-anthrax death" populations did not alter the statistically significant survival benefit observed with both active treatment groups.

With respect to the secondary efficacy endpoint, survival time was significantly longer in the levofloxacin group (FDA Primary p<0.0001) and the levofloxacin/raxibacumab group (p<0.0001) relative to the placebo group. The difference in survival times between the 2 active treatment groups was not statistically significant (p = 0.986 as determined by log-rank test). The median survival time in the placebo group was 78.5 hours. Median survival time could not be determined for the levofloxacin and levofloxacin/raxibacumab groups because the medians extended beyond 28 days. Again, in the "As treated" and "As treated excluding non-anthrax death" populations, the statistically significantly longer survival time in the levofloxacin and the levofloxacin/raxibacumab groups relative to the placebo group were maintained.

All survivors tested positive for anti-PA antibodies at Day 28. All survivors tested positive for TNA at Day 28, with the exception of 1 survivor in the levofloxacin/raxibacumab group (K99224) which tested negative for TNA at baseline and at Day 28. This animal did not test positive for PA toxemia or bacteremia during the study; treatment, however, was triggered by body temperature increase, and the animal was positive for anti-PA antibodies at Day 28. The reason for these discrepant findings is likely related to the poor specificity of the anti-PA assay as discussed in Section 4.2 Clinical Microbiology and in the complete microbiology reviews by Drs. Maureen Davidson and Lynette Berkeley.

Clinical Observations

Rabbits in this study generally experienced outward clinical signs consistent with inhalational anthrax, and those that succumbed to disease followed the hallmark progression pattern characteristic of inhalational anthrax. Hematology parameters were consistent with systemic infection, with additional evidence of anthrax lesions upon necropsy and histopathology evaluation in those that died of anthrax. The vast majority (86%) of rabbits found dead or that were euthanized were positive for bacteremia on terminal blood culture.

Necropsy and Histopathology

All 52 animals, survivors and non-survivors, had their tissues examined microscopically. Gross lesions found in rabbits which died prior to study termination included enlargement, discoloration and/or foci of the adrenal glands, appendix, brain, lung and multiple lymph nodes; fluid (effusion) in the pericardial and thoracic cavities, and fluid/thickening (edema) of the skin and thymus. These correlated histologically with necrosis, inflammation, hemorrhage, edema and the visualization of anthrax bacteria. In contrast, gross lesions found in rabbits surviving to study termination were determined to be unrelated to anthrax infection.

Microscopic findings consistent with anthrax were present in all rabbits dying spontaneously or becoming moribund following anthrax challenge except for animal K99246 (levofloxacin/raxibacumab group), which was not bacteremic at death, and died due to gavage error. In contrast to the placebo animals which died, all of which had evidence of CNS involvement, the one animal in the levofloxacin (K99203) and one animal in the levofloxacin/raxibacumab (K99246) groups that died had no CNS involvement on either gross or microscopic examinations.

6.1 Indication

(b) (4)

Under the Animal Rule, the use of animal efficacy data was used to support the licensure of raxibacumab for therapeutic treatment of inhalational anthrax. Demonstration of effectiveness in more than 1 animal species expected to react with a response predictive for humans and use of an endpoint that is clearly related to the desired benefit in humans.

The pivotal animal efficacy studies, 682-G005758 (NZW rabbit) and 724-G005829 (NHP) have been reviewed. Because raxibacumab would likely be administered in combination with antibiotics in the event of an acute exposure to anthrax, the Applicant was asked to conduct studies 781-G923701 (NZW rabbit) and 789-G923702 (NHP) to confirm that raxibacumab administration does not interfere with the efficacy of levofloxacin in rabbits or ciprofloxacin in monkeys with symptomatic inhalational anthrax. These supportive efficacy studies are reviewed in this BLA to complete the efficacy review of raxibacumab. This following efficacy review is of Study 781-G923701 (NZW rabbit). For the review of Study 789-G923702 (NHP), please refer to the review by Dr. Susan McCune in the Appendix of this review

In a rabbit model characterization study (Report 615-N104504), the onset of abnormal values in physiological parameters (e.g., temperature, bacteremia, serum PA, and hematology) and clinical signs to characterize disease progression post-anthrax-challenge were evaluated. Based on this study, it was concluded that first body temperature increase or detectable serum PA were appropriate trigger symptoms for therapeutic intervention in the rabbit model, and were thus used as therapeutic trigger symptoms in the current study. For details of the model characterization studies in NZW rabbits and cynomolgus monkeys, please refer to microbiology review by Dr. Maureen Davidson.

Study 781-G923701: Evaluating the efficacy of raxibacumab in combination with levofloxacin for post-exposure treatment in the New Zealand White Rabbit inhalational anthrax model

6.1.1 Methods

Objectives:
To evaluate the efficacy of raxibacumab when administered in combination with levofloxacin as a therapeutic treatment against lethality due to inhalation exposure to *B. anthracis* in New Zealand White (NZW) rabbits.

To determine the pharmacokinetics (PK) of intragastric (IG) levofloxacin doses and of an intravenous (IV) raxibacumab dose when co-administered, as well as the kinetics of *B. anthracis* protective antigen (PA), in rabbits with inhalational anthrax.

Study Design:
This was a parallel-group, randomized, double-blind, placebo-controlled GLP study to evaluate the efficacy of raxibacumab in combination with levofloxacin for therapeutic treatment in the NZW rabbit inhalational anthrax model. PA kinetics and raxibacumab and levofloxacin PK were also examined.

Location:
This study was performed at Battelle Memorial Institute, BBRC, located at State Route 142, West Jefferson, OH 43162. The Study Director at BBRC was Kristopher Van Zandt, PhD. The Sponsor representative for this study at HGS was Thi-Sau Migone, PhD.

Methods:
Sixty New Zealand White rabbits (*Oryctolagus cuniculus*) (b) (4) weighing 2.97 to 4.02 kg at randomization were obtained for the study. Fifty-two rabbits were randomized into 1 group of 12 rabbits and 2 groups of 20 rabbits (each group approximately 50% male, 50% female) and assigned to 1 of 3 aerosol challenge days and a challenge order. The 8 remaining rabbits were to serve as replacements.

Rabbits were to be aerosol challenged on Day 0 with a targeted 200x the median lethal dose (LD50) (1.24 x 10^7 spores) of *B. anthracis* (Ames strain) spores. Treatment was administered based on a positive plasma PA result via ECL screening assay or 2 consecutive time points of a body temperature ≥2°F higher than the baseline average temperature, whichever was noted first. Beyond 36 hours post challenge until 72 hours post challenge, only temperature was used as a trigger for treatment. If an animal had not been treated by 72 hours post challenge (negative for PA and did not meet the temperature criteria), the animal was to be treated after its last hourly temperature assessment.

The following 3 treatment groups were evaluated:

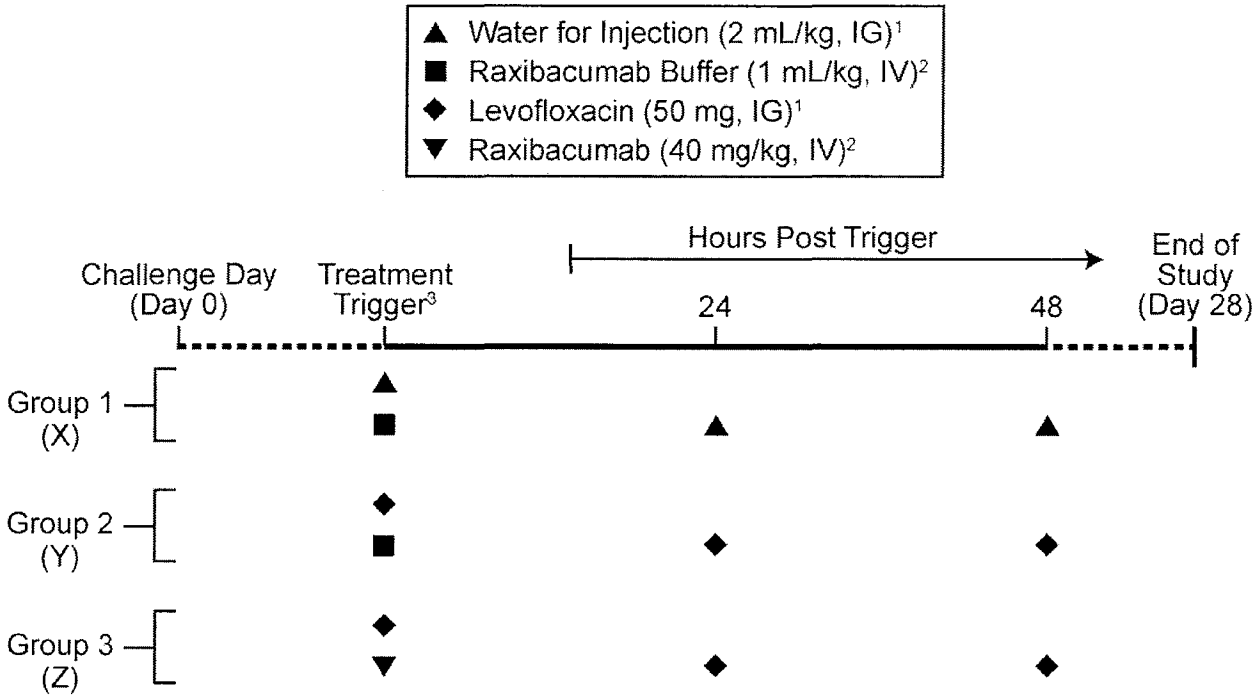
- Group 1 (X): Individual rabbits received sterile WFI (2 mL/kg) IG upon trigger symptom detection, immediately followed by a single IV dose of raxibacumab buffer (1 mL/kg), and then 1 dose of sterile WFI (2 mL/kg) at 24 and 48 hours after trigger symptom detection.

- Group 2 (Y): Individual rabbits received levofloxacin (50 mg/kg) IG upon trigger symptom detection, immediately followed by a single IV dose of raxibacumab buffer (1 mL/kg), and then 1 dose of levofloxacin (50 mg/kg) at 24 and 48 hours after trigger symptom detection.

- Group 3 (Z): Individual rabbits received levofloxacin (50 mg/kg) IG upon trigger symptom detection, immediately followed by a single IV dose of raxibacumab (40 mg/kg), and then 1 dose of levofloxacin (50 mg/kg) at 24 and 48 hours after trigger symptom detection.

Treatment was to be administered based on either the first positive PA result (via ECL screening assay), or the first 2 consecutive time points of a body temperature ≥2°F higher than the baseline average, whichever occurred first. After 36 hours, only temperature was to be used as a trigger for intervention. The study schema is shown in the Figure below.

Figure: Study Schema (from Applicant's submission)



[1]Administered upon detection of trigger symptoms
[2]Administered immediately following the 1st dose of WFI or levofloxacin
[3]Trigger for treatment intervention was either the 1st positive PA result (via ECL assay), or the first 2 consecutive timepoints of a body temperature ≥ 2°F higher than the baseline average (whichever occurred first); after 36 hours post-challenge, only body temperature was to be used as the trigger for treatment

HGS# 000-7092

Clinical Observations

Animals were to be monitored by laboratory animal personnel for abnormal clinical signs (such as respiratory distress, inappetence, inactivity, seizures, and moribundity) for 28 days post challenge. Monitoring was to occur at the same time (within 1 hour) as each post challenge, pre-treatment blood sample. After a rabbit received its treatment, or after 36 hours post challenge if not treated, monitoring was to occur 3 times per day, at least 4 hours apart, through Day 6 post challenge; on the day of treatment, rabbits treated prior to 10 AM, between 10 AM and 2 PM, and after 2 PM were to have 3, 2, and 1 clinical observations, respectively. On Day 7 and after, monitoring was to be conducted twice daily (near the beginning and end of each workday). All pre-challenge monitoring was to be conducted twice daily. Should any animal have been documented as having respiratory distress or as being unresponsive, more frequent observations

may have been performed. Should additional evaluations have been necessary, the resulting observations were to be documented at the time of observation.

Necropsy and histopathology
Complete gross necropsies were to be conducted on all rabbits found dead or that were euthanized to evaluate death or illness due to *B. anthracis* infection. Complete gross necropsies were also to be conducted on all surviving rabbits at the end of the in-life phase of the study. Histopathology was to be conducted on gross lesions, brain/meninges, heart, lungs, spleen, liver, kidney, and if possible, mediastinal and bronchial lymph nodes. Representative samples of spleen, liver, and if possible, mediastinal or bronchial lymph nodes were to be collected during gross necropsy from all animals that died early.

In rabbits, fulminant anthrax disease (i.e. no protracted illness) has been observed regardless of the spore dose, with clinical disease not generally apparent until within 24 hours of death. The following criteria were pre-established for euthanasia: presence of any seizure (denoting meningitis or encephalitis), respiratory distress, dyspnea, or forced abdominal respirations, unresponsiveness to touch or external stimuli, and moribundity. Rabbits that were judged to be moribund by a highly trained life sciences technician, Battelle veterinarian, or by the study director were to be euthanized immediately.

Blood work
Blood samples were collected for hematology and C-reactive protein (CRP) analysis, as well as to measure bacteremia by culture, plasma PA levels, anti-PA antibody and toxin neutralization activity (TNA) levels, plasma raxibacumab levels and levofloxacin levels. The study director and all personnel performing the dosing and assessments of the rabbits were blinded to the treatment.

Bacteremia
To verify the presence of bacteremia in the bloodstream of study animals, *B. anthracis* was to be cultured from blood samples obtained on Day -7, at 16 hours post challenge followed by every 4 hours up to the time of treatment (or up to 36 hours post challenge), immediately prior to treatment, at 24 hours post treatment (immediately prior to the 2nd levofloxacin/sterile WFI treatment), at 48 (immediately prior to the 3rd levofloxacin/sterile WFI treatment), 72, and 96 hours post treatment, and at 7, 14, 21, and 28 days post challenge. If possible, terminal blood samples were also to be cultured for *B. anthracis*. Qualitative bacteremia assessments were also to be performed on sections of spleen, liver, and if possible mediastinal or bronchial lymph nodes collected during gross necropsies.

In addition, samples for quantitative bacteremia analysis were to be obtained immediately prior to treatment, at 24 hours post treatment (immediately prior to the 2nd

levofloxacin/sterile WFI treatment, and at 48 (immediately prior to the 3rd levofloxacin/sterile WFI treatment) and 72 hours post treatment.

Temperature and body weight
Body temperatures were to be measured twice daily except from approximately 16 to 72 hours post challenge, when temperatures were to be measured once every hour. The hourly monitoring period for all rabbits was to commence relative to the challenge end time of the 1st rabbit challenged (± 10 minutes), and continue every hour through 72 hours post challenge end time for the last rabbit. Relative to this start time every hour, the temperature of all rabbits was to be measured in cage order. Body temperatures were to be recorded via two implantable programmable temperature transponders (IPTT-300, BMDS, Seaford, DE). Recording of baseline body temperature from both transponders was to begin on or before the morning of Day -3 and continue until the morning of Day 0 (pre-challenge). A baseline average temperature was to be calculated from pre-challenge measurements for each rabbit. Each rabbit's critical temperature was to be calculated. The critical temperature was to be the first 2 consecutive time points of a body temperature 2 or more °F higher than the baseline average temperature.

Post challenge body temperatures were to be monitored from a single transponder; the 2nd transponder location was only to be monitored if the 1st transponder failed (i.e., temperature could not be read by the telemetry device when chip was scanned). The transponder location selected for post challenge temperature readings was to be the one that provided the least variable baseline readings pre-challenge that were consistent with historic normal temperatures (recorded using this system) for healthy rabbits housed at the BBRC. If a transponder failed, either a) the remaining functioning transponder was to be used for all further measurements; b) a replacement transponder was to be implanted; or c) temperature data collection was to cease for that animal, at the study director's discretion. Although cage doors had to be opened for body temperature readings, rabbits were not to be removed from cages for temperature readings unless the transponder(s) could not be read otherwise.

Main Criteria for Inclusion: Following a quarantine period of a minimum of 7 days, specific pathogen-free (SPF) New Zealand White rabbits that were in good health, free of malformations, and exhibiting no signs of clinical disease, as evaluated by a veterinarian at quarantine release, were used on study. Age was not used as a criterion for placement on study.

The animals were screened for plasma PA and anti-PA antibodies prior to selection for the study.

Study drugs
Raxibacumab was supplied by HGS, with appropriate storage between 2-8°C and protected from light during dosage periods. Raxibacumab lot 71044 was used

(certificate of analysis and stability data provided). The following table summarizes the lot numbers used for all study agents in this protocol.

Table: Study agent and placebo lot identification (from Applicant's submission)

Treatment	Groups	Concentration (mg/mL)	Lot Number	Date of Manufacture	Date of Expiration	Manufacturer
Raxibacumab	3	50	71044	13OCT06	OCT08	HGS
Raxibacumab control[1]	1,2	NA	71043	04OCT06	OCT09	
Levofloxacin	2,3	25	7DB4J00	-[3]	MAR09	(b) (4)
Levofloxacin control[2]	1	NA	C727909	-[3]	DEC08	(b) (4)

[1] Raxibacumab buffer administered IV at 1 mL/kg.
[2] Sterile WFI administered IG at 7.5 mL as a control for levofloxacin (see Section 5.3.11).
[3] Not available at this time.

Statistical Methods/Efficacy:
The primary efficacy endpoint was to compare the percent of animals alive at study Day 28 post challenge between the placebo group and the levofloxacin/raxibacumab group in the FDA primary population (As treated, bacteremic animals). As a secondary analysis of the primary efficacy endpoint, the point estimate for the difference in the survival at Day 28 between the levofloxacin group and the levofloxacin/raxibacumab combination group was presented with a 95% confidence interval (CI).

The secondary efficacy endpoint was survival time, defined as the time from the beginning of spore challenge to death during the 28-day post challenge period.

The Analysis Populations were as follows:

- *Applicant's ITT*: all animals that were randomized and challenged with *B. anthracis* spores in the study. The ITT analysis was based on the intended treatment group planned at randomization rather than the actual treatment received.

- *FDA Primary*: all animals that had bacteremia (defined as positive blood culture) at treatment initiation. This analysis was based on the actual treatment received (As-treated).

The Applicant's primary efficacy endpoint in this study was survival at Day 28 in the ITT population. The FDA used 28 day survival rate for animals that were bacteremic at the time of intervention, because the PA assay used as diagnostic of disease and trigger for intervention was not considered reliable. There was high variability of the assay in the same animal, and the assay was also operator dependant. For these reasons, the FDA examined the FDA Primary population, which was defined as animals that were

bacteremic at the time of therapy and based on the actual treatment received (As treated).

Study Amendments
The original protocol is dated 22 May 2008. Six amendments to the protocol were issued as described below. Amendments 01 and 02 were issued prior to challenge and Amendment 03 was issued after challenge. Amendments 04 to 06 were issued after the end of the study. All amendments consisted of text edits and minor changes to study procedures that did not negatively impact the study.

Amendment 01 (02 June 2008)
• Per FDA request, quantitative bacteremia assessments were added to the study schedule.
• Text was added to Section 6.9, page 11 to specify the inclusion of quantitative bacteremia assessments in the study, and to outline the methods and rationale. Section 6.12, page 12 was altered to specify that blood collected for anti-PA antibody and TNA analyses was to be processed to plasma prior to shipment to the sponsor. HGS decided to use plasma for consistency with the other samples collected during the study.
• Wording in Section 6.1, page 4, was altered to specify that the 8 additional replacement rabbits were not to be randomized.
• Wording in Section 3, page 4, was altered to specify that plasma samples rather than serum samples were to be collected and shipped to HGS.

Amendment 02 (06 June 2008)
To accommodate the extensive number of blood samples required for the study, the total volume of each blood sample diluted in PBS for the purpose of quantitative bacteremia assessment was reduced from 500 μL in 4.5 mL to 100 μL in 900 μL.

Amendment 03 (17 June 2008)
The number of diluted blood samples to be plated for bacteremia assessment was reduced. In addition, whole blood cultures (undiluted) were added to the schedule to increase the likelihood that countable bacterial growth would be observed.

Amendment 04 (16 September 2008)
The name of the Sponsor Representative was corrected on the cover page.

Amendment 05 (20 October 2008)
Anti-raxibacumab immunogenicity assessments were added to the study schedule to allow for determination of the impact of any observed anti-raxibacumab immunogenicity on plasma raxibacumab levels and survival.

Amendment 06 (02 December 2008)
A sentence was added to Section 3.0 to specify that the PK and kinetic analyses were

performed by First-Stop Consulting, LLC.

Changes in Planned Analysis
The following additional analyses (not pre-specified in the analytical plan) were performed:
• Analysis of survival at Day 28 in the as-treated population, excluding rabbit K99246 (levofloxacin/raxibacumab group), which died as the result of gavage error (anthrax death population)
• Survival time in the As-treated population
• Association between survival at Day 28 with treatment trigger, anti-PA antibody status at baseline, TNA status at baseline, and time to treatment
• Survival at Day 28 by anti-raxibacumab antibody status

6.1.2 Demographics

Sixty New Zealand White rabbits (*Oryctolagus cuniculus*) (b) (4) were obtained for the study. Fifty-two rabbits were randomized into three treatment groups of 1 group of 12 rabbits (placebo) and 2 groups of 20 rabbits (group 2 – levofloxacin; group 3 – levofloxacin/raxibacumab). Each group had approximately 50% male and 50% female animals weighing between 2.97 and 4.02 kg. Groups were assigned to 1 of 3 aerosol challenge days and a challenge order. The 8 remaining rabbits were to serve as replacements.

As shown in the table below, the treatment groups were comparable with regard to sex, weight, and age at randomization. One rabbit (K99234) in the levofloxacin group tested positive for anti-PA antibodies at baseline; the levels were just above the LLOD. All rabbits in the levofloxacin/raxibacumab group tested negative for anti-PA antibodies at baseline. All rabbits in the active treatment groups tested negative for toxin neutralization activity (TNA) at baseline.

Table: Summary of demographics and baseline characteristics in the ITT population (from Applicant's submission)

	Placebo N = 12	Levofloxacin N = 20	Levofloxacin/ Raxibacumab N = 20	All Active N = 40	All Groups N = 52	P Value[1]
Sex	-	-	-	-	-	1.0000
Male	6 (50.0%)	10 (50.0%)	10 (50.0%)	20 (50.0%)	26 (50.0%)	-
Female	6 (50.0%)	10 (50.0%)	10 (50.0%)	20 (50.0%)	26 (50.0%)	-
Prechallenge weight (kg)	-	-	-	-	-	0.5937
Mean ± SD	3.5 ± 0.2	3.4 ± 0.2	3.5 ± 0.3	3.5 ± 0.2	3.5 ± 0.2	-
Median	3.5	3.4	3.5	3.5	3.5	-
Range	(3.3, 3.8)	(3.1, 3.9)	(3.0, 4.0)	(3.0, 4.0)	(3.0, 4.0)	-
Age at randomization (days)	-	-	-	-	-	1.0000
Mean ± SD	274.5 ± 18.3	274.5 ± 18.0	274.5 ± 18.0	274.5 ± 17.7	274.5 ± 17.7	-
Median	274.5	274.5	274.5	274.5	274.5	-
Range	(257.0, 292.0)	(257.0, 292.0)	(257.0, 292.0)	(257.0, 292.0)	(257.0, 292.0)	-
Anti-PA antibody positive at baseline[2]	-	1/19 (5.3%)	0/19 (0.0%)	1/38 (2.6%)	1/38 (2.6%)	1.0000
TNA positive at baseline[2]	-	0/19 (0.0%)	0/19 (0.0%)	0/38 (0.0%)	0/38 (0.0%)	-

[1] P value for comparison of the 3 groups, based on Fisher's exact test for categorical variables, and ANOVA for continuous variables.

[2] Data on anti-PA antibody and TNA was available for survivors only.

All surviving rabbits in this study were anti-PA antibody positive at Day 28. All surviving rabbits were TNA positive at Day 28, with the exception of 1 rabbit (K99224) in the levofloxacin/raxibacumab group. This animal did not test positive for PA toxemia or bacteremia while on study, but had its treatment triggered by temperature increase at 21.6 hours following challenge.

6.1.3 Subject Disposition

The numbers of animals planned for enrollment were all randomized and treated as shown in the table below.

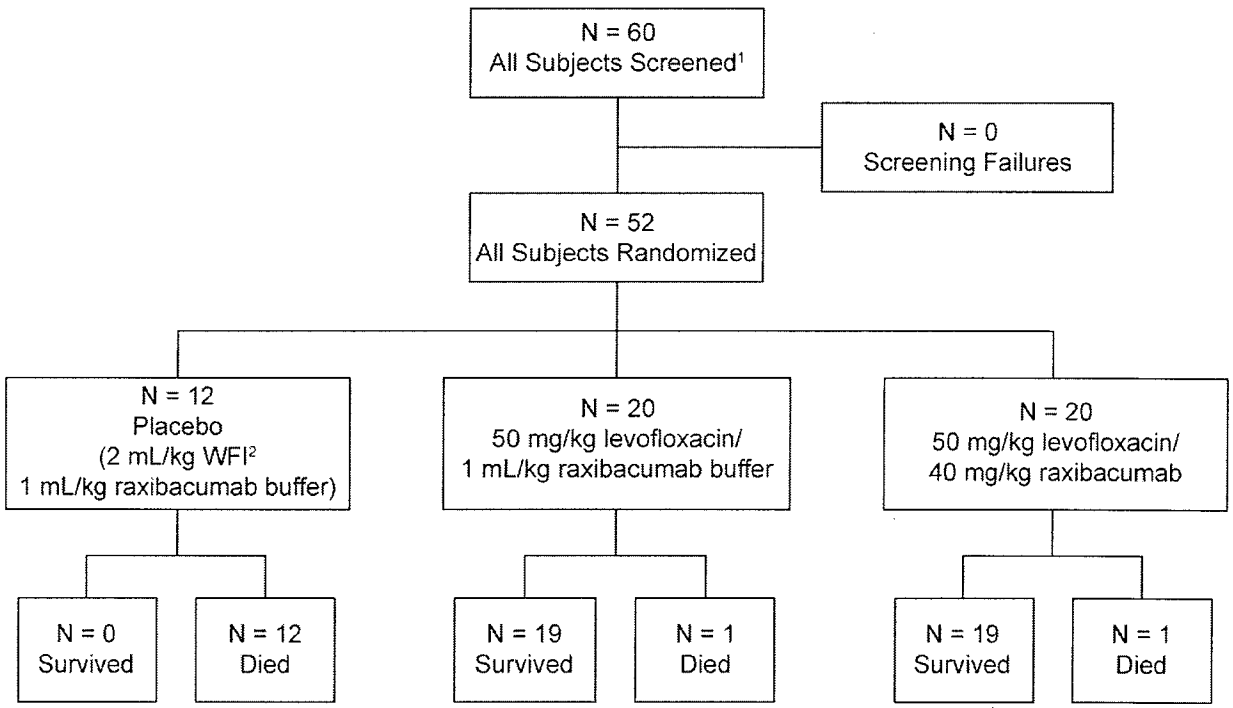
Table: Animal Disposition in Study 781-G923701

Dose Groups	Number of rabbits		
	Planned	Randomized	Treated
Group 1 – placebo	12	12	12
Group 2 – levofloxacin	20	20	20
Group 3 – levofloxacin/raxibacumab	20	20	20

A diagram of animal disposition by treatment group is provided in the Figure below. A total of 60 rabbits were screened, with 52 planned for use in the study plus 8 to use as

replacements if necessary. Four rabbits were replaced: 2 due to unusually high plasma PA values prior to challenge (Day -7), 1 due to a malfunctioning temperature transponder chip, and 1 due to a malfunctioning VAP. All 52 rabbits were randomized and challenged with anthrax spores (12 in the placebo group and 20 each in the levofloxacin group and the levofloxacin/raxibacumab group). All rabbits received treatment according to their assigned group, with the exception of rabbit K99251 (randomized to the levofloxacin/raxibacumab group), for which the PK data suggested was not dosed with raxibacumab. Rabbit K99251 was thus assigned to the levofloxacin group in the "As treated" population.

Figure: Summary of animal disposition in the ITT population (from Applicant's submission)



[1]Sixty animals (52 for study, 8 extra animals for replacement if necessary) were screened.
[2]Sterile water for injection (control for levofloxacin)

HGS# 000-7093

Extent of Anthrax Exposure

Exposure by treatment group is summarized in the table below. The mean inhaled spore doses among the 3 treatment groups all exceeded the targeted dose of 200 x LD_{50}. The difference in mean spore dose among the 3 treatment groups was not statistically significant ($p = 0.3411$ based on 1-way ANOVA). Similarly, doses administered on the 3 challenge days were comparable and exceeded the target dose of 200 x LD_{50} (data not shown).

Table: Summary of extent of anthrax exposure in the ITT population (from Applicant's submission)

	Placebo N = 12	Levofloxacin N = 20	Levofloxacin/ Raxibacumab N = 20	All Groups N = 52	P value[1]
Anthrax exposure (x LD_{50})	-	-	-	-	0.3411
Mean ± SD	324.7 ± 110.1	285.8 ± 64.5	282.5 ± 82.3	293.5 ± 83.6	-
Median	317.4	284.0	285.2	291.5	-
Range	(190.0, 625.8)	(167.3, 394.3)	(171.0, 469.5)	(167.3, 625.8)	-

[1] P value based on 1-way ANOVA for comparison among all treatment groups.

6.1.4 Analysis of Primary Endpoint(s)

The table below outlines time to treatment and trigger event status, and summarizes the occurrence of toxemia, bacteremia, and temperature increase among treatment groups. The 3 treatment groups had similar mean time to treatment: placebo group (28.1 hours), levofloxacin group (26.9 hours), and levofloxacin/raxibacumab group (28.7 hours), with a median time to treatment of 27.3 hours across all groups. Across the 3 treatment groups there were no statistically significant differences in time to treatment, trigger event, or the onset of bacteremia, toxemia, or temperature increase. The majority of animals in each group had their treatment triggered by detection of PA toxemia. Forty-seven of 52 (90.4%) rabbits exhibited positive plasma PA levels by 36 hours post challenge as determined by the screening assay. The 5 remaining animals had their treatment triggered by body temperature increase within 54 hours post challenge. Two of these 5 (K99255 and K99257 in the placebo group) were bacteremic within 76 hours post challenge but died of anthrax.

The groups were similar with respect to signs and symptoms around the time of treatment. The vast majority of rabbits in all groups (47/52, 90.4%) were toxemic and bacteremic at or before treatment initiation. There were no rabbits that were bacteremic but not toxemic at treatment initiation. The incidence of temperature increase at treatment across all groups was low (6/52, 11.5%), reflecting the predominance of PA toxemia as the treatment trigger symptom in the rabbit model.

Table: Summary of signs and symptoms in the ITT population (from Applicant's submission)

	Placebo N = 12	Levofloxacin N = 20	Levofloxacin/ Raxibacumab N = 20	All Groups N = 52	P value[1]
Time to treatment[2] (hour)	-	-	-	-	0.8704
Mean ± SD	28.1 ± 5.7	26.9 ± 3.5	28.7 ± 8.7	27.9 ± 6.3	-
Median	27.4	27.4	26.9	27.3	-
Range	22.6-44.2	22.4-35.3	18.6-54.4	18.6-54.4	-
Trigger event	-	-	-	-	0.8333
Detectable PA	11 (91.7%)	19 (95.0%)	17 (85.0%)	47 (90.4%)	-
Significant temperature increase[3]	1 (8.3%)	1 (5.0%)	3 (15.0%)	5 (9.6%)	-
Toxemia[4] at or before treatment initiation	12 (100.0%)	19 (95.0%)	18 (90.0%)	49 (94.2%)	0.7828
Toxemia at the time of treatment initiation	12 (100.0%)	19 (95.0%)	18 (90.0%)	49 (94.2%)	0.7828
Toxemia at any time	12 (100.0%)	20 (100.0%)	18 (90.0%)	50 (96.2%)	0.3363
Bacteremia[4] at or before treatment initiation	10 (83.3%)	19 (95.0%)	18 (90.0%)	47 (90.4%)	0.6228
Bacteremia at the time of treatment initiation	10 (83.3%)	19 (95.0%)	18 (90.0%)	47 (90.4%)	0.6228
Bacteremia at anytime	12 (100.0%)	19 (95.0%)	18 (90.0%)	49 (94.2%)	0.7828
Toxemia and bacteremia at or before treatment initiation	10 (83.3%)	19 (95.0%)	18 (90.0%)	47 (90.4%)	0.6228
Toxemia and bacteremia at the time of treatment initiation	10 (83.3%)	19 (95.0%)	18 (90.0%)	47 (90.4%)	0.6228
Toxemia and bacteremia at anytime	12 (100.0%)	19 (95.0%)	18 (90.0%)	49 (94.2%)	0.7828
Toxemia and/or bacteremia at or before treatment initiation	12 (100.0%)	19 (95.0%)	18 (90.0%)	49 (94.2%)	0.7828
Toxemia and/or bacteremia at the time of treatment initiation	12 (100.0%)	19 (95.0%)	18 (90.0%)	49 (94.2%)	0.7828
Toxemia and/or bacteremia at anytime	12 (100.0%)	20 (100.0%)	18 (90.0%)	50 (96.2%)	0.3363
Bacteremia without toxemia at or before treatment initiation	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Temperature increased at or before treatment initiation	1 (8.3%)	2 (10.0%)	3 (15.0%)	6 (11.5%)	1.0000
Temperature increased but with no toxemia or bacteremia at or before treatment initiation	0 (0.0%)	0 (0.0%)	2 (10.0%)	2 (3.8%)	0.3363

1 P value for comparison of the 3 groups, based on Log-rank test for time to treatment and 2-sided Fisher's exact test for other parameters.

2 Time to treatment was calculated relative to the start of challenge.

3 First 2 consecutive timepoints of a body temperature ≥ 2°F higher than the baseline average temperature.

4 Throughout this table toxemia was determined by quantitative ECL assay and bacteremia was determined by culture. Only postchallenge bacteremia and toxemia were counted. Toxemia was defined as detectable PA by quantitative ECL assay.

Primary efficacy results: Survival at Day 28 in the ITT Population
The primary efficacy endpoint was to compare the percent of animals alive at study Day 28 post challenge between the placebo group and the levofloxacin/raxibacumab group in the FDA Primary population.

Both the Applicant' ITT and FDA Primary results are shown in the table below. As previously mentioned, the majority of animals in the study were bacteremic before treatment initiation (90.4%, 47/52). In both analyses, statistically higher 28-day survival in the levofloxacin/raxibacumab group relative to placebo was observed. The same survival benefit was also observed with the levofloxacin group when compared to placebo. No statistically significant difference was observed between the 2 active treatment groups (95% CI of Levo/Raxi – Levo for the FDA Primary population -23.9%, 19.6%).

The 1 non-survivor in the levofloxacin group (K99203) was a 3.3 kg male that received a spore dose of 283.03 x LD_{50}. The animal became toxemic by 24.5 hours post challenge, bacteremic by 27.3 hours post challenge, and received its levofloxacin treatment 27.3 hours post challenge triggered by PA detection. The animal was discovered moribund and was euthanized 11 days post challenge. The 1 non-survivor in the levofloxacin/raxibacumab group (K99246) was a 3.6 kg female that received a spore dose of 369.35 x LD_{50}. The animal became toxemic and bacteremic by 20 hours post challenge, and received its levofloxacin/raxibacumab treatment 23.2 hours post challenge triggered by PA detection. This animal died as the result of a dosing accident (inadvertent intratracheal gavage of study agent).

Table: Survival at Day 28 in the ITT and FDA Primary populations

Study population	Placebo	Levofloxacin	Levofloxacin/Raxibacumab
		ITT population	
	N=12	**N=20**	**N=20**
Survival n (%); (95% CI)	0	19 (95); (69.5, 99.9)	19 (95); (69.5, 99.9)
p-value*	-	<0.0001	<0.0001
95% CI** of Levo/raxi – Levo (%)	-	-	-20.4, 20.4
		FDA Primary	
	N=10	**N=20**	**N=17**
Survival n (%);(95% CI)	0	19 (95.0); (65.9, 99.9)	16 (94.1); (64.2, 99.9)
p-value*		<0.0001	<0.0001
95% CI** of Levo/raxi –	-	-	-23.9, 19.6

Levo (%)			

*based on 2-sided Fisher's exact test for comparisons between each active group and placebo
**CIs are exact confidence intervals

Sensitivity Analyses of the Primary Efficacy Endpoint
Sensitivity analyses were performed on several defined populations, with the corresponding efficacy results shown in the Table below.

- *As treated*: PK analysis indicated that Rabbit K99251, which was randomized to the levofloxacin/raxibacumab group, was not dosed with raxibacumab, although it was dosed with levofloxacin. This animal was therefore assigned to the levofloxacin group in the As-treated population, defined as all animals that received a study agent with the assignment to treatment group based on the actual treatment that the animals received.

- *As treated excluding non-anthrax death*: The As treated population excluding animal K99246, which died as a result of intratracheal gavage and not from anthrax.

- *FDA Primary excluding non-anthrax death*: The FDA Primary population (animals that were bacteremic at the time of intervention and as treated) and excluding animal K99246, which died as a result of intratracheal gavage and not from anthrax.

- *Toxemic at treatment*: The ITT population according to the detection of toxemia at treatment, defined as the detection of PA by quantitative ECL.

Table: Sensitivity Analyses of the Primary Efficacy Endpoint

Study population	Placebo	Levofloxacin	Levofloxacin/Raxibacumab
As treated			
	N=12	**N=21**	**N=19**
Survival n (%); (95% CI*)	0	20 (95.2); (70.0, 99.9)	18 (94.7); (68.9, 99.9)
p-value*	-	<0.0001	<0.0001
95% CI** of Levo/raxi – Levo (%)	-	-	-22.2, 18.7
As treated excluding non-anthrax death			
	N=12	**N=21**	**N=18**
Survival n (%); (95% CI)	0	20 (95.2); (70.0, 99.9)	18 (100); (73.5, 100)
p-value*	-	<0.0001	<0.0001
95% CI** of Levo/raxi – Levo (%)	-	-	-14.1, 23.8
FDA Primary excluding non-anthrax death			
	N=10	**N=20**	**N=16**

Survival n (%); (95% CI)	0	19 (95); (65.9, 99.9)	16 (100); (69.2, 100)
p-value*	-	<0.0001	<0.0001
95% CI** of Levo/raxi – Levo (%)	-	-	-15.2, 25.6
Toxemic at treatment			
	N=12	**N=19**	**N=18**
Survival, n (%)	0	18 (94.7); (68.9, 99.9)	17 (94.4); (68.3, 99.9)
p-value*		<0.0001	<0.001
95% CI** of Levo/raxi – Levo (%)	-	-	-22.2, 21.2

*based on 2-sided Fisher's exact test for comparisons between each active group and placebo
** CIs are exact confidence intervals

Sensitivity analyses did not alter the conclusions of the primary efficacy analyses. Statistically significant survival benefit for levofloxacin, and levofloxacin/raxibacumab was maintained over placebo in the analysis of all populations. Similarly, the 95% CI of levofloxacin/raxibacumab – levofloxacin for all populations showed no statistical difference between the active treatment groups.

Animals Excluded from the FDA Primary Analysis Population
Five animals were not bacteremic at the time of treatment initiation were excluded from the FDA Primary analysis population and are shown in the table below.

Table: Animals that were not bacteremic prior to treatment initiation

Treatment group	Animal ID	Sex and weight (kg)	Trigger for treatment and time (h)	Time to alternate trigger (h)	Time to treatment (h)	Bacteremic after treatment/ time (h)	Outcome (Alive/ Dead)
Placebo	K99255	Female/3.35	PA/22.5	25.7	22.6	Yes/46.6	Dead
	K99257	Female/3.44	PA/24.1	28.5	27.8	Yes/75.9	Dead
Levofloxacin	K99254	Female/3.74	Temp/21.9	30.9	23.2	No/-	Alive
Levofloxacin/ Raxibacumab	K99202	Male/3.42	Temp/50.4	N/A	54.4	No/-	Alive
	K99224	Male/2.97	Temp/19.6	N/A	22.9	No/-	Alive

Two of the 5 rabbits (K99255 and K99257 in the placebo group) did become bacteremic at 46.6 hours and 75.9 hours post challenge respectively. Both animals in the placebo group had their treatment triggered by PA, became bacteremic after placebo intervention, and subsequently died of anthrax.

In contrast, all animals in the active treatment groups had their intervention triggered by temperature elevation between 22.9 h and 54.4 hours post challenge. Two of the animals never became toxemic, while the third was PA positive at 30.9 hours after challenge but received treatment at 23.2 hours (Animal K99254). None of these animals became bacteremic and all survived.

Analysis of the results in these 5 animals alone would suggest that the development of systemic disease (*i.e.*, bacteremia) may be a predictor of outcome. However, the majority of all study animals were bacteremic at the time of intervention and while all placebo animals died, only 1 animal that received active treatment (levofloxacin) died from anthrax.
It is interesting that both levofloxacin/raxibacumab animals which were not bacteremic at the time of intervention were also not toxemic. In comparison, the single non-bacteremic levofloxacin animal did develop toxemia. These results suggest levofloxacin administered after 24 hours was still able to kill *B. anthracis* to prevent the development of bacteremia. The addition of raxibacumab to levofloxacin administered between ~23 to 54 hours may have prevented the development of toxemia in the combination treatment animals. These results support the findings of the proof-of-concept rat toxin study, which showed that toxin alone (absence of bacteremia) can result in mortality and preventing toxemia can improve survival.

Triggers for intervention: Toxemia and temperature elevation
For most animals (90.4%, 47/52), the trigger for treatment was toxemia, defined as PA positive by quantitative ECL assay. Five animals received treatment triggered by temperature elevation (2 consecutive time points of a body temperature 2 or more °F higher than the baseline average temperature) rather than toxemia, and are shown in the table below.

Table: Animals with temperature elevation as the trigger for treatment

Treatment group	Animal ID	Time to 2 consecutive 2°F increase in temp (h)	Time to PA by ECL (h)	Bacter-emia prior to treatment	Time to bacter-emia (h)	Time to treatment (h)	Outcome (Alive/ Dead)
Placebo	K99213	42.1	44.1	Positive	44.1	44.2	Dead
Levofloxacin	K99254	21.9	30.9	Negative	N/A	23.2	Alive
Levofloxacin/ Raxibacumab	K99202	50.4	N/A	Negative	N/A	54.4	Alive
	K99204	41.4	43.5	Positive	43.5	43.6	Alive
	K99224	19.6	N/A	Negative	N/A	22.9	Alive

N/A: animal was not positive for this test to have a recorded time

The mean times to toxemia and temperature elevation in the three treatment groups are shown in the table below.

Table: Comparison of mean times to toxemia and temperature elevation for treatment groups in the ITT population

Treatment group	Placebo N=12	Levofloxacin N=20	Levofloxacin/ Raxibacumab N=20
Mean time to toxemia[1] in h (range)	24.9±6.5 (19.8-44.1)	24.1±4.1 (16.0-32.3)	24.1±4.1 (16.0- 32.3)
Mean time to 2 consecutive 2°F increase in temperature in h (range)	29.2±4.8 (22.7-42.1)	39.2±52.7 (21.9-262.8)[2]	31.6±8.7 (21.6-53.3)

[1] Toxemia defined as PA positive by quantitative ECL assay
[2] One animal had an elevated temperature at 262.8 hours

The mean time to two consecutive temperature elevations was longer compared to the mean time to toxemia for all groups.

The table below compares the mean times to treatment for animals in the ITT population stratified by treatment trigger (PA or temperature). Although the analysis is limited by the small number of animals who received treatment based on temperature elevation (5 animals: placebo K99213, levofloxacin K99254, levofloxacin/raxibacumab K99202, K99204, K99224), the mean time to treatment was longer for placebo and levofloxacin/raxibacumab groups where treatment was triggered by temperature elevation compared to animals in the same treatment group who received therapy based on toxemia. However, once the trigger was identified, animals in the placebo and levofloxacin groups who were identified based on temperature had faster mean and median times to treatment than animals identified by PA. The mean and median times to treatment in the levofloxacin/raxibacumab group were similar regardless of trigger event. In addition, the mode of treatment trigger did not appear to have an impact on outcome in the two active treatment groups, as the statistically significant survival benefit relative to placebo was maintained regardless of the trigger for treatment, although the sole death in this study limits the strength of this conclusion.

Table: Mean times to treatment for animals in the ITT population stratified by treatment trigger

Treatment group	Placebo N=12		Levofloxacin N=20		Levofloxacin/ Raxibacumab N=20	
	PA n=11	Temp n=1	PA N=19	Temp N=1	PA n=17	Temp n=3
Mean time to treatment in h	26.6±2.7 (22.6-31.9)	44.2	27.1±3.5 (22.4-35.3)	23.2	26.7±5.3 (18.6-39.0)	40.3±16.0 (22.9-54.4)
Mean time from trigger to treatment in h	3.4	2.1	3.4	1.4	2.9	3.1
Median time from trigger to treatment in h	2.8	2.1	3.8	1.4	3.0	3.3
Number of	0	0	18 (94.7)[1]	1 (100)	16 (94.1)[1]	1 (100)

survivors (%)						

[1]If non-anthrax deaths excluded, there were no deaths in the levofloxacin/raxibacumab group

6.1.5 Analysis of Secondary Endpoints(s)

The secondary efficacy endpoint was survival time, defined as the time from the beginning of spore challenge to death during the 28-day post challenge period. This is shown for the ITT, FDA Primary and other populations in the table below. For animals that were alive at the end of the 28-day post challenge period, survival times were to be censored on the date of study completion at Day 28 (672 hours). As shown in the table and figure below, survival time was significantly longer in both the levofloxacin group and the levofloxacin/raxibacumab group (FDA Primary $p < 0.001$ for both) relative to the placebo group. The difference in survival times between the 2 active treatment groups was not statistically significant ($p = 0.986$ as determined by log-rank test). The median survival time in the placebo group was 78.5 hours, median survival time could not be determined for the levofloxacin and levofloxacin/raxibacumab groups because the medians extended beyond 28 days.

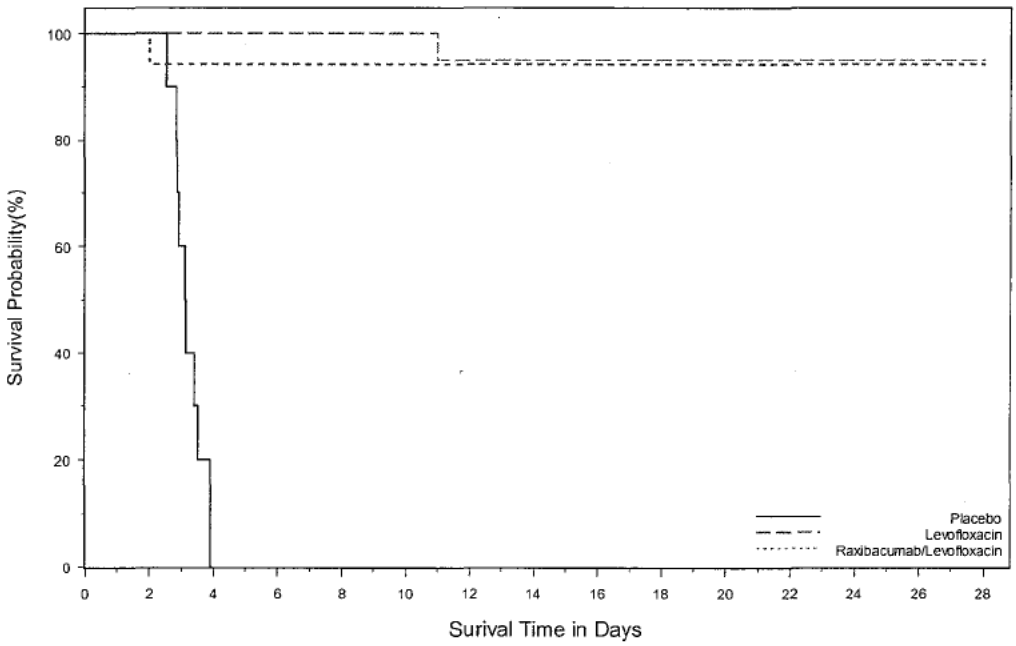
Table: Survival time (time from challenge to death) for ITT, FDA Primary and other sensitivity analyses populations

Study population	Placebo	Levofloxacin	Levofloxacin/ Raxibacumab
ITT population			
	N=12	**N=20**	**N=20**
Number (%)	12 (100)	1 (5.0)	1 (5.0)
Mean survival time in h (range)	80.6±13.5 (60.5-100.7)	263.2 (263.2, -)	47.4 (47.4, -)
Median survival time in h	78.5	-	-
p-value*		<0.0001	<0.0001
FDA Primary			
	N=10	**N=20**	**N=17**
Number (%)	10 (100)	1 (5.0)	1 (5.9)
Mean survival time in h (range)	76.8 (60.6-93.4)	263.2 (263.2, -)	47.4 (47.4, -)
Median survival time in h	74.6	-	-
p-value*		<0.0001	<0.0001
As treated			
	N=12	**N=21**	**N=19**
Number (%)	12 (100)	1 (4.8)	1 (5.3)
Mean survival time in h (range)	80.6±13.5 (60.5-100.7)	263.2 (263.2, -)	47.4 (47.4, -)
Median survival time in h	78.5	-	-
p-value*		<0.0001	<0.0001

As treated excluding non-anthrax death			
	N=12	**N=21**	**N=18**
Number (%)	12 (100)	1 (4.8)	0
Mean survival time in h (range)	80.6±13.5 (60.5-100.7)	263.2 (263.2, -)	-
Median survival time in h	78.5	-	-
p-value*		<0.0001	<0.0001
FDA Primary and excluding non-anthrax death			
	N=10	**N=20**	**N=16**
Number (%)	10 (100)	1 (5.0)	0
Mean survival time in h (range)	76.8 (60.6-93.4)	263.2 (263.2, -)	-
Median survival time in h	74.6	-	-
p-value*		<0.0001	<0.0001

*based on 2-sided Log-rank test for comparisons between each active group and placebo
Survival time based on start of spore challenge. Animals that survived to Day 28 were censored at Day 28
ITT: p-value for 2-sided Log-rank test for comparison between Levofloxacin and Levofloxacin/Raxibacumab is 0.986
FDA Primary: p-value for 2-sided Log-rank test for comparison between Levofloxacin and Levofloxacin/Raxibacumab is 0.891
As treated: p-value for 2-sided Log-rank test for comparison between Levofloxacin and Levofloxacin/Raxibacumab is 0.928

Figure: Survival time in the FDA Primary Population



Exploratory Analyses

Time to Onset of Symptoms in Survivors and Non-Survivors

The table below summarizes treatment trigger, exposure to anthrax, and time to onset of symptoms among survivors and non-survivors in the ITT population by treatment group. There were no survivors in the placebo group. All 3 groups were comparable with regard to anthrax exposure. The 1 non-survivor n the levofloxacin/raxibacumab group (K99246) died as the result of a gavage accident. All 3 groups were comparable with regard to treatment trigger and number of animals with detectable PA. Time to PA detection (screening and quantitative), time to bacteremia, and time to treatment were also comparable among survivors and non-survivors across groups.

Table: Summary of exposure, treatment trigger, and time (hours) to onset of symptoms between survivors and non-survivors at Day 28 in the ITT population
(from Applicant's submission)

	Placebo	Levofloxacin		Levofloxacin/Raxibacumab	
	All Non-Survivors N = 12	Survivors N = 19	Non-Survivors N = 1	Survivors N = 19	Non-Survivors N = 1
Anthrax exposure (x LD_{50})	12	19	1	19	1
Mean ± SD	324.7 ± 110.1	285.9 ± 66.2	283.0 ± NA[1]	277.9 ± 81.9	369.3 ± NA
Median (min, max)	317.4 (190.0, 625.8)	285.0 (167.3, 394.3)	283.0 (283.0, 283.0)	271.7 (171.0, 469.5)	369.3 (369.3, 369.3)
Treatment trigger	-	-	-	-	-
n	12	19	1	19	1
Detectable PA	11 (91.7%)	18 (94.7%)	1 (100.0%)	16 (84.2%)	1 (100.0%)
Significant temperature increase	1 (8.3%)	1 (5.3%)	0 (0.0%)	3 (15.8%)	0 (0.0%)
Anti-PA antibody positive at baseline[2]	-	-	-	-	-
n	0	19	0	19	0
Positive	-	1 (5.3%)	-	0 (0.0%)	-
TNA positive at baseline[2]					
n	0	19	0	19	0
Positive	-	0 (0.0%)	-	0 (0.0%)	-
Time to[3] PA detection (quantitative ECL)	-	-	-	-	-
n	12	19	1	17	1
Mean ± SD[3]	24.9 ± 6.5	23.9 ± 4.2	27.3 ± NA	_[4]	20.1 ± NA
Median (min, max)[3]	23.9 (19.8, 44.1)	23.9 (16.0, 32.3)	27.3 (27.3, 27.3)	23.9 (18.5, _[4])	20.1 (20.1, 20.1)
Time to PA detection (Screening)	-	-	-	-	-
n	11	18	1	16	1
Mean ± SD	_[4]	_[4]	24.5 ± NA	_[4]	20.1 ± NA
Median (min, max)	23.9 (19.9, _[4])	23.9 (19.5, _[4])	24.5 (24.5, 24.5)	23.9 (15.7, _[4])	20.1 (20.1, 20.1)

	Placebo	Levofloxacin		Levofloxacin/Raxibacumab	
	All Non-Survivors N = 12	Survivors N = 19	Non-Survivors N = 1	Survivors N = 19	Non-Survivors N = 1
Time to bacteremia (culture)	-	-	-	-	-
n	12	18	1	17	1
Mean ± SD	33.0 ± 15.7	_[4]	27.3 ± NA	_[4]	20.1 ± NA
Median (min, max)	27.1 (22.9, 75.9)	24.1 (19.7, _[4])	27.3 (27.3, 27.3)	24.2 (18.5, _[4])	20.1 (20.1, 20.1)
Time to temperature increase	-	-	-	-	-
n	12	19	0	19	0
Mean ± SD	30.9 ± 4.6	29.5 ± 3.5	_[4]	30.8 ± 8.1	_[4]
Median (min, max)	31.0 (24.6, 43.1)	29.1 (24.7, 37.4)	_[4] (_[4], _[4])	28.8 (21.6, 53.3)	_[4] (_[4], _[4])
Time to treatment	-	-	-	-	-
n	12	19	1	19	1
Mean ± SD	28.1 ± 5.7	26.9 ± 3.6	27.3 ± NA	29.0 ± 8.8	23.2 ± NA
Median (min, max)	27.4 (22.6, 44.2)	27.4 (22.4, 35.3)	27.3 (27.3, 27.3)	27.0 (18.6, 54.4)	23.2 (23.2, 23.2)

1 Not applicable (throughout table).

2 Baseline anti-PA antibody and baseline TNA were tested for survivors only.

3 Throughout this table all "time to" values were calculated relative to the start of challenge. Rabbit K99240 had a baseline mean PA value of 0.768 ng/mL and the time to PA was computed using the first timepoint relative to start of challenge at which PA was positive.

4 Throughout this table mean, SD and maximum are not be applicable for events with censored animals. In addition, median and min are not applicable for events with all animals in the population censored.

To further expand on the analysis performed by the Applicant above, additional variables were compared between the single non-survivor which died from anthrax and survivors in the levofloxacin arm. These results are shown in the table below. No differences were seen in the exploratory variables.

Table: Comparison of selected variables between survivors and non-survivors in the levofloxacin group (ITT population)

Event	Levofloxacin	
	Survivors n=19	Non-survivor n=1
Weight	3.5 kg	3.3kg
Mean spore dose x LD_{50} (range)	285.9±66.2 (167.3, 394.3)	283.03
Mean time to toxemia in h (range)	23.5±3.6 (19.5, 32.3)	24.5
Mean time to bacteremia in h (range)	59.5±148.1 (19.7, 671.0)	27.3
Mean time to treatment in h (range)	26.9±3.6 (22.4, 35.3)	27.3
Mean time to death	28 days	11 days

Survival at Day 28 by Baseline anti-PA and TNA status in the ITT population
Since anti-PA and TNA assays were conducted on surviving animals only, these results are only available for the active treatment groups. All animals in the active treatment groups were TNA negative at baseline, and all were anti-PA antibody negative at baseline with the exception of 1 survivor in the levofloxacin group (K99234) that tested just above LLOQ positive for anti-PA antibodies at baseline. The table below shows anti-PA antibody and TNA status among survivors in the ITT population. All survivors tested positive for anti-PA antibodies at Day 28. Similarly, all survivors tested positive for TNA at Day 28, with the exception of 1 survivor in the levofloxacin/raxibacumab group (K99224). This animal did not test positive for PA toxemia or bacteremia during the study; treatment, however, was triggered by body temperature increase, and the animal was positive for anti-PA antibodies at Day 28.

Table: Summary of anti-PA antibody and TNA among survivors in the ITT population (from Applicant's submission)

	Placebo N = 12	Levofloxacin N = 20	Levofloxacin/Raxibacumab N = 20
Anti-PA antibody positive[1] at Day 28	0/0	19/19 (100.0%)	19/19 (100.0%)
TNA positive[2] at Day 28	0/0	19/19 (100.0%)	18/19 (94.7%)
Anti-PA antibody converted from negative to positive	0/0	18/18 (100.0%)	19/19 (100.0%)
TNA converted from negative to positive	0/0	19/19 (100.0%)	18/19 (94.7%)

[1] Anti-PA antibody was tested for survivors only. One survivor: K99234 was positive both at baseline and at Day 28.

[2] TNA was tested for survivors only. One survivor: K99224 was negative both at baseline and at Day 28.

Necropsy and Histopathology
All 52 animals, survivors and non-survivors, had their tissues examined microscopically. Gross lesions found in rabbits which died prior to study termination included enlargement, discoloration and/or foci of the adrenal glands, appendix, brain, lung and multiple lymph nodes; fluid (effusion) in the pericardial and thoracic cavities, and fluid/thickening (edema) of the skin and thymus. These correlated histologically with necrosis, inflammation, hemorrhage, edema and the visualization of anthrax bacteria. In contrast, gross lesions found in rabbits surviving to study termination were determined to be unrelated to anthrax infection. Lung discoloration in the only animal in the levofloxacin/raxibacumab group to die spontaneously (K99246) correlated with a gavage accident and not with microscopic evidence of anthrax.

Microscopic findings consistent with anthrax were present in all rabbits dying spontaneously or becoming moribund following anthrax challenge except for animal K99246 discussed above, which was not bacteremic at death.

All placebo animals had microscopic findings of CNS involvement; most (10/12) had bacteria in the brain and brain vasculature, 2/12 animals had gross findings of meningitis and 5/12 animals had CNS hemorrhage. Using the same grading criteria for

histopathologic changes as that done for the pivotal animal studies (high grade lesion defined as the presence of bacteria, hemorrhage or inflammation ≥level 2; presence of necrosis regardless of the grades of bacteria, hemorrhage and inflammation indicated a high grade lesion), only 1/12 placebo animals had a high grade lesion. Rabbit K99213 had both grade 3 bacteria and hemorrhage and necrosis as shown in the following table.

Table: Gross and microscopic brain findings in Rabbit K99213 (placebo group)

Record #	Observation Type	Severity Grade	Observation
1	Gross		DISCOLORATION(S), MENINGES, RED, G1.
2	microscopic	3	BACTERIA.
3	microscopic		Comment: G1 = HEMORRHAGE, MENINGITIS AND NECROSIS ON SLIDES 1-3.
4	microscopic	2	FIBRINOHETEROPHILIC MENINGITIS.
5	microscopic	3	HEMORRHAGE.
6	microscopic	1	NEUROPIL NECROSIS

In contrast, of the animals that died in the active treatment groups, 0/1 in the levofloxacin group (rabbit K99203, died of gavage accident) and 0/1 in the levofloxacin/raxibacumab group (rabbit K99246) had any CNS lesions (and therefore no high grade CNS lesions) found on gross or microscopic exam.

The pathological findings of rabbit K99203, the only animal to die of anthrax in an active treatment group, are listed below.

- Gross exam:
  - Pericardial fluid
- Microscopic exam:
  - Lung: bacteria, hemorrhage, bronchial-associated lymphoid tissue necrosis, fibrin exudation, heterophilic inflammation
  - Kidney: mineralization, tubules
  - Liver: mixed cell infiltrate, sinusoidal leukocytosis
  - Lymph node bronchi: fibrin exudation, heterophilic inflammation, lymphocyte necrosis/depletion
  - Lymph node mediastinal: lymphocyte necrosis/depletion

Similarly, none of the survivors in the levofloxacin and levofloxacin/raxibacumab treatment groups which were sacrificed at Day 28 had brain lesions on gross or microscopic examination.

CNS findings

In the pivotal efficacy studies 682-G005758 (NZW rabbit) and 724-G005829 (cynomolgus monkey), an unexpected finding was that raxibacumab-treated animals that died had more CNS pathology consisting of hemorrhage, necrosis, inflammation and bacteria compared to placebo animals that died. Gross findings in the brains of monkeys that died were seen in 3/12 placebo, 6/7 in the 20 mg/kg group, 3/5 in the 40 mg/kg group. The numbers of animals with inflammation and CNS pathology from studies 724-G005829 and 682-G005758 are reported in the following tables.

Table: Comparison of CNS findings in necropsized animals by treatment group in Study 724-G005829 (cynomolgus monkey) (from Statistics review)

Treatment	Number of Necropsized Monkeys	No. (%) of CNS findings	P-value*
Moderate/Marked Brain Inflammation			
Placebo	n = 12	1 (8.3)	
20 mg/kg Raxibacumab	n = 7	6 (85.7)	0.0017
40 mg/kg Raxibacumab	n = 5	2 (40.0)	0.1912
FDA CNS Pathology Level**			
Placebo	n = 12	1 (8.3)	
20 mg/kg Raxibacumab	n = 7	6 (85.7)	0.0017
40 mg/kg Raxibacumab	n = 5	3 (60.0)	0.0525

* P values are based on two-sided Fisher's exact test for comparisons between the raxibacumab group and placebo.

** The pathology level of CNS pathology findings was defined based on the following criteria: 1) For bacteria, inflammation and hemorrhage, the highest grade recorded was selected, 2) disregard grading of congestion as this represents intravascular hyperemia rather than a true pathologic findings, 3) grades 0-2 constitutes low level of pathology while 3-4 are considered high level, and 4) if necrosis is present, then elevate the pathology level to high if it was initially recorded as low.

Table: Comparison of CNS Findings in Necropsized Animals by Treatment Group in Study 628-G005758 (NZW rabbit) (from Statistics review)

Treatment	Number of Necropsized Rabbits	No. (%) of CNS findings	P-value*
Moderate/Marked Brain Inflammation			
Placebo	*n = 16*	*0*	
20 mg/kg Raxibacumab	*n = 12*	*3 (25.0)*	*0.0672*
40 mg/kg Raxibacumab	*n = 11*	*3 (27.3)*	*0.0564*
FDA CNS Pathology Level*			
Placebo	*n = 16*	*2 (12.5)*	

20 mg/kg Raxibacumab	*n = 12*	*9 (75.0)*	*0.0015*
40 mg/kg Raxibacumab	*n = 11*	*6 (54.5)*	*0.0332*

* P values are based on two-sided Fisher's exact test for comparisons between the raxibacumab group and placebo.
** The pathology level of CNS pathology findings was defined based on the following criteria: 1) For bacteria, inflammation and hemorrhage, the highest grade recorded was selected, 2) disregard grading of congestion as this represents intravascular hyperemia rather than a true pathologic findings, 3) grades 0-2 constitutes low level of pathology while 3-4 are considered high level, and 4) if necrosis is present, then elevate the pathology level to high if it was initially recorded as low.

The hypothesis proposed by the Applicant was that this difference was related to longer survival of the raxibacumab-treated animals rabbits compared to the placebo group was carefully examined. Among the 12 placebo monkeys that died (Table below), 11/12 had no brain inflammation, low pathology level, time from challenge to bacteremia of 1.32-5.34 days, time from treatment to death of 0.18-8.4 days, and a time from challenge to death of 1.92-10.75 days. The single monkey that had moderate brain inflammation and a high grade of pathology, the time from challenge to bacteremia was 1.48 days (no different from low pathology animals), time from treatment to death of 2.99 days (slightly longer than low pathology animals), and a time from challenge to death of 4.47 days (slightly longer than low pathology animals).

Table: Pathologic findings, and various time measures in the placebo animals that died in Study 724-G005829 (from Statistics review)

#	N=normal m=moderate M=marked Brain Inflammation	L=Low H=High Pathology Level	Time from challenge to bact. (days)	Time from bact. to treat (days)	Time from treatment to death (days)	Time from challenge to death (days)
C23601	N	L	1.32	0.43	0.18	1.92
C23630	N	L	1.73	0.15	0.75	2.63
C23036	N	L	1.16	0.003	1.69	2.85
C23404	N	L	1.40	0.003	1.55	2.96
C24793	N	L	1.46	0.17	1.56	3.20
C24851	N	L	2.09	0.16	1.10	3.35
C24858	N	L	1.31	0.18	2.10	3.59
C24853	N	L	1.08	0.18	2.59	3.85
C24056	m	H	1.48	0.002	2.99	4.47
C24784	N	L	1.48	0.18	7.46	9.12
C23052	N	L	5.34	-3.00	8.40	10.75
C24824	N	L	1.94	-0.50	1.80	3.25

Of the 7 monkeys in the raxibacumab 20 mg/kg IV group that died, 6/7 had moderate brain inflammation and a high pathology level, with time from challenge to bacteremia of 1.28-1.97 days, time from treatment to death of 1.54-3.36 days, and a time from challenge to death of 3.16-5.47 days. The single monkey that died with no brain inflammation had a shorter time from challenge to bacteremia of 1.30 days, time from treatment to death of 0.82 days, and a time from challenge to death of 2.31 days, which could support the hypothesis that animals with more severe CNS findings lived longer in order to develop the more severe pathology.

Table: Pathologic findings, and various time measures in the Raxibacumab 20 mg/kg animals that died in Study 724-G005829 (from Statistics review)

#	N= normal m=moderate M=marked Brain Inflammation	L=Low H=High Pathology Level	Time from challenge to bact. (days)	Time from bact. to treat (days)	Time from treatment to death (days)	Time from challenge to death (days)
C24856	N	L	1.30	0.19	0.82	2.31
C24755	m	H	1.44	0.19	1.54	3.16
C24066	m	H	1.33	0.15	2.09	3.57
C24878	m	H	1.52	0.16	2.23	3.91
C24840	m	H	1.28	0.20	2.62	4.10
C20255	m	H	1.54	0.39	2.52	4.46
C21466	m	H	1.97	0.14	3.36	5.47

Lastly, of the 5 monkeys in the raxibacumab 40 mg/kg IV group that died, 3/5 had a high pathology level. The time intervals were as follows: time from challenge to bacteremia 1.47-2.02 days, time from treatment to death 2.04-3.64 days, and time from challenge to death 3.70-5.16 days. The 2 monkeys that died with no brain inflammation and low pathology did not have shorter times from challenge to bacteremia (1.30-1.49 days), although they did have shorter times from treatment to death 0.35-1.01 days, and shorter time from challenge to death 2.26-2.73 days.

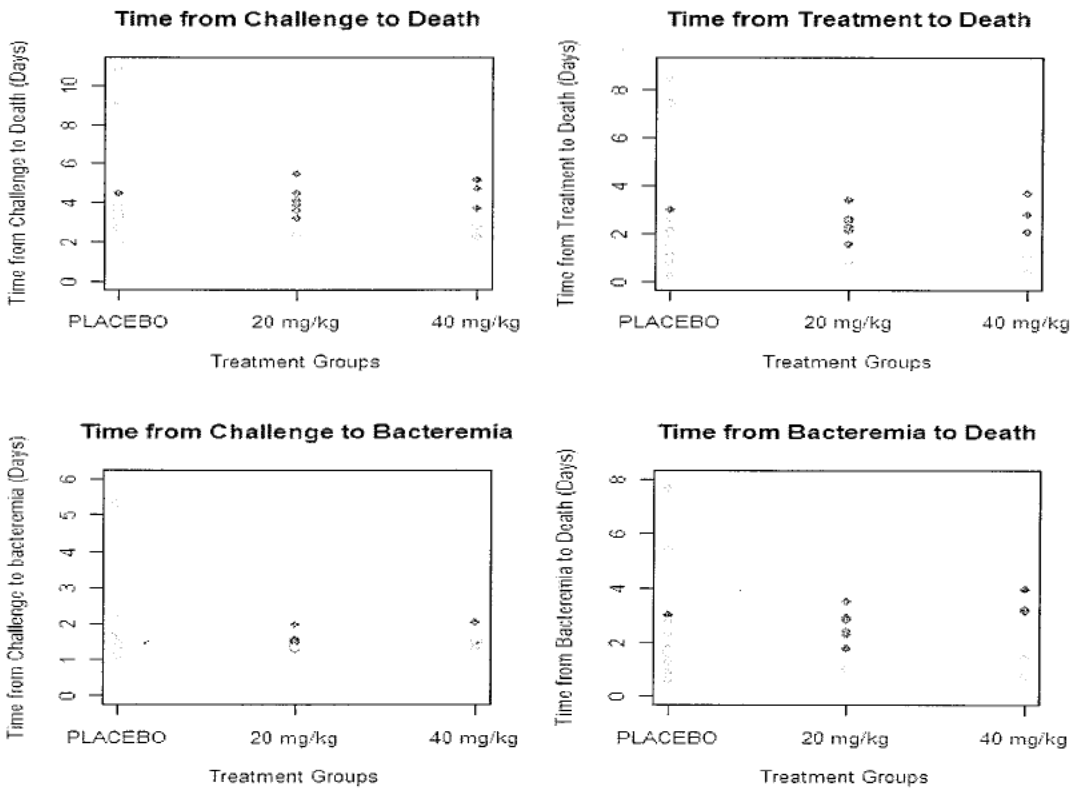
Table: Pathologic findings, and various time measures in the Raxibacumab 40 mg/kg animals that died in Study 724-G005829 (from Statistics review)

#	N=normal m=moderate M=marked Brain Inflammation	L=Low H=High Pathology Level	Time from challenge to bact. (days)	Time from bact. to treat (days)	Time from treatment to death (days)	Time from challenge to death (days)
C23640	N	L	1.49	0.41	0.35	2.26
C24847	N	L	1.30	0.42	1.01	2.73
C23655	N	H	1.47	1.90	2.04	3.70

C24928	m	H	1.50	0.41	2.79	4.71
C23994	m	H	2.02	-0.501	3.64	5.16

Within their respective groups, raxibacumab treated animals that died with more severe CNS findings had longer times from treatment to death, and from challenge to death, than animals with no CNS findings. These findings are less clear in the placebo group, where these same times of treatment to death and challenge to death in the 1 animal with high pathology overlapped with the animals with low pathology. Furthermore, when all these times are compared across groups, as shown in the figure below, there is significant overlap in the various times for animals with low and high pathology levels.

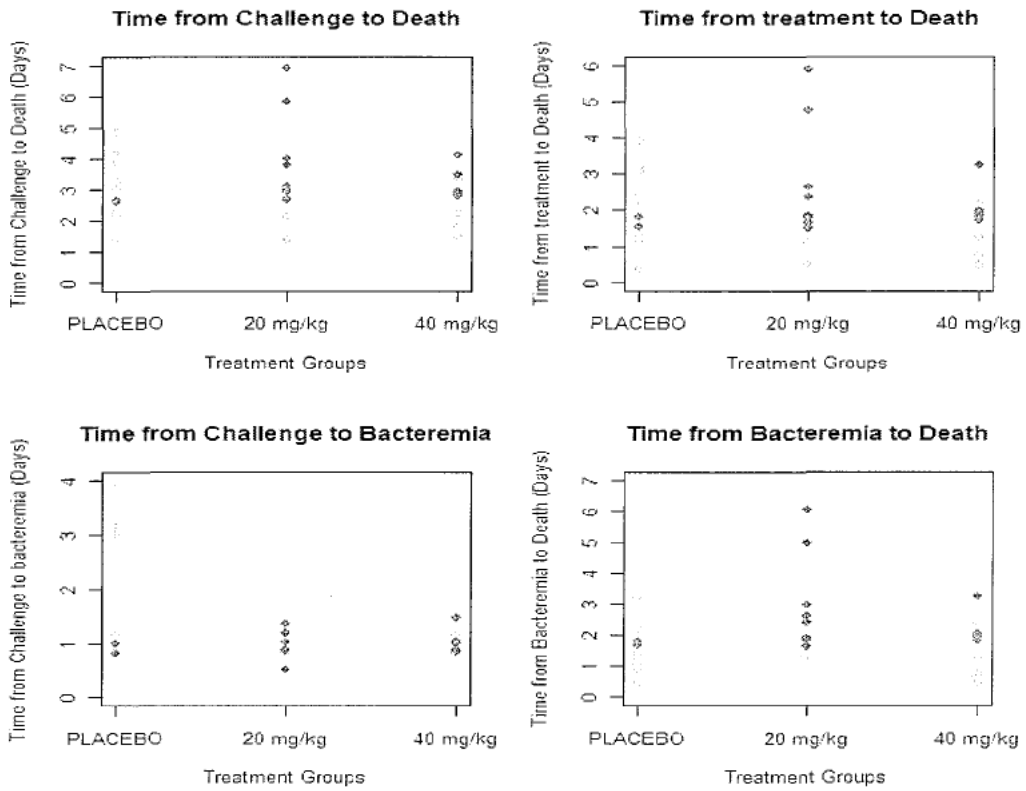
Figure: Comparison of various time analyses relative to pathology grade in Study 724-G005829 (from Statistics review)



Note: Red = High Pathology Level
Gray = Low Pathology Level

Similar results are seen with the pivotal rabbit study. While the raw data is not presented, the corresponding figures are shown below. Please see the statistical review by Dr. Hongling Zhou for additional details.

Figure: Comparison of various time analyses relative to pathology grade in Study 682-G005758 (from Statistics review)



Note: Red = High Pathology Level
Gray = Low Pathology Level

In summary, placebo animals with comparable survival times to those in the active treatment groups did not have CNS findings; time of death was similar across the placebo and raxibacumab animals that died for both the monkey and the rabbit studies. However, within the raxibacumab groups, it appears the animals with CNS findings live longer than those without CNS findings.

Therefore longer survival time does not account for the increased CNS findings observed in raxibacumab treated animals. This suggests a possible study agent-disease interaction, with CNS findings associated with raxibacumab treatment in those animals which develop anthrax disease.

As exploratory analyses only for Study 781-G92370, survival time (time from challenge to death), duration of bacteremia prior to treatment and duration of bacteremia prior to death stratified by outcome were examined and the results are shown in the table below.

Table: Time from challenge to treatment stratified by outcome for FDA Primary population

Treatment group	Placebo N=10		Levofloxacin N=20		Levofloxacin/ Raxibacumab N=17	
	Alive N=0	Dead N=10	Alive N=19	Dead N=1	Alive N=16	Dead[1] N=1
Time from treatment to death in hours[2]						
Mean (range)	-	52.5±14.2 (33.1, 78.2)	-	235.9 (235.9, -)	-	24.2 (24.2, -)
Median	-	51.0	-	235.9	-	24.2
p-value						
Survival time in hours[2]						
Mean (range)	-	76.8±11.2 (60.5, 93.4)	-	263.2 (263.2, -)	-	47.4 (47.4, -)
Median	-	74.6	-	263.2	-	47.4
p-value				<0.0001		<0.0001
Time from aerosol challenge to treatment in hours						
Mean	-	28.6±6.0 (23.2, 44.2)	27.1±3.6 (22.4, 35.3)	27.3 (27.3, -)	27.9±6.6 (18.6, 43.6)	23.2 (23.2, -)
Median	-	27.4	27.5	27.3	27.0	23.2
p-value						
Time from bacteremia to treatment in hours						
Mean (range)	-	1.32±1.6 (0.03, 3.6)	1.58±2.3 (0.03, 8.1)	0.03 (0.03, -)	1.4±2.1 (0.02, 7.4)	3.1 (3.1, -)
Median	-	0.08	0.05	0.03	0.03	3.1
p-value						
Time from bacteremia to death in hours[2]						
Mean (range)	-	49.4±11.3 (33.2, 69.5)	-	235.9 (235.9, -)	-	27.3 (27.3, -)
Median	-	47.5	-	235.9	-	27.3
p-value						

[1] If non-anthrax deaths excluded, there were no deaths in the levofloxacin/raxibacumab group
[2] Survivors in the active treatment arms were all sacrificed at the end of study at 672 hours (28 days) and therefore any time where death is the final time point has not been determined

All analyses were performed in the FDA Primary population and were limited by the few number of deaths (2) in the active treatment groups. Furthermore, if non-anthrax deaths are excluded, there was only 1 anthrax death in an active treatment group (levofloxacin). The levofloxacin treatment group is the only relevant population as it was

the only treatment group to have both survivors and anthrax deaths, and therefore the following discussion focuses primarily on this group.

Time from treatment to death: This was longer for the one animal that died in the levofloxacin treatment group (mean 235.9 hours) compared to the animals in the placebo group (mean 52.5 hours).

Survival time (time from challenge to death): This was longer for the one animal that died in the levofloxacin treatment group (mean 263.2 hours) compared to the animals in the placebo group (mean 76.8 hours).

Time from aerosol challenge to treatment: With respect to mean and median times from challenge to treatment in the levofloxacin group, these times did not differ in the one animal that died compared to the animals that survived. Although there appears to be some difference in the levofloxacin/raxibacumab group, if non-anthrax deaths are excluded, the finding is not applicable. The times from challenge to treatment were similar across both treatment groups.

Time from bacteremia to treatment: Within the levofloxacin group, the mean time from bacteremia to treatment was longer in animals that survived compared to the one animal that died, 1.58 hours compared to 0.03 hours although the median times were similar. These findings are in contrast to pivotal study 682-G005758, which found that the mean and median time between positive culture and treatment was longer in animals that died (median 3.4 hours, mean 3.1) compared to animals that survived (median 1.4 hours, mean 2.6 hours). However, the current analysis is limited by only one death from which data was obtained.

Time from bacteremia to death: The animal that died in the levofloxacin group had a longer duration of bacteremia before death compared to placebo animals, 235.9 hours compared to mean 49.4 hours (median 47.5 hours).

In summary, for the FDA Primary analysis population, the animal in the levofloxacin treatment group had longer time from treatment to death, survival time and duration of bacteremia before death compared to animals that died in the placebo group. It is difficult to interpret this finding as it was the only death in an active treatment group. As mentioned previously in the Necropsy and Histopathology discussion, this animal did not have any CNS findings on gross or microscopic examinations.

Exposure-Response CNS Analysis

The Clinical Pharmacology team performed exposure-response CNS analyses to explore the observation that raxibacumab-treated animals not only had a higher incidence of histopathologic findings, but also had lesions of higher severity grade compared to placebo-treated animals. However, the finding that the raxibacumab 20 mg/kg dose group had a higher incidence and severity of CNS findings compared to

the 40 mg/kg group suggests the absence of a clear dose-response relationship for brain histopathology.

Please see the full Clinical Pharmacology review by Dr. Kim Bergman for details. In brief, her review concluded that the potential for raxibacumab treatment to result in adverse CNS findings was not likely related to prolonged survival time. No clear relationship between exposure and response for CNS pathology grade severity could be determined since these differences between grades were attributed to the small numbers of animals.

Consults Regarding CNS Findings
Consults were requested from the Division of Neurology Products, DNP (pharmacology/toxicology) and from Dr. Marc Walton, a neurologist previously with DNP with experience with the regulation of monoclonal antibodies. Summaries of these consults follow, please refer to DFS for complete details.

Dr. Walton:

The CNS lesions observed in these disease model animal studies seem to be similar to known manifestations of anthrax infection. There is not a necessity to conclude that some anthrax-unrelated process has occurred.

A direct and sole causal relationship of the antibody and the CNS lesions appears to be of lower likelihood than other mechanisms, given the good safety experience of the product in healthy animals and humans, the unconcerning cross-reactivity studies, and the relative good safety profile of other humanized monoclonal antibody products that are marketed. Nonetheless, following up on the cross-reactivity studies to confirm there is no cross-reactivity with animal or human meninges is worthwhile.

The possibility of antibody cross-reactivity with CNS/meninges ligands expressed only in presence of anthrax infection should be discussed with personnel with greater expertise in such phenomena prior to pursuing, and may be deemed low likelihood after greater reflection. If not deemed sufficiently unlikely, perhaps cross-reactivity tests using tissue samples from moribund raxibacumab-untreated disease-model animals, and/or examining brain and meninges from treated and surviving disease model animals for signs of CNS lesions would offer some useful information. If no evidence to support this process were found in these examinations, concern that this is the operative process may be decreased.

The hypothesis that requires the least new and unknown phenomena to explain the CNS findings is that proposed by the sponsor. The sponsor's data are insufficient to be convincing, but have some suggestive elements. It appears that it would be possible, although potentially difficult to provide stronger evidence for this being the process leading to the differing incidence of CNS lesions. If this is the operative process,

however, it does not pose a significant new safety issue for use in human infection. The CNS lesions appear to occur only when there is ultimately insufficient treatment to prevent demise, but that the patient would have died sooner without treatment. While certainly not a desired effect, it would not outweigh the clear expectation that the product would offer benefit to a substantial fraction of patients with anthrax infection at high risk of death without treatment.

The consult from the Division of Neurology Products Pharmacology/Toxicology reviewer recommended the following:

- We recommend that the sponsor repeat the animal efficacy studies.
    - Histopathology should be performed on all study animals, including survivors.
    - In light of the meningitis findings, the repeat studies should include measurements of raxibacumab in CSF and brain tissue.
    - Monitoring for development of anti-drug antibodies should be included in the repeat studies.
    - Animals used in the repeat studies should be verified as treatment naïve and anti-drug antibody naive prior to initiation of the study.
- We suggest, if possible, immunohistochemical staining of retained tissue samples of brain and meninges from Study # 724-G005829 and 682-G005758 for presence of raxibacumab to address the question of blood brain barrier penetration by the monoclonal antibody.

Other investigations

Mean Inhibitory Concentration (MIC)
The MIC results confirmed that bacteria cultured from the blood of challenged animals were inhibited by similar concentrations of levofloxacin and ciprofloxacin as the challenge material.

Laboratory investigations

White Blood Cells (WBCs)
WBC levels were generally stable through 24 hours post challenge and exhibited an overall increase at 48 hours post challenge. WBC levels also exhibited a less pronounced increase at 7 to 14 days post challenge, returning to comparable baseline levels by Day 28 post challenge. This pattern is consistent with the initial recruitment of WBC to the site of infection followed by the increase in circulating WBC observed in bacterial infections. For the majority of placebo-treated rabbits this pattern was not observed; most animals died before the increase in WBCs could take place. WBCs for survivors in both the levofloxacin and levofloxacin/raxibacumab groups declined to baseline levels by the end of the study, with a 2nd, less pronounced increase at 7 to 14

days post challenge. The 1 levofloxacin non-survivor also showed a similar pattern of WBC increase and decline to baseline levels prior to death, whereas the 1 levofloxacin/raxibacumab non-survivor died from a gavage error during the period of WBC increase.

WBC Differentials
With respect to WBC types, the percent of neutrophils and basophils generally increased following challenge, but subsided in surviving animals. Concurrently, the lymphocyte percentage trended in the opposite direction. Much like the lymphocyte percentages, the monocyte and eosinophil percentages were observed to decrease subtly following challenge. N/L ratios were unusually high in a large number of animals from just prior to treatment through 24 hours post challenge. Surviving animals had relatively normal N/L ratios between 7-14 days post challenge. The hematology data collected on this study suggests the animals were demonstrating non-specific indicators of infection post challenge.

Platelets
Platelets were relatively stable for the majority of animals through the pre-challenge period and up to approximately 24 hours post challenge, after which there was a clear decline. For the majority of placebo-treated rabbits (all non-survivors) and for the 1 levofloxacin/raxibacumab-treated non-survivor, the platelet counts remained depressed until time of death, whereas among levofloxacin- and levofloxacin/raxibacumab-treated survivors, the decline period was followed by a clear upward trend, with platelet levels in the majority of survivors stabilizing to at or above baseline levels by the end of the study. The thrombocytopenia secondary to bacteremia was likely due to a combination of intravascular destruction and hemophagocytosis in the bone marrow.

Red Blood Cells (RBCs)
RBC levels were variable among all rabbits during the pre-challenge period, and displayed a pattern of decline post challenge through 72 hours post challenge. These findings are consistent with the anemia frequently seen in severe bacteremia and sepsis. Anemia due to hemorrhage and decreased RBC production may be caused in part by altered erythropoiesis in the presence of mediators of sepsis and/or frequent blood sampling; extensive blood sampling in the first72 hours may have contributed to the RBC declines. This was followed, among survivors, by a period of stabilization between 3 and 7 days post challenge, and then a period of increase to baseline values through the end of the study. The 1 non-survivor in the levofloxacin group had increasing RBCs through death, whereas the 1 non-survivor in the levofloxacin/raxibacumab group died during the RBC decline phase.

C-reactive protein (CRP)
CRP levels, an indicator of systemic inflammation, were stable through the pre-challenge period, and then increased sharply between 24 and 48 hours post challenge. The majority of placebo-treated rabbits (all non-survivors) died during this period of CRP

rise, with the remaining placebo-treated rabbits demonstrating a decline in CRP through death. Levofloxacin- and levofloxacin/raxibacumab-treated survivors' CRP levels declined to baseline values by the end of the study. One survivor in the levofloxacin group (K99254) had a late rise in CRP at 7 days post challenge, which was followed by a decline to baseline levels. This rabbit did not test positive for bacteremia or toxemia (screening assay) during the study, and had its treatment triggered by temperature increase. Three survivors (K99201, K99209, K99219) in the levofloxacin/raxibacumab group had a late rise in CRP at 7 days post challenge, followed by a decline to baseline levels; all had their treatment triggered by PA toxemia and all became bacteremic. CRP levels for the 1 non-survivor in the levofloxacin group were elevated at death, whereas the 1 non-survivor in the levofloxacin/raxibacumab group died during the initial period of CRP increase.

Other Observations
Rabbits in this study generally experienced outward clinical signs consistent with inhalational anthrax, and those that succumbed to disease followed the typical progression pattern characteristic of inhalational anthrax. Hematology parameters were consistent with systemic infection, with additional evidence of anthrax lesions upon necropsy and histopathology evaluation.

Plasma PA, Anti-PA Antibodies, TNA, and Anti-Raxibacumab Antibodies

Plasma PA
The majority of rabbits tested negative for PA toxemia prior to challenge. PA values rose sharply by 24 hours post challenge. Among placebo-treated rabbits, PA values showed a slight plateau during the period from 24 to 48 hours post challenge followed by a 2nd rapid increase until death. There were no plateaus of PA levels among levofloxacin- and levofloxacin/raxibacumab-treated survivors. The majority of these rabbits had a rapid decline in PA levels following the initial rise which continued until the end of the study. The magnitude of the PA increase in the 1 levofloxacin- treated non-survivor was far less than among the majority of rabbits in the study, and PA declined to baseline by 72 hours post challenge. This was followed by a late period of PA increase starting 7 days post challenge and 2 days post termination of antibiotic dosing and continuing until death. This rabbit had its treatment triggered by PA toxemia at 24.5 hours post challenge and became bacteremic but did not develop elevated body temperature prior to death. The 1 non-survivor in the levofloxacin/raxibacumab group did not display a plateau in PA levels after the initial rise; rather the PA levels declined until death.

Anti-PA Antibodies
Due to lack of material and high raxibacumab concentrations that could affect accurate measurement of anti-PA levels, only baseline and Day 28 samples were tested. Since 2 of the rabbits in the levofloxacin group (K99205 and K99212) appeared to be outliers, the Applicant's analysis in the table below excluded the values for these 2 animals. The

mean plasma anti-PA antibody concentration at 28 days post challenge for the levofloxacin group was numerically higher (48%) than that for the levofloxacin/raxibacumab dose group, although the difference did not attain statistical significance. For the analysis which included the 2 outlier animals, the difference between dose groups was numerically larger (about 2-fold), but did not attain statistical significance. It should be noted that difference in Day 28 anti-PA antibody concentrations did not result in altered survival between the 2 groups, suggesting that the anti-PA antibody response in rabbits administered both levofloxacin and raxibacumab was adequate for survival.

Table: Summary of plasma anti-PA antibody concentrations in surviving rabbits
(from Applicant's submission)

		Plasma Anti-PA Antibody Concentration (µg/mL)[1]	
		Prechallenge	28 Day Postchallenge
Levofloxacin	N	17	17
	Mean ± SD	0.0148 ± 0.0609	78.237 ± 64.875
Levofloxacin/raxibacumab	N	19	19
	Mean ± SD	0.000 ± 0.000	52.851 ± 40.799
	P-value[2]	0.3322	0.1643

1 From Table 5.10, Appendix 2 of the PK report (Report AB50409.INF.0.043).
2 From an unpaired t-test.

Toxin Neutralization Assay (TNA)
Due to lack of material and high raxibacumab concentrations that could affect accurate measurement of TNA levels, only baseline and Day 28 samples were tested. The plasma TNA titer results for the active treatment groups are summarized in the table below. The mean plasma TNA titer at 28 days post challenge for the levofloxacin group was significantly higher (approximately 3-fold, p = 0.0050) than that for the levofloxacin/raxibacumab dose group. Like anti-PA antibodies, the difference in TNA titres did not result in altered survival between the 2 groups, suggesting that the TNA response in rabbits administered both levofloxacin and raxibacumab was adequate for survival.

Table: Summary of plasma TNA titers in surviving rabbits (from Applicant's submission)

		Plasma TNA Titer[1]	
		Prechallenge	28 Day Postchallenge
Levofloxacin	N	19	19
	Mean ± SD	0 ± 0	2099.8 ± 1944
Levofloxacin/raxibacumab	N	19	19
	Mean ± SD	0 ± 0	648.84 ± 505.98
	P-value[2]	-	0.0050

[1] From Table 5.11, Appendix 2 of the PK report (AB50409.INF.0.043).
[2] From an unpaired t-test.

Anti-Raxibacumab Antibodies
Fifteen of 18 rabbits (83%) in the levofloxacin/raxibacumab group tested positive for anti-raxibacumab antibodies on Day 28. Six of 19 (31.6%) rabbits in the levofloxacin group also tested positive for anti-raxibacumab antibodies on Day 28 although the levofloxacin only treatment group did not receive raxibacumab. The Applicant suggested the anti-PA response may have included antibodies that cross-reacted in the anti-human anti-PA assay, and given the modest incidence of anti-raxibacumab immunogenicity at low titers compared with the raxibacumab-treated animals, this explanation is plausible. The emergence of potential anti-raxibacumab immunogenicity did not negatively impact survival.

6.1.7 Subpopulations

The three treatment groups each consisted of 50% male and 50% female rabbits. Given the small numbers of animals, subpopulation analysis according to sex was not performed.

6.1.8 Analysis of Clinical Information Relevant to Dosing Recommendations

N/A

6.1.9 Discussion of Persistence of Efficacy and/or Tolerance Effects

As discussed previously, anti-PA and TNA assays were conducted on surviving animals only. All animals with the exception of one (K99234 in the levofloxacin group) were anti-PA antibody negative at baseline, and all tested positive for anti-PA antibodies at Day

28. All animals in the active treatment groups were TNA negative at baseline and tested positive for TNA at Day 28, with the exception of 1 survivor in the levofloxacin/raxibacumab group (K99224) which tested negative for TNA at baseline and at Day 28. This animal did not test positive for PA toxemia or bacteremia during the study but was positive for anti-PA antibodies at Day 28.

While surviving animals were not challenged with a second anthrax exposure after recovering from the initial exposure to test the persistence of efficacy, the presence of anti-PA antibody at 28 days suggests that protective efficacy should theoretically be present at 28 days and for the duration the antibody persists. However no formal studies have assessed the longevity or functionality of anti-PA antibody at defined time points after the initial exposure.

No studies on tolerance have been conducted.

6.1.10 Additional Efficacy Issues/Analyses

Summary of Study 789-G923702
Evaluation of the Efficacy of Raxibacumab in Combination with Ciprofloxacin for Therapeutic Treatment in the Cynomolgus Monkey Inhalation Anthrax Model

Please see the complete review by Dr. Susan McCune in the Appendix of this review. A brief summary follows:

This was a parallel-group, double-blind, randomized, placebo-controlled GLP study in 40 healthy male and female cynomolgus monkeys to evaluate the efficacy of raxibacumab in combination with ciprofloxacin as a therapeutic treatment in cynomolgus monkeys with inhalation anthrax

Efficacy conclusions:

- Both the ciprofloxacin treated group and the ciprofloxacin/raxibacumab treated group showed statistically significant superiority over placebo in both the sponsor's ITT analysis and the FDA primary analysis.
- For the FDA primary analysis, the survival rate in the placebo group was 0%, the survival rate in the ciprofloxacin treated group was 100% and the survival rate in the ciprofloxacin/raxibacumab treated group was 84.6%. The treated groups were superior to placebo ($p<0.0001$).
- There were no differences in survival rates between the two treated groups.

Safety conclusions:

- Safety data were limited in the study to findings on gross necropsy, histopathology, and serial CBCs.

- In the placebo animals, 11 animals had evidence of low CNS pathology while 1 animal had high CNS pathology. That animal inadvertently was exposed to ciprofloxacin. Only one treated animal died of anthrax disease and that animal had evidence of hemorrhagic meningitis that affected the entire brain. No surviving animals were sacrificed.
- Based on the available analyses, the pathophysiologic basis for the increased CNS inflammation seen in the pivotal efficacy trial in cynomolgus monkeys is not clear. The number of animals analyzed is small. While it is reassuring that no gross neurologic findings were seen in the surviving animals, and no brain abnormalities were seen in toxicologic studies, a potential interaction between anthrax and raxibacumab contributing to CNS inflammation and hemorrhage cannot be ruled out. It will be important to examine the histopathology in animals that die in any subsequent studies and it would be reassuring to examine the brains of those animals that survive in order to establish normal brain histology in survivors. If it is not possible to sacrifice survivors, then more formal neurologic assessments including imaging studies should be performed in subsequent studies.

7 Review of Safety

Safety Summary

The safety database consisted of 326 healthy subjects treated with the recommended dose of raxibacumab of 40 mg/kg manufactured by the M11 process (the version intended for licensure), with 303 subjects receiving a single dose (studies HGS1021-C1064 and HGS1021-C1063), 23 subjects receiving a 2nd dose 2 weeks after their first dose (study HGS1021-1063), and 20 subjects from study HGS1021-C1064 who received a 2nd dose more than 4 months after their initial dose (study HGS1021-C1069). In Study HGS1021-C1064, 48 subjects who received raxibacumab also received ciprofloxacin as part of this drug interaction study.

The M11 formulation of raxibacumab is safe for use in the adult population at the intended single IV dose of 40 mg/kg when administered after diphenhydramine pre-medication. There was only 1 death in a placebo-treated subject from injuries sustained in a motor vehicle accident which was not related to raxibacumab. In addition to this death, there were 2 additional SAEs reported, 1 case of cholecystitis and 1 case of schizophrenia, both of which were likely related to underlying conditions. There were 4 subjects (1.2%) who discontinued raxibacumab during infusion because of AEs. Two of these were cases of urticaria in subjects who did not receive premedication with diphenhydramine. AEs of severe intensity were infrequently reported (1.9%). The most frequently reported AE was headache; other commonly reported AEs included upper respiratory tract infection and rash.

As the safety database consisted of healthy volunteers, a study agent/disease interaction could not be directly assessed. While CNS AEs were reviewed, it appears from the animal models that systemic anthrax disease must be present for CNS pathology to be observed. A review for CNS safety in humans was therefore limited. Other limitations with respect to safety include the exclusion of pediatric subjects and pregnant patients from the safety database. While subjects greater than 65 years of age were enrolled in the study, they represented only 6.4%(21/326) of the population which is insufficient to make any conclusions as to whether these subjects respond differently to raxibacumab compared to subjects less than 65 years of age. Similarly, non-white subjects represented 28% of the safety database. Therefore, it is unclear how generalizable the results of raxibacumab safety assessments are to the above populations.

7.1 Methods

A review of the integrated summary of safety (ISS) database was performed for this BLA. The safety data on raxibacumab product manufactured using the M10 manufacturing process was not included in this analysis; only safety studies that used raxibacumab manufactured by the M11 process intended for licensure, were considered.
Therefore the safety database consists of 326 subjects treated with the recommended dose of raxibacumab of 40 mg/kg manufactured by the M11 process, with 303 subjects receiving a single dose (studies HGS1021-C1064 and HGS1021-C1063), 23 subjects receiving a 2nd dose 2 weeks after their first dose (study HGS1021-1063), and 20 subjects from study HGS1021-C1064 who received a 2nd dose more than 4 months after their initial dose (study HGS1021-C1069).

7.1.1 Studies/Clinical Trials Used to Evaluate Safety

Studies HGS1021-C1064, HGS1021-C1063 and HGS1021-C1069 were included in the safety database. These are briefly summarized in the table below.

Table: Clinical trials included in the safety database

Study ID	Design Control Type	Group: Entered/ Completed	Indication	Sex M/F; Median age (range); Race	Duration	Study and Control Drug, Route, Regimen, Dosing frequency
HGS-1021-C1063	P3, R, SB, PC, RD	Double dose raxibacumab:24/23 Double dose placebo: 8/5 Single dose raxibacumab: 216/206 Single dose placebo: 72/68	Safety and PK in healthy subjects with single and repeat injection	156M/164F; 39.8 (18-88); 259W, 30B, 24A, 13NL, 10MR, 5NH	Single-dose (day 0) Double-dose (days 0 and 14) with 56 day follow-up	Raxibacumab, IV Placebo, IV; Single dose (day 0), double dose (days 0 and 14)
HGS-1021-C1069	P2/3, SG. OL, RD	All 20 subjects treated with a single 40 mg/kg IV raxibacumab dose at least 4 months after an initial 40 mg/kg. Single dose administered IV during study HGS-1021-C1064	Safety and PK in healthy subjects with repeat injection	12M/8F; 38.5 (22-61); 13W, 7B, 4HL	Single-dose with 56 day follow-up	Raxibacumab, IV; single dose
HGS-1021-C1064	P2/3, R, OL, single-dose	PO cipro, IV raxi : 32/26 IV raxi: 28/22 IV cipro, IV raxi :28/22	Safety and PK in healthy subjects with concomitant use with ciprofloxacin	43M/45F; 34.4 (18-61); 53W, 31B, 3NL, 1BNH, 21HL	7 days with 56 day follow-up	Raxibacumab IV; Ciprofloxacin PO; Ciprofloxacin IV Raxibacumab IV single dose; Cipro PO Q12h, 15 doses, Cipro IV 2 doses 12 h apart

A, Asian; AI, American India; B, Black; BNH, Black and Native Hawaiian; HL Hispanic or Latino; MR, Multiracial; NH Native Hawaiian; NL Not listed; W, White
CPL, Cipro, Ciprofloxacin
OL, Open label; P1, Phase 1; P2/3, Phase 2/3; P3, Phase 3

P, Placebo; PC, Placebo-controlled; R, Randomized; RD Repeat dose; SB, Single blind; SG, Single Group; Tx, Number of subjects treated

7.1.2 Categorization of Adverse Events

Usual definitions were used in the definition and categorization of adverse events.

An adverse event was defined as any unfavorable or unintended sign, symptom, or disease that is temporally associated with the use of a study agent but is not necessarily caused by the study agent. This included worsening (e.g., increase in frequency or severity) of pre-existing conditions. All AEs that were identified from the start of study agent administration on Day 0 through Day 56 (single-dose subjects) or Day 70 (double-dose subjects) were to be recorded on the AE CRF. The record of each AE on the CRF included AE description or diagnosis, start date, end date, whether the AE was ongoing, seriousness, severity, action taken with study agent, relationship to study agent (causality), and outcome. It was required that all data fields on the AE CRF be completed.

The severity of an AE was graded using the modified DMID toxicity tables. For AEs not included in these toxicity tables, the following guidelines applied:
Mild - causing no limitation of usual activities
Moderate - causing some limitation of usual activities
Severe - causing inability to carry out usual activities
Life-threatening - potentially life threatening or disabling

A serious adverse event was defined as any AE resulting in any of the following outcomes:
- Death
- Was life threatening (i.e., was an immediate threat to life)
- Inpatient hospitalization
- Prolongation of an existing hospitalization
- Persistent or significant disability or incapacity
- Congenital anomaly or birth defect
- Medically important

The causality assessment was to be made by the investigator based on information available at the time that the SAE worksheet was completed.

7.1.3 Pooling of Data Across Studies/Clinical Trials to Estimate and Compare Incidence

All single dose exposures were pooled for analysis. This included subjects from studies HGS1021-C1064 and HGS1021-C1063. Double dose subjects were presented both separately (study HGS1021-1063, where subjects received a second dose 2 weeks after their initial dose, and study HGS1021-C1069, where subjects received a second dose more than 4 months after their initial dose) and pooled for analysis.

7.2 Adequacy of Safety Assessments

7.2.1 Overall Exposure at Appropriate Doses/Durations and Demographics of Target Populations

Subjects enrolled in the 3 analyzed safety studies all received a single target therapeutic dose of raxibacumab 40 mg/kg IV. Forty-six additional subjects received a second 40 mg/kg IV dose to investigate the immunogenicity of the product.

In study HGS1021-C1064, 5 of the first 25 subjects (20%) dosed with raxibacumab developed dermatologic events following IV raxibacumab administration. Three subjects responded to oral diphenhydramine, and 2 had discontinuation of their treatment with resolution of the event (1 with diphenhydramine, 1 spontaneous). Following these observations, the protocol was amended (9 May 2007) to specify administration of diphenhydramine 50 mg PO prior to dosing with raxibacumab. At the completion of HGS1021-C1064, rash was reported in 8 subjects (9.1%, 8/88). The incidence of rash among subjects who did not receive diphenhydramine was 6/27 (22.2%), while the incidence of rash among subjects who received diphenhydramine was 2/61 (3.3%). All subjects enrolled in the subsequent Studies HGS1021-C1063 and HGS1021-C1069 received diphenhydramine prior to raxibacumab infusion. In Study HGS1021-C1063, the rate of rash (preferred terms rash and urticaria) was 2.5% in both placebo and raxibacumab-treated subjects.

Demographics for all subjects are shown in the table below. Most subjects were white, within a median age of 38 years (range 18 to 88 years) with a similar number of males and females enrolled.

Table: Demographics of Safety Population (from Applicant's submission)

	Single-Dose (HGS1021-C1063 + HGS1021-C1064[1]) N = 263	Double-Dose ≥ 4 Months Apart (HGS1021-C1066) N = 20	Double-Dose 14 Days Apart (HGS1021-C1063) N = 23	All Raxibacumab Treated (HGS1021-C1064/ HGS1021-C1069[2] +HGS1021-C1063) N = 326
Sex				
Male	130 (45.9 %)	12 (60.0 %)	10 (43.5 %)	152 (46.6 %)
Female	153 (54.1 %)	8 (40.0 %)	13 (56.5 %)	174 (53.4 %)
Race[3]				
White	207 (73.1 %)	14 (70.0 %)	21 (91.3 %)	242 (74.2 %)
Asian	16 (5.4 %)	-	-	18 (5.5 %)
Black or African American	48 (17.0 %)	6 (30.0 %)	1 (4.3 %)	55 (16.9 %)
American Indian or Alaska Native	1 (0.4 %)	-	-	1 (0.3 %)
Native Hawaiian or Other Pacific Islander	5 (1.8 %)	-	-	5 (1.5 %)
Not Listed	14 (4.9 %)	-	1 (4.3 %)	15 (4.6 %)
Multiracial	9 (3.2 %)	-	-	9 (2.8 %)
Hispanic or Latino origin	45 (15.3 %)	3 (15.0 %)	1 (4.3 %)	50 (15.3 %)
Age (years)[4]				
n	263	20	23	326
Mean ± SD	29.0 ± 15.4	40.5 ± 13.3	46.5 ± 14.6	32.9 ± 15.3
Median	36.5	38.5	50.5	37.4
Range	(18.1, 87.9)	(22.4, 60.6)	(22.1, 76.2)	(18.1, 87.9)
Age group				
< 65 years	244 (93.3 %)	20 (100.0 %)	21 (91.3 %)	305 (93.6 %)
> = 65 years	19 (6.7 %)	-	2 (8.7 %)	21 (6.4 %)
Weight (kg)				
n	263	20	23	326
Mean ± SD	76.3 ± 17.3	78.4 ± 13.9	82.6 ± 19.2	76.9 ± 17.3
Median	74.7	77.2	79.9	75.6
Range	(44.6, 155.9)	(56.5, 98.6)	(54.8, 121.5)	(44.6, 155.9)

1 Excluded the 20 subjects who received a 2nd Raxibacumab dose in HGS1021-C1069.

2 Subjects participating in both HGS1021-C1064 and HGS1021-C1069 were counted once only with race, age, and weight based on HGS1021-C1064.

3 Subjects who checked more than one race category were counted under individual race categories as well as the multiracial category.

4 Age was calculated from the date of birth to the date of randomization.

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7.2.2 Explorations for Dose Response

One dose escalation study was performed in the raxibacumab development program. Study PAM-NH-01, which used the M10 formulation of the product, was a phase 1 single-blind, placebo-controlled, single-injection study which evaluated the safety and pharmacokinetics of 3 different single doses of PA mAb [0.3 mg/kg, 1 mg/kg and 3 mg/kg given intramuscularly (IM in 2 different sites – gluteus and vastus lateralis)] and 5 different single doses of PA mAb [1 mg/kg, 3 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg given intravenously (IV)] in healthy subjects. While this first in man study predominantly used doses well below the putative therapeutic dose and assessed the safety of a developmental product rather than the to-be-marketed scale-up product, it did not find any dose-related AEs. Dose response exploration for the M11 formulation was limited only to subjects who received 2 doses of raxibacumab 14 days apart (Study HGS1021-C1063), but would not include subjects enrolled in Study HGS1021-1069 who received

their second dose more than 4 months after the first dose, as washout was complete by this time.

The table below shows the number of treatment emergent AEs in the groups relative to the number of doses received. Note that placebo rates are not shown

Table: Number of raxibacumab-treated subjects with treatment emergent AEs

	Placebo C1063[1] N=80 (%)	Single Dose C1063+C1064[2] N=283 (%)	Double Dose C1063 Post 1st dose prior to 2nd dose N=23 (%)	Double Dose C1069 Post 1st dose prior to 2nd dose N=20 (%)	Double Dose C1063 Post 2nd dose N=23 (%)	Double Dose C1069 Post 2nd dose N=20 (%)	Total Raxibacumab Subjects[3] N=326 (%)
At least 1 AE	38 (47.5)	143 (50.5)	4 (17.4)	6 (30.0)	9 (39.1)	8 (40.0)	170 (52.1)
At least 1 SAE	-	1 (0.4)	0	0	1 (4.3)	0	2 (0.6)
At least 1 severe AE	3 (1.4)	5 (1.8)	0	0	1 (4.3)	0	6 (1.8)

[1] Includes both single (n=74) and double (n=6) dose placebo subjects.
[2] Excluded the 20 subjects who received a 2nd raxibacumab dose in HGS1021-C1069.
[3] Subjects participating in both HGS1021-C1064 and HGS1021-C1069 were counted only once.

The number of subjects reporting at least 1 AE was higher in the single dose group (50.5%) compared to either of the two double-dose groups prior to their 2nd dose (17.4% and 30%). This observation may be related to the small study numbers in the double dose groups. The number of subjects reporting at least 1 AE post 2nd dose was also lower (approximately 40% in both groups), which again could be related to small study numbers, or the absence of a dose-adverse event relationship when a second raxibacumab dose is administered 2 weeks or later following the first dose. Overall, the numbers of SAEs and AEs of severe intensity were infrequently reported.

7.2.3 Special Animal and/or In Vitro Testing

Three relevant nonclinical studies were reviewed in detail by the Pharmacology/Toxicology team and are briefly summarized here:

The initial *ex vivo* tissue cross-reactivity study in human and cynomolgus monkey tissue showed specific cytoplasmic (non-membrane) binding of raxibacumab (M10 manufacturing process) in skeletal muscle and thyroid (both species), endometrium (human only) and breast and prostate (monkey only). These tissues were identified as possible target organs for toxicity.

The second *ex vivo* tissue cross-reactivity study was performed on tissues of NZW rabbit, cynomolgus macacque, and man and tested raxibacumab from to-be-licensed M11 manufacturing process. This study showed no thyroid histopathology or abnormal levels of circulating thyroid hormones. Please refer to Dr. Terry Miller's summary in Section 4.3 Preclinical Pharmacology/Toxicology for additional information on these studies.

Regarding safety, three non-clinical studies were reviewed in detail for the purposes of the CDC IND and are briefly summarized here:

The 120-day monkey study evaluated raxibacumab 40 mg/kg given on days 0, 13, and 25, either IM or IV and again at day 69 by IM route. No difference in weight or feeding between the placebo and raxibacumab arms, and no gross pathological changes in the study arms were reported. Local inflammation resulting from trauma at site of IM injection was noted. Some animals had microcysts in the thyroid. Some animals had chronic inflammation in the prostate. These findings were consistent with the tissue cross-reactivity study above. CPK and thyroid hormones were reported to be unremarkable. Although the histological findings were not judged to be significant, these organs were mentioned as possible targets of toxicity. No anti Fab or Fc antibodies were found in these animals. There were no gross or histopathological CNS changes reported.

The rabbit embryo-fetal toxicity study tested raxibacumab 0, 40, 120 mg/kg IV on gestation days 7 and 14. No adverse effects on the fetus or mother were identified, and the NOAEL was determined to be 120 mg/kg, the highest dose tested. Minor skeletal variations were noted, but these were not considered as adverse effects, and were stated to be within the rate for such variations seen in rabbits. It was briefly noted that in this study, 4 animals (2/20 in 40 mg/kg arm, 2/20 in 120 mg/kg arm) with anti-raxibacumab antibodies had reduced serum raxibacumab levels compared to animals without such antibodies. However, caution should be exercised when interpreting the significance of these findings, as detection of the anti-raxibacumab antibodies is hindered when the raxibacumab concentration exceeds 150 mcg/mL.

7.2.4 Routine Clinical Testing

Routine physical exams, vital signs, and laboratory tests were collected for all studies.

7.2.5 Metabolic, Clearance, and Interaction Workup

Please refer to the Clinical Pharmacology review by Dr. Kimberly Bergman for details.

7.2.6 Evaluation for Potential Adverse Events for Similar Drugs in Drug Class

N/A as there are no other drugs in this drug class.

7.3 Major Safety Results

7.3.1 Deaths

There was only 1 death reported among the 326 subjects. Subject US001-002 was enrolled in study HGS1021-C1063 and randomized to the placebo double-dose group. This subject died from injuries sustained in a motor vehicle accident. A narrative of the event follows:

Subject US001-002, was a 45-year-old white female in the placebo double-dose group, and she received the infusions of study drug per protocol on Day 0 and Day 14. No relevant medical history or concomitant medications were reported. The subject was involved in a motorcycle accident that occurred 25 days after the 2nd placebo dose (Day 39), and died as a result of injuries sustained in the accident. The investigator indicated that the injuries sustained in the accident (life-threatening) were not related to study agent.

7.3.2 Nonfatal Serious Adverse Events

Three subjects (0.9%) experienced an SAE which are shown in the table below.

Table: SAEs reported across all studies

Study	Treat-ment received	Subject ID	A/S/R	MedDRA system organ class	Preferred Term	AE Start Day/ Duration (days)	Outcome	Relationship to study drug	Action taken with study drug
HGS1021-C1063	Placebo double dose	US001-002	45/F/W	Injury, poisoning and procedural	Injury	39/1	Fatal	Not related	None
HGS1021-C1063	Raxibacumab double dose	US002-017	52/F/W	Hepatobiliary disorders	Cholecystitis	24/3	Recovered/ resolved	Possibly related	None
HGS1021-C1064	Raxibacumab single dose	US001-005	48/B/M	Psychiatric disorders	Schizophrenia	5/ongoing	Not recovered/ not resolved	Probably not related	None

A/S/R: age/sex/race

There was 1 death in Study HGS1021-C1063 described in Section 7.3.1.

The narratives for the remaining SAES are provided below:

Subject US001-017
Subject US001-017, a 52-year-old white female in the raxibacumab double-dose group, developed upper abdominal pain, nausea, and low grade fever 10 days after her 2nd raxibacumab dose (Day 24). The subject was hospitalized and underwent a cholecystectomy on Day 26; the post-operative diagnosis was confirmed to be cholecystitis. Dosing was completed and no action was taken with regard to raxibacumab. The investigator indicated that the cholecystitis (severe) was possibly related to raxibacumab, and most likely caused by progression of concurrent disease as the subject had multiple risk factors for cholelithiasis including female gender, age, obesity (body weight of 111.5 kg), elevated serum glucose and elevated triglycerides.

Subject US001-005
A 48-year-old black male received his 1st dose of ciprofloxacin (500 mg PO) on Day 0 (31Jan 2007) and his raxibacumab dose (40 mg/kgIV) on Day 5 per protocol. Relevant medical history which was not disclosed at screening included diagnoses of schizophrenia, depression, and bipolar disorder. There were no concomitant medications taken. The subject completed ciprofloxacin dosing as planned on Day 7. At the Day 47 follow-up visit, the subject reported auditory hallucinations occurring 2 to 3 times per week. He stated that the auditory hallucinations began after receiving raxibacumab; however, he did not report this to the clinical staff at that time. The subject denied suicidal ideation. The subject denied prior history of symptoms before entering the study and denied any history of psychiatric illness for himself or his family. A physical examination was within normal limits with no evidence of agitation, anxiety or depression. The subject left the clinical unit prior to completing further psychiatric evaluation. The subject later returned for evaluation and disclosed a past medical history of depression, bipolar disorder, and schizophrenia dating back to his early 20s. On Day 61, the subject was diagnosed with schizophrenia and prescribed olanzapine. As the subject received all 15 ciprofloxacin doses and the single raxibacumab dose per study schedule prior to disclosing the SAE, no action was taken with regard to study agents. The investigator revised the event term to schizophrenia with a severity grading of life-threatening, and indicated that it was probably not related to raxibacumab or ciprofloxacin. The schizophrenia was ongoing at the end of the study.

MO comment: The SAEs of cholecystitis and schizophrenia are unlikely to be due to raxibacumab based on temporal presentation and the presence of pre-existing conditions which could account for the SAEs.

7.3.3 Dropouts and/or Discontinuations

There were no subject withdrawals due to AEs. However, there were 4 subjects (1.2%) who received active treatment who experienced AEs and discontinued treatment, as shown in the table below.

Table: Subjects with AEs resulting in discontinuation of treatment

Study	Treatment received	Subject ID	A/S/R	Preferred Term	Serious/ Severity	Study Day	Relationship to study drug	Notes
HGS1021-C1064	Cipro/raxibacumab	US003-006	36/F/W	Urticaria	No/mild	5	Probably related	No premedication with diphenhydramine
HGS1021-C1064	Cipro/raxibacumab	US003-028	56/F/W	Clonus	No/mild	5	Probably related	"Left arm and leg clonic muscular contractions" and "facial flushing", both mild and resolved in 1 day; no change in vital signs
HGS1021-C1064	Raxibacumab single dose	US003-002	33/F/W	Urticaria	No/mild	1	Probably related	No premedication with diphenhydramine
HGS1021-C1063	Raxibacumab single dose	US002-011	26/M/B	Dyspnea	No/moderate	0	Probably not related	"Difficulty breathing" of moderate severity during infusion; no symptoms of anaphylaxis or rash, no changes in vital signs; investigator attributed the subject's symptoms as most likely due to esophageal spasm caused by rapid eating prior to the infusion and/or anxiousness
HGS1021-C1063	Placebo double dose	US001-022	59/M/W	Skin infection	No/moderate	12	-	

A/S/R: age/sex/race

There were 2 subjects who discontinued treatment due to urticaria. Both of these subjects were enrolled in Study HGS1021-C1064 and received partial doses of raxibacumab due to mild urticaria at the time of raxibacumab dosing. Neither of these subjects received premedication with diphenhydramine prior to raxibacumab infusion. Subject US003-006 subsequently was given diphenhydramine for generalized urticaria

with resolution of the AE, while subject US003-002 had resolution of the urticaria without any intervention.

The other subject from Study HGS1021-C1064 who discontinued treatment was Subject US003-028. This was a 56 year old white female who received a PO Cipro/IV raxibacumab and developed "left arm and leg clonic muscular contractions" and "facial flushing", both coded as mild and non-serious. The clonus led to discontinuation of the raxibacumab infusion, and both AEs resolved in 1 day.

There were 2 subjects enrolled in Study HGS1021-C1063 who had treatment discontinuation. Subject US002-011 was randomized to a single dose of raxibacumab, and described the event "difficulty breathing" of moderate severity and non-serious during infusion and only received a partial dose. There were no other associated AEs of anaphylaxis or rash, and no changes in vital signs. The subject recovered within 20 minutes of discontinuation of the drug. The investigator attributed the subject's symptoms as most likely due to esophageal spasm caused by rapid eating prior to the infusion and/or anxiousness. This subject completed follow-up. Subject US001-022 had the AE of moderate skin infection on his left hand on Day 12 which was not related to raxibacumab and resolved. This subject did not receive his second placebo dose but completed follow-up.

MO comment: Urticaria was likely related to raxibacumab, as neither subject received premedication with diphenhydramine as recommended.

With respect to the clonic muscular contractions and facial flushing in Subject US003-028, one condition which would relate the two AEs is serotonin syndrome. Typically symptom onset is rapid, with a triad of abnormalities seen: Cognitive effects (headache, agitation), autonomic effects (tachycardia, diaphoresis, hypertension, hyperthermia) and somatic effects (clonus, tremor). The subject's vital signs were unchanged 30 minutes post-dose and no other symptoms were recorded. Although her symptoms resolved upon discontinuation of raxibacumab without additional intervention suggesting a possible relationship to raxibacumab, the investigator also noted anxiety could account for these symptoms.

The AE of dyspnea was unlikely related to raxibacumab as per the investigator's assessment.

7.3.4 Significant Adverse Events

AEs of severe intensity were reported in 1.9% of raxibacumab-treated subjects and are shown in the table below.

Table: Severe AEs across studies

MedDRA system organ class	MedDRA Preferred Term	Single Dose C1063+C1064 N=283 (%)	Double Dose C1063 N=23 (%)	Total raxibacumab subjects* N =326
Hepatobiliary disorders	Cholecystitis	0	1 (4.4)	1 (0.2)
Infections and infestations	Influenza	1 (0.4)	0	1 (0.2)
Investigations	Blood amylase increased	1 (0.4)	0	1 (0.2)
	Blood creatine phosphokinase increased	1 (0.4)	0	1 (0.2)
	Prothrombin time prolonged	1 (0.4)	0	1 (0.2)
Psychiatric disorders	Schizophrenia	1(0.4)	0	1 (0.2)

*There were no AEs of severe intensity reported in the double dose separated by ≥4 months group (Study HGS1021-1069) although they are included in the Total Subject count

There were 2 severe and serious AEs in raxibacumab treated subjects (cholecystitis, schizophrenia) which were described in Section 7.3.2. In placebo-treated subjects, there was a single event of severe leukocytosis reported.

Influenza

Subject US004-000030 was a 22 year old white male enrolled in Study HGS-1021-C1063 randomized to the single dose raxibacumab arm. The subject had the following AEs recorded: rhinitis (study day 7), clavicle fracture (study day 25), influenza (study day 36), headache (study day 36), vomiting and pyrexia (study day 41). All were non-serious (all coded as mild except clavicle fracture which was moderate and influenza which was severe), no action was taken for any of the AEs and all resolved at the end of study except for the clavicle fracture. Given the delayed onset of influenza, it is unlikely to be related to study drug.

Blood amylase increased

Subject US003-00002 was a 54 year old white male enrolled in Study HGS-1021-C1063 randomized to the single dose raxibacumab arm. His screening amylase was elevated at 159 U/L(range 28-100 U/L) but was normal on days 0 and 1. On study day 14, it was elevated again at 208 U/L, but was normal again by study day 28. The subject had several readings of elevated glucose throughout the study. No action was taken for the elevated amylase level.

MO comment: Given the episodic elevations in amylase prior to enrollment in the study and after discharge, it is possible that the subject had chronic pancreatitis. While this could have been due to raxibacumab, the elevated level at screening and again at day 14 suggests a different etiology.

Blood creatine phosphokinase increased
Subject US003-000042 was a 22 year old black male enrolled in Study HGS-1021-C1064 randomized to the IV Cipro/IV Raxibacumab/PO Cipro arm. On study day 42, the subject had elevated blood creatine phosphokinase (5206 U/L, normal 24-204 U/L), as well as elevated AST (91 IU/L, normal 0-40 IU/L) although the ALT, ALP and GGT were normal (45 U/L, range 0-55 U/L; 25 IU/L, range 25-150 IU/L; and 12 IU/L, range 0-65 IU/L respectively), as well as amylase, total bilirubin and LDH. The CK elevation was deemed severe while the liver enzyme elevation was coded as mild. These AEs were noted for 15 days, on day 56 the CK was 87 U/L. No action was taken regarding these laboratory findings and the investigator thought these AEs were probably not related to the study drug.

MO comment: The Sponsors' Integrated Summary of Safety stated that the CK elevation was temporally related to increased physical activity and not accompanied by any clinical symptoms indicating an acute cardiac event.

Prothrombin time prolonged
Subject US003-000001 was a 26 year old white female enrolled in Study HGS-1021-C1063 randomized to the single dose raxibacumab arm. The subject had a normal PT ratio (INR) on study day 0 (1.2, normal 0.9-1.3), but was found to be prolonged on study day 28 at 2.5. All other hematologic parameters were normal. No action was taken and the investigator did not think this was related to the study drug.

In summary, none of the AEs of severe intensity were likely related to raxibacumab.

7.3.5 Submission Specific Primary Safety Concerns

Central Nervous System
Based on the increased CNS findings in raxibacumab-treated animals that died of anthrax in the pivotal efficacy studies, CNS AEs were examined more closely in the human safety database.

In the SOC "Nervous system disorders", the following AEs were reported.

Table: Nervous system disorders across all studies

MedDRA system organ class	MedDRA Preferred Term	Placebo* C1063 N=80 (%)	Single Dose C1063+ C1064 N=283 (%)	Double Dose C1063 N=23 (%)	Double Dose C1069 N=20 (%)	Total Raxiba cumab Subjects N=326 (%)
Nervous system	Clonus	0	1 (0.4)	0	0	1 (0.2)
	Dizziness	1 (1.3)	3 (1.1)	0	0	3 (0.6)

	Headache	8 (10)	25 (8.8)	1 (4.3)	5 (25)	31 (9.5)
	Lethargy	1 (1.3)	0	0	1 (5)	1 (0.2)
	Paraesthesia	0	1 (0.4)	0	0	1 (0.2)
	Somnolence	0	4 (1.4)	1 (4.3)	0	5 (1.5)
	Syncope vasovagal	0	2 (0.7)	0	0	2 (0.4)
	Tension headache	2 (2.5)	0	0	0	0

* Includes both single (n=74) and double (n=6) dose placebo subjects

The main CNS finding in non-survivors on necropsy in the animal studies was meningitis, with the pathologic findings of inflammation, hemorrhage and necrosis. There were no CNS infections reported in the SOC "Infections and infestations" across all studies. Symptoms suggestive of meningitis are headache, fever and meningismus. Headache is nonspecific and was reported in similar numbers of placebo and raxibacumab treated subjects (10% and 9.5%, respectively). There were an additional 2 reports of tension headache in the placebo group.

With respect to subjects reporting any combination of the triad of symptoms suggestive of meningitis, there were no reports of meningismus across studies. There was 1 raxibacumab treated subject (US004-000030) enrolled in study HGS1021-C1063 who reported fever (mild) on day 41 in conjunction with vomiting (mild), both of 1 day duration with no action taken. Similarly, there was 1 placebo subject US005-000046 enrolled in Study HGS1021-C1063 with pyrexia and headache reported on Study day 36, again of 1 day's duration with no intervention. It is unlikely that either of these represented cases of meningitis.

Rash

In Study HGS1021-C1064, 5 of the first 25 subjects (20%) enrolled developed Grade 1 to Grade 2 rashes following IV raxibacumab administration which resolved with PO diphenhydramine or without medication. Three subjects responded to oral diphenhydramine, and 2 had discontinuation of their treatment with resolution of the event (1 with diphenhydramine, 1 spontaneous). Following a protocol amendment, subsequent subjects were to be treated with PO diphenhydramine (50 mg) up to 60 minutes prior to the infusion of raxibacumab. Subjects who developed a rash following infusion were to be treated with PO diphenhydramine (25 to 50 mg) Q8h as needed.

Following this amendment, only 3 additional cases of rash were reported, for a total of 8/88 (9.1%) subjects with mild to moderate infusion related rashes, none of which were above Grade 2 severity. The overall incidence of rash among subjects who did not receive diphenhydramine was 6/27 (22.2%), while the incidence of rash among subjects who received diphenhydramine was 2/61 (3.3%). All subjects enrolled in the subsequent Studies HGS1021-C1063 and HGS1021-C1069 received diphenhydramine prior to raxibacumab infusion. In Study HGS1021-C1063, the rate of rash (preferred terms rash and urticaria) was 2.5% in both placebo and raxibacumab-treated subjects.

The Applicant attempted to explore the pathophysiology of these infusion related rashes by assessing a panel of immune mediators of allergic response, including histamine, immunoglobulin E levels, eosinophil counts, and a broad panel of cytokines produced by Th1 and/or Th2 cells. There was no consistent pattern indicative of a histamine-mediated allergic response in subjects who experienced rash, nor any pattern suggestive of immune stimulation or an immune complex-mediated process based on the absence of any significant differences in complement levels or cytokine panels.

The Applicant concluded that the precise etiology or pathophysiology of the observed rashes remained unclear, but argued that the clinical presentation of mild infusion-related rashes with no recurrence suggested an urticarial process associated with localized histamine release. Lastly, they correctly noted that premedication with an antihistamine was able to reduce the incidence of rash.

7.4 Supportive Safety Results

7.4.1 Common Adverse Events

Adverse events according to System Organ Class (SOC) are shown in the table below.

Table: AEs by System Organ Class

MedDRA system organ class	Placebo* N=80 (%)	Single Dose C1063+ C1064 N=283 (%)	Double Dose C1063 N=23 (%)	Double Dose C1069 N=20 (%)	Total double dose N=43 (%)	Total raxibacumab subjects N=326 (%)
Blood and lymphatic system disorders	3 (3.8)	8 (2.8)	0	0	0	8 (2.5)
Cardiac disorders	1 (1.3)	2 (0.7)	0	0	0	2 (0.6)
Ear and labyrinth disorders	1 (1.3)	3 (1.1)	1 (4.4)	0	1 (2.3)	4 (1.2)
Eye disorders	0	3 (1.1)	0	0	0	3 (0.9)
Gastrointestinal disorders	9 (11.3)	26 (9.2)	2 (8.7)	2 (10.0)	4 (9.3)	30 (9.2)
General disorders and administration site conditions	3 (3.8)	22 (7.8)	0	2 (10.0)	2 (4.7)	24 (7.4)
Hepatobiliary disorders	0	0	1 (4.4)	0	1 (2.3)	1 (0.3)
Infections and infestations	15 (18.8)	30 (10.6)	5 (21.7)	2 (10.0)	7 (16.3)	37 (11.3)
Injury, poisoning and procedural complications	7 (8.8)	12 (4.2)	3 (13.0)	2 (10.0)	5 (11.6)	17 (5.2)
Investigations	1 (1.3)	12 (4.2)	1 (4.4)	0	1 (2.3)	13 (4.0)
Metabolism and nutrition disorders	1 (1.3)	1 (0.4)	1 (4.4)	0	1 (2.3)	2 (0.6)
Musculoskeletal and connective tissue disorders	6 (7.5)	20 (7.1)	2 (8.7)	1 (5.0)	3 (7.0)	23 (7.1)
Nervous system disorders	11 (13.8)	35 (12.4)	2 (8.7)	6 (30.0)	8 (18.6)	43 (13.2)
Psychiatric disorders	0	4 (1.4)	0	0	0	4 (1.2)
Reproductive system and breast disorders	1 (1.3)	3 (1.1)	0	0	0	3 (0.9)
Respiratory, thoracic and mediastinal disorders	5 (6.3)	15 (5.3)	0	0	0	15 (4.6)
Skin and subcutaneous tissue disorders	4 (5.0)	29 (10.3)	0	1 (5.0)	1 (2.3)	30 (9.2)
Social circumstances	0	1 (0.4)	0	0	0	1 (0.3)
Vascular disorders	2 (2.5)	5 (1.8)	0	0	0	5(1.5)

* includes both single (n=74) and double (n=6) dose placebo subjects

The most frequently reported SOCs among raxibacumab-treated subjects were Nervous system disorders (13.2%), Infections and infestations (11.3%), and Gastrointestinal disorders and Skin and subcutaneous tissue disorders (both 9.2%). These were all higher in the raxibacumab-treated subjects compared to placebo.

Adverse events by preferred terms (PT) occurring at a frequency >0.5% are shown the table below.

Table: AEs by PT occurring at frequency >0.5% across all studies

MedDRA system organ class	MedDRA Preferred Term	Placebo N=80 (%)	Single Dose C1063+ C1064 N=283 (%)	Double Dose C1063 N=23 (%)	Double Dose C1069 N=20 (%)	Total double dose N=43 (%)	Total raxibacumab subjects N=326 (%)
Blood and lymphatic system disorders	Anemia	0	2 (0.7)	0	0	0	2 (0.4)
	Leukocytosis	2 (2.5)	0	0	0	0	0
	Leukopenia	0	2 (0.7)	0	0	0	2 (0.4)
	Lymphadenopathy	0	2 (0.7)	0	0	0	2 (0.4)
Cardiac disorders	Palpitations	0	2 (0.7)	0	0	0	2 (0.4)
Ear and labyrinth disorders	Tympanic membrane perforation	0	0	1 (4.4)	0	1 (2.3)	1 (0.2)
	Vertigo	0	3 (1.1)	0	0	0	3 (0.6)
Gastrointestinal disorders	Abdominal pain	2 (2.5)	4 (1.4)	0	1 (5.0)	1 (2.3)	5 (1.5)
	Abdominal pain upper	0	2 (0.7)	0	0	0	2 (0.4)
	Diarrhea	2 (2.5)	4 (1.4)	2 (8.7)	0	2 (4.7)	6 (1.8)
	Gastroesophageal reflux disease	0	0	0	1 (5.0)	1 (2.3)	1 (0.2)
	Nausea	3 (3.8)	8 (2.8)	0	0	0	8(2.5)
	Stomach discomfort	0	2 (0.7)	0	0	0	2 (0.4)
	Toothache	0	3 (1.1)	0	0	0	3 (0.6)
	Vomiting	2 (2.5)	1 (0.4)	0	0	0	1 (0.3)
General disorders and administration site conditions	Fatigue	0	3 (1.1)	0	0	0	3 (0.6)
	Infusion site extravasation		2 (0.7)	0	0	0	2 (0.4)
	Infusion site pain	0	3 (1.1)	0	0	0	3 (0.6)
	Edema peripheral	0	3 (1.1)	0	0	0	3 (0.6)
	Pain	0	0	0	1 (5.0)	1 (2.3)	1 (0.2)
Hepatobiliary disorders	Cholecystitis	0	0	1 (4.4)	0	1 (2.3)	1 (0.2)
Infections and infestations	Gastroenteritis	1 (1.3)	2 (0.7)	0	0	0	2 (0.4)
	Nasopharyngitis	0	2 (0.7)	0	0	0	2 (0.4)

	Oral herpes	0	2 (0.7)	0	0	0	2 (0.4)
	Pharyngitis streptococcal	4 (5.0)	2 (0.7)	0	0	0	2 (0.4)
	Pyelonephritis	0	0	0	1 (5.0)	1 (2.3)	1 (0.2)
	Rhinitis	1 (1.3)	3 (1.1)	0	0	0	3 (0.6)
	Sinusitis	1 (1.3)	4 (1.4)	1 (4.4)	0	1 (2.3)	5 (1.5)
	Tooth abscess	0	2 (0.7)	0	0	0	2 (0.4)
	Upper respiratory tract infection	4 (5.0)	11 (3.9)	4 (17.4)	1 (5.0)	5 (11.6)	16 (4.9)
	Urinary tract infection	2 (2.5)	0	0	0	0	0
Injury, poisoning and procedural complications	Excoriation	1 (1.3)	3 (1.1)	0	0	0	3 (0.6)
	Incision site pain	0	0	0	1 (5.0)	1 (2.3)	1 (0.2)
	Joint sprain	0	4 (1.4)	1 (4.4)	0	1 (2.3)	5 (1.5)
	Muscle strain	0	2 (0.7)	0	0	0	2 (0.4)
	Procedural pain	0	0	1 (4.4)	0	1 (2.3)	1 (0.2)
	Skin laceration	2 (2.5)	0	0	0	0	0
	Sunburn	0	0	1 (4.4)	0	1 (2.3)	1 (0.2)
Investigations	Blood amylase increased	0	3 (1.1)	1 (4.4)	0	1 (2.3)	4 (0.8)
	Blood creatine phosphokinase increased	0	3 (1.1)	0	0	0	3 (0.6)
	Blood pressure increased	0	2 (0.7)	0	0	0	2 (0.4)
	Prothrombin time prolonged	0	2 (0.7)	0	0	0	2 (0.4)
Metabolism and nutrition disorders	Hyperglycemia	0	0	1 (4.4)	0	1 (2.3)	1 (0.2)
Musculoskeletal and connective tissue disorders	Arthralgia	3 (3.8)	3 (1.1)	0	0	0	3 (0.6)
	Back pain	0	2 (0.7)	2 (8.7)	0	2 (4.7)	4 (0.8)
	Bone pain	0	0	1 (4.4	0	1 (2.3)	1 (0.2)
	Joint stiffness	0	0	0	1 (5.0)	1 (2.3)	1 (0.2)
	Muscle spasms	0	1 (0.4)	1 (4.4)	0	1 (2.3)	2 (0.4)
	Myalgia	1 (1.3)	4 (1.4)	0	0	0	4 (0.8)
	Pain in extremity	1 (1.3)	7 (2.5)	0	0	0	7 (2.1)

Nervous system disorders	Dizziness	1 (1.3)	3 (1.1)	0	0	0	3 (0.6)
	Headache	8 (10)	25 (8.8)	1 (4.4)	5 (25.0)	6 (14)	31 (9.5)
	Lethargy	0	0	0	1 (5.0)	1 (2.3)	1 (0.2)
	Somnolence	0	4 (1.4)	1 (4.4)	0	1 (2.3)	5 (1.5)
	Syncope vasovagal	0	2 (0.7)	0	0	0	2 (0.4)
	Tension headache	2 (2.5)	0	0	0	0	0
Psychiatric disorders	Insomnia	0	2 (0.7)	0	0	0	2 (0.4)
Reproductive system and breast disorders	Dysmenorrhea	0	2 (0.7)	0	0	0	2 (0.4)
Respiratory, thoracic and mediastinal disorders	Cough	3 (3.8)	5 (1.8)	0	0	0	5 (1.5)
	Nasal congestion	0	4 (1.4)	0	0	0	4 (0.8)
	Pharyngolaryngeal pain	2 (2.5)	0	0	0	0	0
	Rhinitis allergic	0	2 (0.7)	0	0	0	2 (0.4)
	Sinus congestion	0	2 (0.7)	0	0	0	2 (0.4)
Skin and subcutaneous tissue disorders	Acne	0	3 (1.1)	0	0	0	3 (0.6)
	Dermatitis	1 (1.3)	2 (0.7)	0	0	0	2 (0.4)
	Dry skin	0	0	0	1 (5.0)	1 (2.3)	1 (0.2)
	Pruritus	0	7 (2.5)	0	0	0	7 (2.1)
	Rash	1 (1.3)	6 (2.1)	0	0	0	6 (1.8)
	Rash erythematous	0	2 (0.7)	0	0	0	2 (0.4)
	Urticaria	1 (1.3)	4 (1.4)	0	0	0	4 (0.8)
Vascular disorders	Hypertension	0	3 (1.1)	0	0	0	3 (0.6)

Central Nervous system

In the Nervous system disorders SOC, headache was by far the most frequently reported AE and was responsible for the difference in rates observed between the placebo and raxibacumab treated subjects. Headache was reported in 8.8% of single dose subjects, 14% of double dose subjects, and 10% of placebo subjects.

Infections

In contrast, within the Infections and infestations SOC, upper respiratory tract infection occurred in 11.6% of double dose raxibacumab subjects, compared to 3.9% of single dose raxibacumab subjects and 5.0% of placebo subjects. However, other infections were not reported more frequently in the active treatment groups to suggest a uniform immune effect of raxibacumab. All AEs in the raxibacumab treated subjects ranged in frequency from 0.2% to 4.9%.

Gastrointestinal system

In the Gastrointestinal disorders SOC, rates of abdominal pain, diarrhea, nausea and vomiting were all higher in the placebo group compared to all raxibacumab subjects.

Skin and subcutaneous tissue system
In the Skin and subcutaneous tissue disorders SOC, AEs reported by the raxibacumab treated subjects ranged from 0.2% to 2.1% in frequency. Pruritis and rash were reported in all raxibacumab subjects at 2.1% and 1.8% respectively, and were comparable to placebo rates (pruritis 0%, rash 1.3%). As previously mentioned in Section 7.2.1, 5 of 25 subjects (20%) in Study HGS1021-C1064 developed dermatologic events following IV raxibacumab administration. As a result of these findings, the study protocol was amended so that all subsequent subjects received diphenhydramine before raxibacumab infusion. At the completion of HGS1021-C1064, 27 subjects did not receive diphenhydramine, of which 6/27 (22%) developed rash. A total of 8/88 subjects (9.1%) reported rash in the study overall.

7.4.2 Laboratory Findings

Laboratory tests with toxicity grade greater than or equal to 3 (severe) were infrequently reported. The table below summarizes these results.

Table: Laboratory tests with toxicity grade greater than or equal to 3 (severe)

Lab Test code	Single Dose C1063+C1064 N=283 (%)	Double Dose C1063 N=23 (%)	Double Dose C1069 N=20 (%)	Total raxibacumab subjects N=326 (%)
ALT	1 (0.4)	0	0	1 (0.3)
Amylase	1 (0.4)	0	0	1 (0.3)
Potassium	1 (0.4)	1 (4.4)	0	2 (0.6)
PT	2 (0.7)	0	1 (5.0)	3 (0.9)
WBC	2 (0.7)	0	0	2 (0.6)

Each of these is discussed in its relevant section.

Hematology
Hematologic changes were infrequently reported across studies. The rates of Grade 2 and higher hematology laboratory abnormalities were comparable between placebo-treated subjects and raxibacumab-treated subjects. Among raxibacumab-treated subjects, there was 1 subject each with Grade 3 leukocytosis (Subject US001-069) and Grade 4 neutropenia (Subject US001-029), and 2 subjects with Grade 3 PT (Subjects US006-030, US003-001, and US002-007). All of these were reported in single dose raxibacumab subjects, and each laboratory abnormality resolved on-study.

In the double-dose cohorts, one subject (a 24 year-old white female in study HGS1021-C1069, raxibacumab post-2nd dose) had a Grade 4 PT

Hematology Narratives

Leukocytosis

Subject US001-069 (24 year-old white female in Study HGS1021-C1063, raxibacumab single-dose group) had a Grade 1 (11.44 x 103/μL) white blood cell (WBC) count at baseline, and a transient shift to Grade 3 WBC count (22.17 x 103/μL) on Day 2. This event was recorded as a mild AE (not related, resolved). The subject also had AEs of moderate abdominal pain on Day 3 (not related, resolved), which lasted for 3 days, and mild malaise on Day 4 (possibly related to study agent, resolved), which lasted for 1 day. The investigator indicated that the elevated WBC count was probably not related to raxibacumab and that the most likely cause of the event was a laboratory error.

Neutropenia

Subject US006-029 (35 year-old white female in Study HGS1021-C1063, raxibacumab single-dose group) had a Grade 0 (4.46 x 103/μL) neutrophil count at baseline, which shifted to Grade 4 (0.41 x 103/μL) on Day 28. This subject's neutrophil count returned to baseline levels (1.7 x 103/μL) by Day 33. This event was recorded as a mild AE (resolved) that was possibly related to study agent.

PT

Subject US006-030 (41 year-old white male in Study HGS1021-C1063, raxibacumab single-dose group) had a Grade 0 PT (10.3 seconds) at baseline, and an isolated shift to Grade 3 (25.7 seconds) PT on Day 28. All prior assessments of coagulation studies (PT, PTT, and INR) were within reference range. The subject was asymptomatic. Upon repeat testing 6 days later, the value normalized. The investigator indicated that the elevated PT was probably not related to raxibacumab, and that the most likely cause of the event was a laboratory error. This event was recorded as a mild AE (not related, resolved). No other AEs were reported.

Subject US003-001 (26 year-old white female in Study HGS1021-C1063, raxibacumab single-dose group) had a Grade 0 PT (11.7 seconds) at baseline, and an isolated shift to Grade 3 (23.9 seconds) on Day 28. Concurrent activated PTT (APTT) was Grade 1 (47 seconds). All prior coagulation studies (PT, PTT, and INR) for this subject had been within normal limits. The subject was asymptomatic. On repeat testing 3 days later, the results normalized. This event was recorded as a Grade 3 (severe) AE, which was not related to study agent, and which resolved. The investigator indicated that the most likely cause of the event was an error in specimen collection, processing, or analysis.

Subject US002-007 (24 year-old white female in Study HGS1021-C1069, raxibacumab post-2nd dose) had fluctuating PT and PTT levels. This subject had a Grade 2 (15.0 seconds) PT at screening and Grade 1 (12.7 seconds) upon rescreening 6 days later. The subject's PT shifted to Grade 2 (16.7 seconds) on Day 7, decreased to Grade 0 by Day 14, increased again to Grade 3 on Day 42 (17.6 seconds) and Day 56 (27.5 seconds), and further shifted to Grade 4 (37.9 seconds) on Day 70. PT Grades of 2 and 3 respectively were observed at 2 unscheduled assessments done after the end of the

study period (103 days after raxibacumab dosing). PT had returned to Grade 0 at a post-study, unscheduled assessment performed 112 days after raxibacumab infusion. This subject also had a Grade 0 PTT (32 seconds) at baseline, which shifted to Grade 2 (48 seconds) on Day 70. The PTT had returned to baseline at the unscheduled assessment conducted 112 days after raxibacumab infusion. The subject remained asymptomatic throughout this period and had no complaints of bleeding or easy bruising, and no other clinically significant lab abnormalities. No AEs were reported. The additional laboratory assessments for this subject were conducted to verify the abnormal results, and thus did not trigger the recording of the abnormal laboratory values as AEs at the discretion of the investigator as the subject had no clinical symptoms. No relevant medical history was reported for this subject. The subject moved overseas and was not available for further testing. The laboratory reports were sent to the subject so that she could follow-up with specialists. Relationship to study agent was judged to be probably not related.

Hepatic parameters
There was 1 subject in Study C1063 (US004-000057) with a grade 3 ALT (304 IU/L, range 0-40 IU/L) on day 56 (16 July 2008). This subject also had a Grade 1 AST elevation of 97 IU/L (range 0-40 IU/L) and a Grade 0 elevation in ALP (154 IU/L, range 25-150 IU/L) on the same day. However, when these tests were repeated on 28 July and 4 Aug 2008, the ALT was 146 IU/L and 57 IU/L and the AST was 90 IU/L and 34 IU/L respectively.

Electrolytes and other laboratory tests
Subjects treated with 2 raxibacumab doses generally did not have a higher incidence of electrolyte shifts from baseline compared with those treated with a single dose. Time between the 2 raxibacumab doses did not appear to influence the incidence of electrolyte shifts from baseline.

There were 2 subjects with a shift to Grade 3 or higher laboratory value for electrolytes. Subject US003-004 enrolled in study HGS1021-C1064 was a 51 year-old white male randomized to raxibacumab single dose who had Grade 0 (5.1 mEq/L) potassium level at baseline with an isolated shift to Grade 3 (6.7 mEq/L) on Day 4. This event resolved without treatment and no AEs

Subject US005-000002 enrolled in C1063 randomized to double dose raxibacumab with a baseline potassium of 8.3 mEq/L (Grade 4) on Day 0. On Study day 1, the level was normal at 4.3 mEq/L and the subject continued in the study. No additional information was available.

With respect to other laboratory tests, there was 1 subject with a Grade 3 amylase. The narrative is provided below.

Electrolytes and other laboratory tests narratives

Amylase
Subject US003-002, a 54 year-old white male in Study HGS1021-C1063 (raxibacumab single-dose group) had a Grade 2 amylase level of 159 U/L at screening and a Grade 3 level of 208 U/L on Day 14. The screening value was considered not clinically significant by the investigator. However, the site was not aware of the grading of this value and did enroll the subject; the event was documented as a protocol violation (enrolled but did not meet inclusion/exclusion criteria). The subject was asymptomatic. Repeat assessment on Day 0 was within normal limits (amylase level of 69 U/L). All other relevant laboratory values were within normal limits and the subject was again asymptomatic. On repeat testing, lipase and amylase were within normal limits (lipase 55 U/L, amylase 83 U/L); amylase fractionation was also obtained and found to be within normal limits with no evidence of macroamylasemia. The subject underwent an abdominal ultrasound revealing steatohepatitis, with no stones or wall thickening in the gallbladder, no pericholecystic fluid, and normal pancreas. The findings were determined to be consistent with nonalcoholic steatohepatitis (NASH) and considered by the investigator to be not clinically significant. No action was taken with regard to raxibacumab. The investigator indicated that the elevated amylase (severe) was probably not related to raxibacumab. The investigator indicated that there was no clear cause of the asymptomatic recurring elevation in amylase, but that it likely pre-dated exposure to raxibacumab. This event was recorded as a Grade 3 AE (resolved).

In summary, the incidence of laboratory abnormalities was comparable between raxibacumab-treated and placebo-treated subjects (only active treatment AEs reported here). The incidence was not higher after a second raxibacumab administration than after a single administration. Most lab abnormalities were of low grade (Grade 2 or less), with grade 3 and 4 laboratory abnormalities infrequently reported, isolated and transient.

7.4.3 Vital Signs

Three subjects treated with a single raxibacumab dose in study HGS1021-C1063 had AEs of hypertension, which were judged to be not related to raxibacumab. The hypertension was ongoing at the end of the study for 2 subjects. Two additional subjects in the raxibacumab single-dose group had an AE of mild elevated blood pressure, which resolved. The elevated blood pressure for 1 subject was recorded as possibly related to study agent.

There were 2 reports of fever in study HGS1021-C1063 which were not related to study agent and resolved on study. One case was likely related to a viral infection, as fever was also reported with vomiting and influenza. The remaining case was reported concurrent with headache, both AEs lasted 2 days and resolved without intervention.

Two AEs of mild tachycardia were reported in Study HGS1021-C1063. Subject US003-000030 was a 18 year old white female with 1 day of palpitations and tachycardia reported on the same day as her single-dose raxibacumab infusion. These AEs were both mild and resolved with no intervention. The second report occurred in a double dose placebo group subject, lasted 1 day with no intervention.

7.4.4 Electrocardiograms (ECGs)

Electrocardiograms were obtained prior to enrollment in all studies, and subjects with clinically significant abnormalities were excluded from participation. Telemetric monitoring was performed in Study HGS1021-C1064 prior to dosing of raxibacumab, during the infusion, and for 2 hours following the infusion. No significant arrhythmias during this period were noted. There were no protocol-required subsequent electrocardiograms obtained after the day of raxibacumab infusion in any study.

7.4.5 Special Safety Studies/Clinical Trials

N/A

7.4.6 Immunogenicity

Non-clinical (Combination animal studies):
Anti-raxibacumab immunogenicity assessments were added to Study 781-G923701 (NZW rabbits) as a protocol amendment to allow for determination of the impact of any observed anti-raxibacumab immunogenicity on plasma raxibacumab levels and survival.

Fifteen of 18 rabbits (83%) in the levofloxacin/raxibacumab group tested positive for anti-raxibacumab antibodies on Day 28. In addition, 6/19 (31.6%) rabbits in the levofloxacin group also tested positive for anti-raxibacumab antibodies on Day 28. Although the levofloxacin only treatment group did not receive raxibacumab, the levofloxacin-treated rabbits mounted an anti-PA response which may have included antibodies that cross-reacted in the anti-human anti-PA assay. Given the modest incidence of anti raxibacumab immunogenicity at low titers compared with the raxibacumab-treated animals, this was felt to be a possible explanation proposed by the Applicant. The emergence of potential anti-raxibacumab immunogenicity did not negatively impact survival.

Human
All 4 human safety studies looked for the development of an anti-raxibacumab antibody response, including PAM-NH-01 which used the M10 formulation of the product. The table below summarizes the time points for immunogenicity assessments in Studies

HGS1021-C1064, HGS1021-C1063 (second raxibacumab dose 2 weeks following first dose) and HGS1021-C1069 (second dose ≥4 months following first dose). In all studies, there were no subjects who had a positive anti-raxibacumab antibody response following single or repeat (every 14 days or following ≥4 months) doses of raxibacumab.

Table: Immunogenicity sampling in raxibacumab clinical trials (from Clinical Pharmacology review)

Study	Sampling Schedule for Anti-Raxibacumab Antibodies
PAM-NH-01	Day 0 predose, and Days 14, 28, and 56/exit
HGS1021-C1063*	• Double-dose groups (Group 1 and Group 3): blood samples were collected prior to dosing on Day 0 and Day 14, and on Days 42 and 70/End of Study. • Single-dose groups (Group 2 and Group 4): blood samples were collected prior to dosing on Day 0, and on Days 28 and 56/End of Study.
HGS1021-C1064*	• Group 1: Blood samples were collected on Days 0 (prior to raxibacumab dose), 33, and 61/End of Study. • Group 2 and Group 3: Blood samples were collected on Days 0 (prior to prior to raxibacumab dose), 28, and 56/End of Study.
HGS1021-C1069*	Screening, prior to raxibacumab dosing on Day 0, and on Days 28, 56, and 70 (End of Study)

*Subjects found to have a positive anti-raxibacumab antibody titer following raxibacumab administration were to have serum collected approximately 6 months after the dose for follow-up immunogenicity assessment.

Immunogenicity testing consisted of screening and confirmatory assays. The screening assay (direct binding) was an electrochemiluminescence (ECL)-based bridging assay, with rabbit polyclonal antibody used as a positive control. Samples identified as positive in the screening assay were to be confirmed positive in a confirmatory assay. The inhibition of binding confirmatory assay was performed identically to the direct binding screening assay with the exception that the samples were tested in parallel with excess unlabeled raxibacumab.

7.5 Other Safety Explorations

Non-clinical

In the first *ex vivo* tissue cross-reactivity studies in humans and cynomolgus monkeys which used the M10 formulation, there was specific binding in skeletal muscle and thyroid in both species, which identified these tissues as possible target organs for toxicity. However, there was no thyroid histopathology or abnormal levels of circulating thyroid hormones in the follow-up study which used the M11 formulation of the product. There were no thyroid AEs reported in the SOC "Metabolism and nutrition disorders".

Clinical
There were 3 AEs of "blood creatine phosphokinase increased", one case which was severe and discussed in section 7.3.4 and attributed to increased physical activity. The remaining cases were both coded as mild with no action taken. Subject US005-000021 was a 39 year old white male who received a single dose of raxibacumab in study HGS1021-C1063, while subject US002-000009 enrolled in HGS1021-C1064 was a 18 year old black male who received PO cipro/IV raxi with "exacerbation of CK" 18 days after raxibacumab infusion. The AE was still ongoing at the end of follow-up for the latter subject.

7.5.1 Dose Dependency for Adverse Events

Non-clinical
There were no dose-dependent safety findings in the embryo-fetal toxicology study in rabbits ((b) (4) Study 6962-173) or in the chronic toxicology study in cynomolgus monkeys (b) (4) Study 6962-140). In the histological examination of survivors and nonsurvivors in the anthrax therapeutic efficacy studies the findings were not dose-dependent. Please refer to the Pharmacology/toxicology review for additional details.

Clinical
With respect to clinical information, data is limited to subjects who got 2 doses of the M11 formulation 2 weeks apart in Study HGS1021-C1063, and those enrolled in PAM-NH-01 (M10 formulation). There were no dose-dependent AEs observed in the former study.

7.5.2 Time Dependency for Adverse Events

There was no specific analysis to look for time dependency for adverse events performed as the proposed therapeutic regimen of raxibacumab consists of a single dose.

7.5.3 Drug-Demographic Interactions

Sex
Overall, females had a numerically higher incidence of AEs (59%) relative to males (39%). The incidences of severe AEs and SAEs, although infrequently reported, were comparable between males and females.

Table: AEs across studies stratified by sex (from Applicant's submission)

	Male Subjects N = 152	Female Subjects N = 174
At least 1 AE	59 (38.8%)	103 (59.2%)
At least 1 related[1] AE	22 (14.5%)	27 (15.5%)
At least 1 serious AE	1 (0.7%)	1 (0.6%)
At least 1 severe[2] AE	4 (2.6%)	2 (1.1%)
At least 1 related serious AE	-	1 (0.6%)
At least 1 related severe AE	-	1 (0.6%)
At least 1 grade 2 or higher AE	17 (11.2%)	28 (16.1%)
At least 1 grade 2 or higher related AE	3 (2.0%)	5 (2.9%)

[1] Possibly, probably or definitely related.
[2] Grades 3, 4, & 5 AEs, including life-threatening.

Table: AEs by SOC reported in raxibacumab treated subjects stratified by sex (from Applicant's submission)

System Organ Class	Male Subjects N = 152	Female Subjects N = 174
Blood and lymphatic system disorders	1 (0.7%)	7 (4.0%)
Cardiac disorders	1 (0.7%)	1 (0.6%)
Ear and labyrinth disorders	-	4 (2.3%)
Eye disorders	-	3 (1.7%)
Gastrointestinal disorders	10 (6.6%)	20 (11.5%)
General disorders and administration site conditions	10 (6.6%)	14 (8.0%)
Hepatobiliary disorders	-	1 (0.6%)
Infections and infestations	15 (9.9%)	22 (12.6%)
Injury, poisoning and procedural complications	6 (3.9%)	11 (6.3%)
Investigations	9 (5.9%)	4 (2.3%)
Metabolism and nutrition disorders	1 (0.7%)	1 (0.6%)
Musculoskeletal and connective tissue disorders	7 (4.6%)	16 (9.2%)
Nervous system disorders	16 (10.5%)	27 (15.5%)
Psychiatric disorders	1 (0.7%)	3 (1.7%)
Reproductive system and breast disorders	-	3 (1.7%)
Respiratory, thoracic and mediastinal disorders	4 (2.6%)	11 (6.3%)
Skin and subcutaneous tissue disorders	8 (5.3%)	22 (12.6%)
Social circumstances	1 (0.7%)	-
Vascular disorders	4 (2.6%)	1 (0.6%)

Similar to the AE observations summarized for the entire safety population earlier, the most frequent AEs among both males and females were in the Nervous system disorders SOC. While note shown, the AEs by PT were also similar, with headache the most frequently reported in both males and females at 7.2% and 11.5% respectively.

Overall, AE incidences across SOC categories were comparable between males and females, although females had a numerically higher incidence of blood and lymphatic disorder AEs (males vs. females, 0.7% vs. 4% respectively), which included leukopenia (0% vs. 1.1%) and lymphadenopathy (0% vs. 1.1%) in addition to lymph node pain and neutropenia (0% vs. 0.6% for both). All these AEs resolved on study with the exception of 1 incidence each of leukopenia and lymphadenopathy. Females also had a numerically higher incidence of skin and subcutaneous disorder AEs (males 5.3%, females 12.6%), with pruritis as the most frequent of these AEs (males 1.3%, females 2.9%).

Race

There were no differences in AE incidences, severe AEs or SAEs when stratified according to race (white and non-white) as shown in the table below. The most frequent AEs among white subjects was headache (9.8%), while in non-white subjects pruritis was the most frequently reported AE (3.3%).

Table: AEs across studies stratified by race (from Applicant's submission)

	White Subjects N = 234	Non-White Subjects N = 92
At least 1 AE	120 (51.3%)	42 (45.7%)
At least 1 related[1] AE	39 (16.7%)	10 (10.9%)
At least 1 serious AE	1 (0.4%)	1 (1.1%)
At least 1 severe[2] AE	4 (1.7%)	2 (2.2%)
At least 1 related serious AE	1 (0.4%)	-
At least 1 related severe AE	1 (0.4%)	-
At least 1 grade 2 or higher AE	34 (14.5%)	11 (12.0%)
At least 1 grade 2 or higher related AE	5 (2.1%)	3 (3.3%)

[1] Possibly, probably or definitely related.

[2] Grades 3, 4, & 5 AEs, including life-threatening.

Table: AEs by SOC reported in raxibacumab-treated subjects stratified by race
(from Applicant's submission)

System Organ Class	White Subjects N = 234	Non-White Subjects N = 92
Blood and lymphatic system disorders	6 (2.6%)	2 (2.2%)
Cardiac disorders	2 (0.9%)	-
Ear and labyrinth disorders	4 (1.7%)	-
Eye disorders	3 (1.3%)	-
Gastrointestinal disorders	22 (9.4%)	8 (8.7%)
General disorders and administration site conditions	17 (7.3%)	7 (7.6%)
Hepatobiliary disorders	1 (0.4%)	-
Infections and infestations	28 (12.0%)	9 (9.8%)
Injury, poisoning and procedural complications	13 (5.6%)	4 (4.3%)
Investigations	9 (3.8%)	4 (4.3%)
Metabolism and nutrition disorders	2 (0.9%)	-
Musculoskeletal and connective tissue disorders	19 (8.1%)	4 (4.3%)
Nervous system disorders	34 (14.5%)	9 (9.8%)
Psychiatric disorders	3 (1.3%)	1 (1.1%)
Reproductive system and breast disorders	2 (0.9%)	1 (1.1%)
Respiratory, thoracic and mediastinal disorders	11 (4.7%)	4 (4.3%)
Skin and subcutaneous tissue disorders	20 (8.5%)	10 (10.9%)
Social circumstances	-	1 (1.1%)
Vascular disorders	4 (1.7%)	1 (1.1%)

Age

The Guidance for Industry: Content and Format for Geriatric Labeling (10) published in 2001 based on the geriatric labeling regulations (CFR 201.57(f)(10) provides standard language for statements in the "Geriatric Use" subsection of the label based on the type of information available and the interpretation of that information. Fewer than 100 geriatric subjects is considered an insufficient number to determine whether these subjects respond differently compared to subjects less than 65 years of age. Of the total number of subjects in clinical studies of raxibacumab, 6.4% (21/326) were 65 and over, while 1.5% (5/326) were 75 and over. Therefore while subset analyses based on age were performed by the Applicant and are presented below, the label will include standard language which states "Clinical studies of raxibacumab did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects".

10 Guidance for Industry: Content and Format for Geriatric Labeling. October 2001

AEs among subjects <65 years of age and ≥65 years of age had similar incidences of AEs as shown in the table below. Subjects < 65 years of age had a higher incidence of Grade 2 AEs compared with subjects ≥65 years of age, although there were many more subjects in the former category (n=305 vs. n=21). There were no severe AEs or SAEs among subjects ≥65 years of age.

The most frequent AE among subjects < 65 years of age was headache (10.2%). AEs among subjects ≥65 years of age were infrequently reported. The most frequent AEs among subjects ≥65 years of age were in the SOC category for skin and subcutaneous tissue disorders and included AEs of skin pain, rash, and urticaria (1 subject each).

Table: AEs across studies stratified by age (from Applicant's submission)

	Subjects < 65 Years of Age N = 305	Subjects ≥ 65 Years of Age N = 21
At least 1 AE	153 (50.2%)	9 (42.9%)
At least 1 related[1] AE	45 (14.8%)	4 (19.0%)
At least 1 serious AE	2 (0.7%)	-
At least 1 severe[2] AE	6 (2.0%)	-
At least 1 related serious AE	1 (0.3%)	-
At least 1 related severe AE	1 (0.3%)	-
At least 1 grade 2 or higher AE	44 (14.4%)	1 (4.8%)
At least 1 grade 2 or higher related AE	8 (2.6%)	-

[1] Possibly, probably or definitely related.

[2] Grades 3, 4, & 5 AEs, including life-threatening.

Table: AEs by SOC reported in raxibacumab-treated subjects stratified by age
(from Applicant's submission)

System Organ Class	Subjects < 65 Years of Age N = 305	Subjects ≥ a ge 65 Years N =21
Blood and lymphatic system disorders	8 (2.6%)	-
Cardiac disorders	2 (0.7%)	-
Ear and labyrinth disorders	4 (1.3%)	-
Eye disorders	3 (1.0%)	-
Gastrointestinal disorders	29 (9.5%)	1 (4.8%)
General disorders and administration site conditions	22 (7.2%)	2 (9.5%)
Hepatobiliary disorders	1 (0.3%)	-
Infections and infestations	37 (12.1%)	-
Injury, poisoning and procedural complications	15 (4.9%)	2 (9.5%)
Investigations	13 (4.3%)	-
Metabolism and nutrition disorders	2 (0.7%)	-
Musculoskeletal and connective tissue disorders	23 (7.5%)	-
Nervous system disorders	42 (13.8%)	1 (4.8%)
Psychiatric disorders	4 (1.3%)	-
Reproductive system and breast disorders	3 (1.0%)	-
Respiratory, thoracic and mediastinal disorders	15 (4.9%)	-
Skin and subcutaneous tissue disorders	27 (8.9%)	3 (14.3%)
Social circumstances	1 (0.3%)	-
Vascular disorders	4 (1.3%)	1 (4.8%)

7.5.4 Drug-Disease Interactions

Non-clinical

In both NZW rabbit and cynomolgus monkey pivotal efficacy studies, raxibacumab-treated animals that died had more CNS pathology consisting of hemorrhage, necrosis, inflammation and bacteria compared to placebo animals that died. A number of hypotheses were considered investigated, although no clear explanation could be found. Longer survival time may have played some role, but in itself did not account for the increased CNS findings observed primarily in raxibacumab-treated animals.

In the consult by Dr. Marc Walton, former Director of ODE VI/CDER, he noted that the CNS lesions observed in the pivotal efficacy studies in animal models of inhalational

anthrax were similar to known manifestations of anthrax infection in animals and humans, and therefore a separate anthrax-unrelated process as the cause of these lesions (*i.e.,* drug-disease interaction) was less likely. In particular, he cited the overall safety of raxibacumab in human studies to date, lack of findings on cross-reactivity studies, and the relative good safety profile of other marketed humanized monoclonal antibody products as evidence against a drug-disease interaction. He further points out that CNS lesions appear to occur only when there is ultimately insufficient treatment to prevent demise, and that death occurs sooner without treatment. For additional analyses and discussion on non-clinical CNS findings, please refer to Section 6.1.5 Analysis of Secondary Endpoints.

7.5.5 Drug-Drug Interactions

FDA requested that Studies 781-G923701 and 789-G923702 be conducted to evaluate the effect of raxibacumab on the fluoroquinolones (levofloxacin and ciprofloxacin respectively). The efficacy results suggested that while raxibacumab did not offer additional benefit compared to antibiotics alone, there was no antagonism observed between administration of raxibacumab and antibiotic.

No other drug-monoclonal interactions have been studied with raxibacumab.

7.6 Additional Safety Evaluations

N/A

7.6.1 Human Carcinogenicity

No studies have been undertaken to evaluate carcinogenicity or genotoxicity for this fully human immunoglobulin G (IgG) antibody, consistent with the recommendations of ICH S6 (1997).

Carcinogenicity or genotoxicity studies have not been conducted with raxibacumab.

7.6.2 Human Reproduction and Pregnancy Data

There have been no human reproductive and pregnancy studies conducted. An embryo-fetal toxicity study has been conducted in NZW rabbits with negative findings, Please refer to the Pharmacology/toxicology review for details.

7.6.3 Pediatrics and Assessment of Effects on Growth

Raxibacumab has not been used in pediatric subjects (subjects ≤16 years of age); therefore there is no information on its effects on growth.

7.6.4 Overdose, Drug Abuse Potential, Withdrawal and Rebound

There is no clinical experience with overdosage of raxibacumab. In toxicity studies in rabbits and cynomolgus monkeys receiving multiple injections of raxibacumab at 3 to 4 times the recommended dose, there was no raxibacumab-related toxicity. In case of overdosage, it is recommended that the patient be monitored for any signs or symptoms of adverse reactions or effects and appropriate symptomatic treatment instituted immediately.

The product abuse and dependence potential of raxibacumab has not been examined in nonclinical or clinical studies but is expected to be negligible.

Withdrawal and rebound are not applicable to raxibacumab as the product is intended for single dose administration.

7.7 Additional Submissions / Safety Issues

Safety Limitations to Animal Models

With respect to the pivotal efficacy studies and CNS findings, the main limitation was that survivors were not sacrificed, and therefore no brain histopathology was available to compare with animals that died. While the high efficacy of antibiotics limited the number of animals that died from anthrax in the active treatment arms in both rabbit and NHP combination studies, the rabbit combination study did sacrifice survivors and necropsy was performed on all enrolled animals. While all placebo animals had microscopic findings consistent with anthrax, only 1 animal in an active treatment group died of anthrax (rabbit K99203, levofloxacin group; note rabbit K99246 in the levofloxacin/raxibacumab group died of a gavage accident). CNS analysis was limited as none of the surviving rabbits in the active treatment groups had lesions attributable to anthrax at sacrifice, or any brain lesions on microscopic examination. Therefore, the pathophysiology behind CNS findings observed in the pivotal studies remains to be elucidated. Second, the efficacy of raxibacumab cannot be directly determined in humans. While the animal models have been carefully and extensively tested and characterized, it is still unknown how well they predict efficacy in humans.

Similar limitations were noted in Study 789-G923702 (ciprofloxacin and raxibacumab in cynomolgus monkeys). No animals in the ciprofloxacin group died during the initial 28

day time period (monkey C30988 died at 36 days from left leg arterial thrombosis). Only one animal in the ciprofloxacin/raxibacumab treatment group died of anthrax disease (monkey C31142, the other death in this group was due to a gavage error in monkey C24791). None of the surviving animals were sacrificed for histopathologic analysis. As with the rabbit study, the high efficacy of antibiotics and absence of a raxibacumab alone arm limit the degree of analysis for this study.

With respect to safety as determined in human subjects, a safety database of 326 subjects cannot exclude a SAE or fatal event occurring at less than 1% frequency. In addition, as the safety database consisted only of healthy volunteers, any possible study agent/disease interaction could not be directly assessed. While CNS AEs were reviewed, it appears from the animal models that systemic anthrax disease must be present for CNS pathology to be observed. A review for CNS safety in humans was therefore limited. Other limitations with respect to safety include the exclusion of pediatric subjects and pregnant patients from the safety database. Similarly, non-white subjects represented 28% of the safety database, while subjects greater than 65 years of age represented only 6.4% of subjects; it is unclear whether results of the raxibacumab safety assessment performed for this BLA are generalizable to such populations.

8 Postmarketing Experience

There is no postmarketing experience available for raxibacumab.

9 Appendices

9.1 Literature Review/References

N/A

9.2 Labeling Recommendations

Revisions to the label are ongoing at the time of submitting this review. The following groups were consulted and their recommendations will be incorporated into the label:

- Study Endpoint and Labeling Development (SEALD)
- Office of Surveillance and Epidemiology (OSE), Divisions of Risk Management (DRISK) and Medication Error Prevention and Analysis (DMEPA)
- Neuropharmacology consult (pending)

Section 6 (Adverse Reactions) was revised according to the "Guidance on Adverse Reactions Section of Labeling for Human Prescription Drug and Biological Products". Summaries of human safety studies HGS1021-C1063, HGS1021-C1064 and HGS1021-C1069 were reported in this section; results from PAM-NH-01, which used the M10 formulation of raxibacumab were not included. Emphasis was placed on infusion reactions with and without diphenhydramine pre-treatment.

Section 14 (Clinical Studies) included all the animal efficacy studies (pivotal and combination).

9.3 Advisory Committee Meeting

As raxibacumab is a new molecular entity/first in class, this monoclonal antibody will be presented at an Advisory Committee meeting on October 27, 2009.

9.4 Individual Study Reports

Study Report HGS1021-C1063: A Randomized, Single-Blind, Placebo-Controlled Study to Evaluate the Safety and Tolerability of Raxibacumab (Human Monoclonal Antibody to *B. Anthracis* Protective Antigen) in Healthy Subjects

Study Phase: 3

Indication: Therapeutic treatment of inhalational anthrax

First Subject Enrolled: 12 March 2008

Last Subject Last Visit: 22 July 2008

Investigators and Study Centers: Multicenter (6) (US)

Study Sponsor: Human Genome Sciences, Inc., 14200 Shady Grove Road Rockville, MD 20850

Primary Objective
• To evaluate the safety and tolerability of IV administered raxibacumab in healthy subjects.

Secondary Objective
• To determine serum raxibacumab concentrations for use in a population PK analysis

Study Design
This was a randomized, single-blind, placebo-controlled study of raxibacumab to evaluate the safety and tolerability of raxibacumab administered IV in healthy subjects. Subjects were randomized to 1 of 2 raxibacumab groups (40 mg/kg double-dose administered 14 days apart via IV infusion, or 40 mg/kg IV single dose) or to 1 of 2 matching placebo groups. Subjects were randomized to a treatment group at a ratio of 3:1 (raxibacumab:placebo). All subjects were to be treated with oral diphenhydramine (25-50 mg) up to 60 minutes prior to infusion of raxibacumab.

Study subjects, investigators and clinical site staff were blinded to treatment assignment. The site pharmacist, study sponsor and Safety Data Monitoring Board were not blinded to treatment assignments.

The planned treatment groups are shown in the table below.

Table: Planned treatment groups (from Applicant's submission)

Table 5-1 Planned treatment groups

Group	# of Subjects	Treatment	Dose	Frequency
1	24	Double-dose raxibacumab	40 mg/kg IV	Dose 1 - Day 0 Dose 2 - Day 14
3	8	Double-dose placebo	Placebo IV	Dose 1 - Day 0 Dose 2 - Day 14
2	216	Single-dose raxibacumab	40 mg/kg IV	Single-dose - Day 0
4	72	Single-dose placebo	Placebo IV	Single-dose - Day 0

Safety Data Monitoring Board (SMB)
The SMB met after 100 and then 200 subjects were dosed and evaluated through Day 7 and also reviewed Grade 3 or 4 events or AEs of interest.

Inclusion Criteria
To be considered eligible to participate in this study, subjects were required to meet the following inclusion criteria:
1. Male or female, ≥18 years of age.
2. Laboratory values that were Grade 0 by the modified Division of Microbiology and Infectious Diseases (DMID) toxicity tables. Subjects with laboratory values that were Grade 1 and were not considered clinically significant by the PI may have been enrolled following consultation with the Medical Monitor.
3. A female subject was eligible for this study if she was:
• Not pregnant or nursing.
• Post menopausal, had a hysterectomy, or documented sterility.
• Of child bearing potential (i.e., a woman with an intact uterus and ovaries and no
documentation of oviductal or uterine dysfunction that would cause sterility). These women must have had a negative blood pregnancy test at screening and on Day 0 prior to randomization and must have agreed to 1 of the following:
• Complete abstinence from intercourse from the date of screening through the duration of follow-up.
• Consistent and correct use of 1 of the following medically accepted methods of birth control, in addition to a male partner who correctly used a condom or was sterile prior to the female subject's entry into the study and was the sole sexual partner for the female subject from the date of screening through the duration of follow-up.
• implants of levonorgestrel;
• injectable progesterone;
• any intrauterine device (IUD) with a documented failure rate of less than 1% per year;
• oral contraceptives (either combined or progesterone only);
• double barrier method: condom, cervical cap or diaphragm with spermicidal agent;
• transdermal contraceptive patch.
4. All males who were not sterile must have agreed to either abstain from intercourse or consistently and correctly use a condom while their female partner

agreed to 1 of the appropriate medically accepted methods of birth control listed above from the date of screening through the duration of follow-up.
5. Have the ability to understand the requirements of the study, provide written informed consent (including consent for the use and disclosure of research-related health information), comply with the study protocol procedures, and agree to return for the required study visits.

Exclusion Criteria
Subjects who met any of the following criteria were excluded:
1. History or clinical evidence of significant, acute or chronic diseases, (i.e., cardiovascular, pulmonary, gastrointestinal, hepatic, renal, neurological, or infectious diseases) which could confound the results of the study or put the subject at undue risk.
2. Prior immunization with AVA, prior treatment with investigational anthrax therapies, prior treatment for anthrax exposure, or a confirmed anthrax infection.
3. History of Type I hypersensitivity reaction to food or drugs, IV contrast agents, antihistamines, or history of urticaria.
4. A current drug or alcohol addiction (subjects who had a documented addiction-free period of at least 1 year may have been enrolled based on the clinical judgment of the investigator).
5. History of or positive test at the screening visit for human immunodeficiency virus (HIV-1), Hepatitis B surface antigen (HBsAg), or Hepatitis C antibody.
6. Clinically significant abnormalities in an electrocardiogram (EKG) that would make the subject (in the opinion of the investigator) unsuitable for the study.
7. Evidence of active or suspected malignancy or history of malignancy within the last 5 years (with the exception of adequately treated basal cell carcinoma of the skin or in situ carcinoma of the cervix).
8. Participation within 60 days of initiating study agent or refusal to refrain from participation during this study in any other clinical trials of an investigational compound.
9. Previous exposure to raxibacumab.

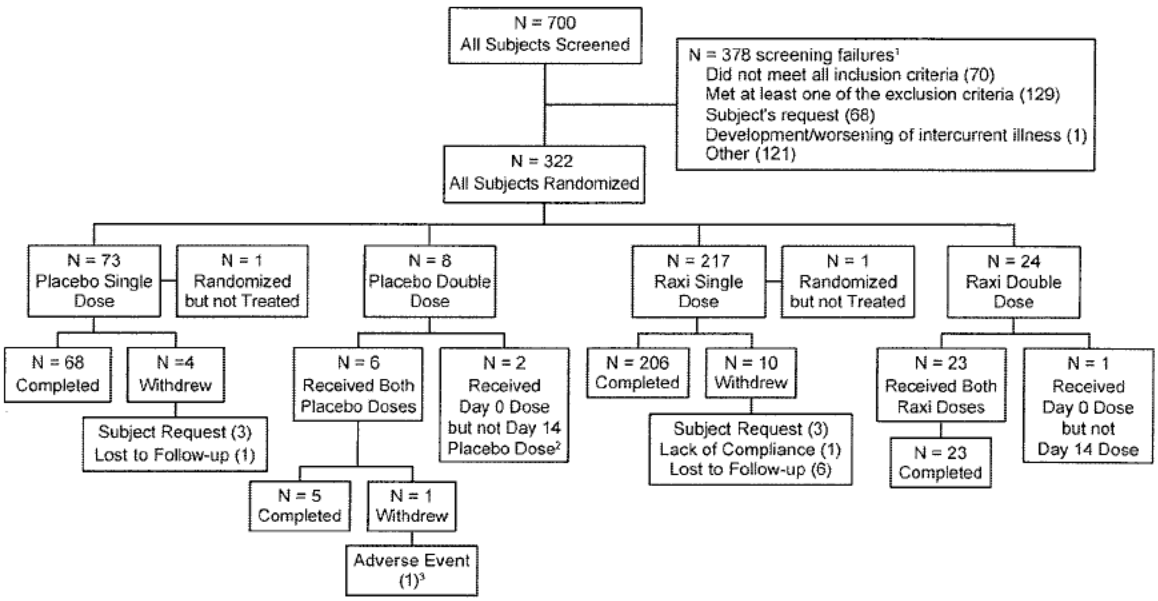
Study Schedule
Subjects who underwent all screening procedures and met the entry criteria were enrolled into the study and assigned a treatment. Randomization was performed on Day 0 prior to administration of the first dose. Subjects randomized to the double dose groups were administered study agent on Days 0 and 14, with follow-up until Day 70 for AEs, while subjects randomized to the single dose groups were administered study agent on Day 0 and followed until Day 56.

AEs were defined as any unfavorable or unintended sign, symptom, or disease that is temporally associated with the use of a study agent but is not necessarily caused by the study agent. This included worsening (increase in frequency or severity) of pre-existing conditions. Relationship of the AE with the study agent was performed, as well as severity grading using the modified DMID toxicity tables. SAEs were defined in the usual regulatory manner.

Subject Disposition

The figure below summarizes subject disposition of the 322 randomized subjects in the study. Of the 322 randomized subjects, 320 were treated (72 in the placebo single-dose group, 8 in the placebo double-dose group, 216 in the raxibacumab single-dose group, and 24 in the raxibacumab double-dose group). One subject (US002-004) randomized to the placebo single-dose group had a positive urine drug screen (UDS) and was not dosed per investigator decision. One subject (US005-014) in the raxibacumab single-dose group refused treatment following an unsuccessful initial attempt at venipuncture for IV dosing.

Figure: Subject disposition for Study HGS1021-C1063 (from Applicant's submission)



[1]Subjects may have multiple reasons for screening failure.

[2]Subject US001-022 had an AE of moderate skin infection on his left hand (not related, resolved) and did not receive his 2nd placebo dose. Per protocol, Subject US002-001 was not given the scheduled 2nd placebo dose due to a positive urine drug screen for opiates.

[3]Subject US001-002 died as a result of injuries sustained in a motor vehicle accident- an event determined to be not related to study agent.

HGS# 000-6993

The following table summarizes study completion and reasons for withdrawal in the As treated population, defined as the set of all subjects who received at least 1 dose of study agent. A total of 304 (95.0%) subjects completed the study. The most common reason for subject withdrawals among single dose placebo-treated subjects was subject request (3/80, 3.8%). One subject (US001-002) in the placebo double-dose group withdrew due to death (motor vehicle accident, unrelated to study agent). A 2nd subject (US001-022) in the placebo double-dose group withdrew from study agent dosing due to an AE of moderate skin infection (not related, resolved) and received only 1 placebo dose. In addition, 1 subject in the placebo double dose group (Subject US002-001) and 1 subject in the raxibacumab double dose group (Subject US001-009) did not receive their 2nd dose of study agent and were assigned to the single dose group in the As-treated

analysis. There were no withdrawals due to AEs among subjects treated with raxibacumab. Subjects lost to follow-up (6/240, 2.5%) constituted the majority of withdrawals among raxibacumab-treated subjects. Other reasons for withdrawals from the study included subject request (4 subjects) and lack of compliance (1 subject), all in the raxibacumab single-dose group.

Table: Reason for withdrawal from the study (As treated population) (from Applicant's submission)

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	Placebo - Single-Dose N = 74	Placebo - Double-Dose N = 6	All Placebo N = 80	Raxibacumab - Single-Dose N = 217	Raxibacumab - Double-Dose N = 23	All Raxibacumab N = 240
Number of subjects completed	70 (94.6%)	5 (83.3%)	75 (93.7%)	206 (94.9%)	23 (100.0%)	229 (95.4%)
Number of subjects not completed	4 (5.4 %)	1 (16.7 %)	5 (6.3 %)	11 (5.1 %)	-	11 (4.6 %)
Subject Request	3 (4.1 %)	-	3 (3.8 %)	4 (1.8 %)	-	4 (1.7 %)
Adverse Event	-	1 (16.7 %)[1]	1 (1.3 %)	-	-	-
Lack of Compliance	-	-	-	1 (0.5 %)	-	1 (0.4 %)
Lost to Follow-up	1 (1.4 %)	-	1 (1.3 %)	6 (2.8 %)	-	6 (2.5 %)
Protocol Violation	-	-	-	-	-	-
Investigator Decision	-	-	-	-	-	-
Other	-	-	-	-	-	-

- Subject US001-002 died as a result of injuries sustained in a motor vehicle accident, an event determined to be not related to study agent.

Extent of exposure

Most subjects got their intended dose, as shown in the table below. In the raxibacumab single-dose group, 2 subjects (US003-016, US003-017) were to have been administered raxibacumab, but no measurable raxibacumab concentrations were obtained at any post dose collections for these subjects. A review of study documentation did not reveal any evidence that these subjects had inadvertently been administered placebo instead of raxibacumab. However, given that high serum raxibacumab levels were attained in other subjects in that dose group, the complete absence of measurable serum raxibacumab concentrations implies that it is very unlikely that these 2 non-evaluable subjects were actually administered a raxibacumab dose.

One subject randomized to the raxibacumab double-dose group and 2 subjects randomized to the placebo double-dose group did not receive their scheduled second infusion on Day 14 per protocol. Subject US001-009 (raxibacumab double-dose group) reported to the site that he would be unable to comply with the study visits due to work-related obligations. He withdrew from the study due to subject request prior to the next follow-up (Day 7) and thus did not receive the scheduled 2nd dose on Day 14.

Table: Summary of exposure (from Applicant's submission)

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Subjects Receiving Study Agent	Placebo - Single-Dose N = 72	Placebo - Double-Dose N = 8	All Placebo N = 80	Raxibacumab - Single-Dose N = 216	Raxibacumab - Double-Dose N = 24	All Raxibacumab N = 240
1 dose	72 (100.0%)	2 (25.0%)	74 (92.5%)	216 (100.0%)	1 (4.2%)	217 (90.4%)
2 doses	-	6 (75.0%)	6 (7.5%)	-	23 (95.8%)	23 (9.6%)

Demographics and Baseline Characteristics

The table below summarizes demographics and baseline characteristics. There were similar numbers of male and female subjects. The majority of subjects in this study were white (259/320, 80.9%), < 65 years old (age range of 18 to 88 years), and had a weight range of 45 to 156 kg. Overall, demographics and baseline characteristics were similar among treatment groups.

Table: Summary of demographics and baseline characteristics (from Applicant's submission)

	Placebo - Single-Dose N = 74	Placebo - Double-Dose N = 6	All Placebo N = 80	Raxibacumab - Single-Dose N = 217	Raxibacumab - Double-Dose N = 23	All Raxibacumab N = 240
Sex						
Male	44 (59.5%)	2 (33.3%)	46 (57.5%)	100 (46.1%)	10 (43.5%)	110 (45.8%)
Female	30 (40.5%)	4 (66.7%)	34 (42.5%)	117 (53.9%)	13 (56.5%)	130 (54.2%)
Race[1]						
White	65 (87.8%)	4 (66.7%)	69 (86.3%)	169 (77.9%)	21 (91.3%)	190 (79.2%)
Asian	6 (8.1%)	-	6 (7.5%)	18 (8.3%)	-	18 (7.5%)
Black or African American	4 (5.4%)	2 (33.3%)	6 (7.5%)	23 (10.6%)	1 (4.3%)	24 (10.0%)
Native Hawaiian or Other Pacific Islander	-	-	-	5 (2.3%)	-	5 (2.1%)
Not Listed	1 (1.4%)	-	1 (1.3%)	11 (5.1%)	1 (4.3%)	12 (5.0%)
Multiracial	2 (2.7%)	-	2 (2.5%)	8 (3.7%)	-	8 (3.3%)
Hispanic or Latino origin	7 (9.5%)	-	7 (8.8%)	28 (12.9%)	1 (4.3%)	29 (12.1%)
Age (years)[2]						
n	74	6	80	217	23	240
Mean ± SD	39.9 ± 18.6	52.2 ± 13.8	40.7 ± 18.8	40.3 ± 18.3	48.5 ± 14.6	41.1 ± 18.3
Median	39.3	48.0	41.5	37.5	50.5	39.4
Range	(18.1, 77.9)	(38.7, 78.0)	(18.1, 78.0)	(18.1, 87.9)	(22.1, 78.2)	(18.1, 87.9)
Age group						
< 65 years	67 (90.5%)	5 (83.3%)	72 (90.0%)	198 (91.2%)	21 (91.3%)	219 (91.3%)
≥ 65 years	7 (9.5%)	1 (16.7%)	8 (10.0%)	19 (8.8%)	2 (8.7%)	21 (8.8%)

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	Placebo - Single-Dose N = 74	Placebo - Double-Dose N = 6	All Placebo N = 80	Raxibacumab - Single-Dose N = 217	Raxibacumab - Double-Dose N = 23	All Raxibacumab N = 240
Weight (kg)						
n	74	6	80	217	23	240
Mean ± SD	77.8 ± 18.2	75.4 ± 12.7	77.6 ± 16.8	78.0 ± 18.2	82.8 ± 19.2	78.5 ± 18.3
Median	78.5	71.7	75.4	78.7	79.9	78.9
Range	(48.1, 120.1)	(62.2, 93.9)	(48.1, 120.1)	(44.8, 155.9)	(54.8, 121.6)	(44.8, 155.9)

- Subjects who checked more than 1 race category were counted in the individual categories as well as the multiracial category.
- Age at randomization (Day 0).

Safety evaluation

Treatment-emergent adverse events (TEAEs)

A summary of all subjects with at least 1 TEAE is shown in the table below. The incidence of subjects who reported at least 1 AE in this study was comparable among placebo (38/80, 47.5%) and raxibacumab (113/240, 47.1%) treated subjects. In addition, the incidence of at least 1 AE was comparable among subjects in the raxibacumab single-dose (103/217, 47.5%) and raxibacumab double-dose groups (10/23, 43.5%).

The incidence of SAEs was similarly comparable among placebo and raxibacumab treated subjects. There were 2 SAEs, 1 in the placebo double-dose group and 1 in the raxibacumab double-dose group. In the placebo double-dose group, the SAE was a death that occurred as a result of injuries sustained in a motor vehicle accident, an event determined to be not related to study agent. In the raxibacumab double-dose group, a Grade 3 event of cholecystitis occurred and thought to be possibly related to raxibacumab.

Table: Number of subjects with treatment-emergent adverse events (from Applicant's submission)

	Placebo - Single-Dose N = 74	Placebo - Double-Dose N = 6	All Placebo N = 80	Raxibacumab - Single-Dose N = 217	Raxibacumab - Double-Dose N = 23	All Raxibacumab N = 240
At least 1 AE	32 (43.2%)	6 (100.0%)	38 (47.5%)	103 (47.5%)	10 (43.5%)	113 (47.1%)
At least 1 related[1] AE	15 (20.3%)	3 (50.0%)	18 (22.5%)	30 (13.8%)	2 (8.7%)	32 (13.3%)
At least 1 serious AE	-	1 (16.7%)	1 (1.3%)	-	1 (4.3%)	1 (0.4%)
At least 1 severe[2] AE	1 (1.4%)	1 (16.7%)	2 (2.5%)	3 (1.4%)	1 (4.3%)	4 (1.7%)
At least 1 related[1] serious AE	-	-	-	-	1 (4.3%)	1 (0.4%)
At least 1 related[1] severe[2] AE	-	-	-	-	1 (4.3%)	1 (0.4%)

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	Placebo - Single-Dose N = 74	Placebo - Double-Dose N = 6	All Placebo N = 80	Raxibacumab - Single-Dose N = 217	Raxibacumab - Double-Dose N = 23	All Raxibacumab N = 240
At least 1 Grade 2 or higher AE	11 (14.9%)	3 (50.0%)	14 (17.5%)	30 (13.8%)	5 (21.7%)	35 (14.6%)
At least 1 Grade 2 or higher related[1] AE	4 (5.4%)	-	4 (5.0%)	4 (1.8%)	1 (4.3%)	5 (2.1%)

- Possibly, probably or definitely related.
- Grade 3, Grade 4, and Grade 5 AEs.

TEAEs by System Organ Class (SOC)

The table below summarizes TEAEs by MedDRA System Organ Class (SOC) classification. The incidences of AEs by SOC among raxibacumab-treated subjects were generally lower than or comparable to those among placebo-treated subjects. The exceptions were in the General disorders, Hepatobiliary disorders, Investigations, Psychiatric disorders, and Skin and subcutaneous tissue disorders SOCs, and all of these were small differences. In both raxibacumab and placebo treatment groups, the highest incidence of AEs occurred in the SOC category of Infections and infestations.

Table: Subjects with TEAEs by SOC

MedDRA system organ class	Single-dose Placebo n=74 (%)	Double-dose Placebo n=6 (%)	Total Placebo subjects N=80 (%)	Single-dose Raxi N=217 (%)	Double-dose Raxi n=23 (%)	Total Raxibacumab subjects N=240 (%)
Blood and lymphatic system disorders	3 (4.1)	0	3 (3.8)	6 (2.8)	0	6 (2.5)
Cardiac disorders	0	1 (16.7)	1 (1.3)	2 (0.9)	0	2 (0.8)
Ear and labyrinth disorders	0	1 (16.7)	1 (1.3)	2 (0.9)	1 (4.4)	3 (1.3)
Eye disorders	0	0	0	2 (0.9)	0	2 (0.8)
Gastrointestinal disorders	7 (9.5)	2 (33.3)	9 (11.3)	20 (9.2)	2 (8.7)	22 (9.2)
General disorders and administration site conditions	3 (4.1)	0	3 (3.8)	12 (5.5)	0	12 (5.0)
Hepatobiliary disorders	0	0	0	0	1 (4.4)	1 (0.4)
Infections and infestations	14 (18.9)	1 (16.7)	15 (18.8)	26 (12.0)	5 (21.7)	31 (12.9)
Injury, poisoning and procedural complications	5 (6.8)	2 (33.3)	7 (8.8)	9 (4.1)	3 (13.0)	12 (5.0)
Investigations	0	1 (16.7)	1 (1.3)	8 (3.7)	1 (4.4)	9 (3.8)

Metabolism and nutrition disorders	1 (1.4)	0	1 (1.3)	1 (0.5)	1 (4.4)	2 (0.8)
Musculoskeletal and connective tissue disorders	4 (5.4)	2 (33.3)	6 (7.5)	11 (5.1)	2 (8.7)	13 (5.4)
Nervous system disorders	10 (13.5)	1 (16.7)	11 (13.8)	27 (12.4)	2 (8.7)	29 (12.1)
Psychiatric disorders	0	0	0	3 (1.4)	0	3 (1.3)
Reproductive system and breast disorders	1 (1.4)	0	1 (1.3)	2 (0.9)	0	2 (0.8)
Respiratory, thoracic and mediastinal disorders	5 (6.8)	0	5 (6.3)	13 (6.0)	0	13 (5.4)
Skin and subcutaneous tissue disorders	2 (2.7)	2 (33.3)	4 (5.0)	15 (6.9)	0	15 (6.3)
Vascular disorders	2 (2.7)	0	2 (2.5)	3 (1.4)	0	3 (1.3)

TEAEs by PT

The table below shows TEAEs by SOC and PT, ordered by frequency in the All raxibacumab subjects group. The incidences of the most common AEs in this study were comparable among raxibacumab and placebo-treated subjects, and administration of the 2nd dose of either agent was not associated with a higher incidence of the most common AEs.

Headache was the most common AE among raxibacumab-treated subjects (20/240, 8.3%), followed by upper respiratory tract infection (13/240, 5.4%). Headache was also the most common AE among placebo-treated subjects (8/80, 10%), followed by upper respiratory tract infection, and pharyngitis (both 4/80, 5.0%). All headaches were mild to moderate in severity, all resolved, and the majority of headaches were not related to study agent. The majority of headaches resolved within 24 hours, and time to onset varied among subjects, with no pattern of clustering around the time of dosing. All AEs of upper respiratory tract infection were mild to moderate in severity, 12 of 13 (92.0%) were not related, and all resolved.

Based on the occurrence of rash in a prior study (Study HGS1021-C1064), all subjects in the current study received premedication with diphenhydramine, which was well-tolerated. Six of the 240 raxibacumab-treated subjects (2.5% all single-dose subjects) in this study had an AE of rash; 3 were possibly or probably related to raxibacumab. Two of 80 (2.5%) placebo-treated subjects had an AE of rash (both were in the placebo double-dose group; 1 was possibly related). All

rashes in this study were mild in severity and no action was taken with study agent dosing. All rashes were mild with 2 exceptions (1 in the raxibacumab single-dose group and 1 in the placebo double-dose group [both not related]). Similarly, all were transient with the exception of 1 rash (not related) in a raxibacumab-treated subject (US005-012), which started on Day 45 and was ongoing at the end of the study, and 1 rash (not related) in a placebo-treated subject (US001-002), which started on Day 21 was ongoing at the end of the study.

Table: Subjects with TEAEs by PT for AEs>0.5% in the raxibacumab arms

MedDRA system organ class	Coding: MedDRA Preferred Term	Single-dose Placebo n=74	Double-dose Placebo n=6	All Placebo n=80	Single-dose Raxi n=217	Double-dose Raxi n=23	All raxibacumab n=240
Nervous system disorders	Headache	7 (9.5)	1 (16.7)	8 (10.0)	19 (8.8)	1 (4.4)	20 (8.3)
	Dizziness	1 (1.4)	0	1 (1.3)	3 (1.4)	0	3 (1.3)
	Somnolence	0	0	0	2 (0.9)	1 (4.4)	3 (1.3)
	Syncope vasovagal	0	0	0	2 (0.9)	0	2 (0.8)
	Paraesthesia	0	0	0	1 (0.5)	0	1 (0.4)
Infections and infestations	Upper respiratory tract infection	3 (4.1)	1 (16.7)	4 (5.0)	9 (4.2)	4 (17.4)	13 (5.4)
	Sinusitis	1 (1.4)	0	1 (1.3)	4 (1.8)	1 (4.4)	5 (2.1)
	Rhinitis	1 (1.4)	0	1 (1.3)	3 (1.4)	0	3 (1.3)
	Gastroenteritis	1 (1.4)	0	1 (1.3)	2 (0.9)	0	2 (0.8)
	Nasopharyngitis	0	0	0	2 (0.9)	0	2 (0.8)
	Oral herpes	0	0	0	2 (0.9)	0	2 (0.8)
	Pharyngitis streptococcal	0	0	0	2 (0.9)	0	2 (0.8)
	Cellulitis	0	0	0	1 (0.5)	0	1 (0.4)
	Conjunctivitis bacterial	0	0	0	1 (0.5)	0	1 (0.4)
	Influenza	0	0	0	1 (0.5)	0	1 (0.4)
	Otitis externa	0	0	0	1 (0.5)	0	1 (0.4)
	Pharyngitis	4 (5.4)	0	4 (5.0)	1 (0.5)	0	1 (0.4)
	Tooth abscess	0	0	0	1 (0.5)	0	1 (0.4)
Gastrointestinal disorders	Nausea	2 (2.7)	1 (16.7)	3 (3.8)	73 (3.2)	0	7 (2.9)
	Diarrhea	2 (2.7)	0	2 (2.5)	4 (1.8)	2 (8.7)	6 (2.5)
	Abdominal pain	2 (2.7)	0	2 (2.5)	4 (1.8)	0	4 (1.7)
	Abdominal pain upper	0	0	0	2 (0.9)	0	2 (0.8)
	Colitis	0	0	0	1 (0.5)	0	1 (0.4)
	Dental caries	0	0	0	1 (0.5)	0	1 (0.4)
	Dental plaque	0	0	0	1 (0.5)	0	1 (0.4)
	Dyspepsia	2 (2.7)	0	2 (2.5)	1 (0.5)	0	1 (0.4)
	Stomach discomfort	0	0	0	1 (0.5)	0	1 (0.4)
	Toothache	0	0	0	1 (0.5)	0	1 (0.4)
	Vomiting	1 (1.4)	1 (16.7)	2 (2.5)	1 (0.5)	0	1 (0.4)

Respiratory, thoracic and mediastinal disorders	Cough	3 (4.1)	0	3 (3.8)	5 (2.3)	0	5 (2.1)
	Nasal congestion	0	0	0	3 (1.4)	0	3 (1.3)
	Sinus congestion	0	0	0	2 (0.9)	0	2 (0.8)
	Dyspnea	1 (1.4)	0	1 (1.3)	1 (0.5)	0	1 (0.4)
	Epistaxis	0	0	0	1 (0.5)	0	1 (0.4)
	Rhinitis allergic	0	0	0	1 (0.5)	0	1 (0.4)
	Rhinorrhea	1 (1.4)	0	1 (1.3)	1 (0.5)	0	1 (0.4)
	Wheezing	0	0	0	1 (0.5)	0	1 (0.4)
Injury, poisoning and procedural complications	Joint sprain	0	0	0	3 (1.4)	1 (4.4)	4 (1.7)
	Excoriation	1 (1.4)	0	1 (1.3)	3 (1.4)	0	3 (1.3)
	Muscle strain	0	0	0	2 (0.9)	0	2 (0.8)
	Back injury	0	0	0	1 (0.5)	0	1 (0.4)
	Clavicle fracture	0	0	0	1 (0.5)	0	1 (0.4)
	Contusion	1 (1.4)	0	1 (1.3)	1 (0.5)	0	1 (0.4)
	Procedural pain	1 (1.4)	0	1 (1.3)	0	1 (4.4)	1 (0.4)
	Sunburn	0	0	0	0	1 (4.4)	1 (0.4)
Musculoskeletal and connective tissue disorders	Myalgia	1 (1.4)	0	1 (1.3)	4 (1.8)	0	4 (1.7)
	Back pain	0	0	0	1 (0.5)	2 (8.7)	3 (1.3)
	Arthralgia	1 (1.4)	2 (33.3)	3 (3.8)	2 (0.9)	0	2 (0.8)
	Muscle spasms	0	0	0	1 (0.5)	1 (4.4)	2 (0.8)
	Bone pain	0	0	0	0	1 (4.4)	1 (0.4)
	Musculoskeletal pain	0	0	0	1 (0.5)	0	1 (0.4)
	Neck pain	0	0	0	1 (0.5)	0	1 (0.4)
	Pain in extremity	1 (1.4)	0	1 (1.3)	1 (0.5)	0	1 (0.4)
	Tendonitis	0	0	0	1 (0.5)	0	1 (0.4)
Skin and subcutaneous tissue disorders	Rash	0	1 (16.7)	1 (1.3)	4 (1.8)	0	4 (1.7)
	Pruritus	0	0	0	3 (1.4)	0	3 (1.3)
	Acne	0	0	0	2 (0.9)	0	2 (0.8)
	Urticaria	0	1 (16.7)	1 (1.3)	2 (0.9)	0	2 (0.8)
	Dermatitis	1 (1.4)	0	1 (1.3)	1 (0.5)	0	1 (0.4)
	Drug eruption	0	0	0	1 (0.5)	0	1 (0.4)
	Pain of skin	0	0	0	1 (0.5)	0	1 (0.4)
	Psoriasis	0	0	0	1 (0.5)	0	1 (0.4)
Investigations	Blood amylase increased	0	0	0	2 (0.9)	1 (4.4)	3 (1.3)
	Blood pressure increased	0	0	0	2 (0.9)	0	2 (0.8)
	Prothrombin time prolonged	0	0	0	2 (0.9)	0	2 (0.8)
	Blood creatine phosphokinase increased	0	0	0	1 (0.5)	0	1 (0.4)
	White blood cell count increased	0	0	0	1 (0.5)	0	1 (0.4)
General disorders and administration site conditions	Fatigue	0	0	0	3 (1.4)	0	3 (1.3)
	Edema peripheral	0	0	0	2 (0.9)	0	2 (0.8)
	Malaise	0	0	0	1 (0.5)	0	1 (0.4)
	Non-cardiac chest pain	0	0	0	1 (0.5)	0	1 (0.4)
	Pyrexia	1 (1.4)	0	1 (1.3)	1 (0.5)	0	1 (0.4)

	Thirst	0	0	0	1 (0.5)	0	1 (0.4)
	Vessel puncture site hematoma	0	0	0	1 (0.5)	0	1 (0.4)
	Vessel puncture site pain	0	0	0	1 (0.5)	0	1 (0.4)
	Vessel puncture site paraesthesia	0	0	0	1 (0.5)	0	1 (0.4)
Vascular disorders	Hypertension	0	0	0	3 (1.4)	0	3 (1.3)
Psychiatric disorders	Insomnia	0	0	0	2 (0.9)	0	2 (0.8)
	Anxiety	0	0	0	1 (0.5)	0	1 (0.4)
Blood and lymphatic system disorders	Leukopenia	0	0	0	2 (0.9)	0	2 (0.8)
	Lymphadenopathy	0	0	0	2 (0.9)	0	2 (0.8)
	Anemia	0	0	0	1 (0.5)	0	1 (0.4)
	Neutropenia	1 (1.4)	0	1 (1.3)	1 (0.5)	0	1 (0.4)
Cardiac disorders	Palpitations	0	0	0	2 (0.9)	0	2 (0.8)
	Tachycardia	0	1 (16.7)	1 (1.3)	1 (0.5)	0	1 (0.4)
Ear and labyrinth disorders	Vertigo	0	0	0	2 (0.9)	0	2 (0.8)
	Tympanic membrane perforation	0	0	0	0	1 (4.4)	1 (0.4)
Eye disorders	Blepharitis	0	0	0	1 (0.5)	0	1 (0.4)
	Conjunctivitis allergic	0	0	0	1 (0.5)	0	1 (0.4)
Hepatobiliary disorders	Cholecystitis	0	0	0	0	1 (4.4)	1 (0.4)
Metabolism and nutrition disorders	Decreased appetite	0	0	0	1 (0.5)	0	1 (0.4)
	Hyperglycemia	0	0	0	0	1 (4.4)	1 (0.4)
Reproductive system and breast disorders	Dysmenorrhea	0	0	0	1 (0.5)	0	1 (0.4)
	Ovarian cyst ruptured	0	0	0	1 (0.5)	0	1 (0.4)

A listing of study agent-related AEs ongoing at the end of the study is provided in the table below. Two placebo-treated subjects and 3 raxibacumab-treated subjects had related AEs ongoing at the end of the study. There were no ongoing AEs that resulted in interruption or discontinuation of study agent. All ongoing AEs were mild to moderate in severity and assessed as not related to study agent.

Table: Ongoing TEAEs at the end of study

Treatment group	Subject ID	Age/Sex/Race	MedDRA Preferred Term	AE start-study day	AE duration	Severity	Relationship to study agent	Action taken with study agent
Placebo - single dose	US003-047	31/F/W	Diarrhea	1	>57	Mild	Not related	None

Placebo - double dose	US005-006	46/M/W	Arthralgia	2	>69	Mild	Not related	None
Raxi - single dose	US004-003	27/M/W	Paresthesia	6	>52	Mild	Not related	None
Raxi - single dose	US005-047	65/M/W	Fatigue	6	>50	Mild	Not related	None
Raxi - single dose	US006-037	26/F/B	Leukopenia	7	>50	Moderate	Not related	None

TEAEs of severe intensity

There was 1 death on study. Subject US001-002 in the placebo double-dose group died from injuries sustained in a motor vehicle accident; the event was considered not to be related to study agent. There was 1 raxibacumab-related severe AE (also serious). Subject US001-017 (raxibacumab double-dose group) had Grade 3 cholecystitis, determined to be possibly related to raxibacumab. Four other subjects had a severe AE not related to study agent.

Table: TEAEs of severe intensity

Treatment group	Subject ID	Age/Sex/Race	Coding: MedDRA Preferred Term	AE start-study day	AE duration	Outcome	Relationship to study agent	Action taken with study agent
Placebo - single dose	US004-036	68/M/W	Leukocytosis	1	14	Recovered	Not related	None
Placebo - double dose	US001-002	45/F/W	Injury	39	1	Fatal	Not related	None
Raxi - single dose	US003-001	26/F/W	Prothrombin time prolonged	28	4	Recovered	Not related	None
Raxi - single dose	US003-002	54/M/W	Blood amylase increased	15	3	Recovered	Probably not related	None
Raxi - single dose	US004-030	22/M/W	Influenza	36	6	Recovered	Not related	None
Raxi - double dose	US001-017	53/F/W	Cholecystitis	24	3	Recovered	Possibly related	None

Discontinuations due to AEs
There were 2 discontinuations of dosing due to AEs. Subject US001-022 in the placebo double-dose group had an AE of moderate skin infection on his left hand on Day 12 (not related, resolved) and did not receive his 2nd placebo dose. This subject completed follow-up. Subject US002-011 in the raxibacumab single-dose group had his raxibacumab dose discontinued due to an AE of moderate dyspnea (not related, resolved), and received a partial dose. There were no significant changes in vital signs or physical examination, and no evidence of rash, erythema, or edema. No other associated AEs were reported and no significant laboratory abnormalities were found. All symptoms resolved within twenty minutes with no change in physical examination or vital signs during or after this period. Per the investigator, the most likely cause of the subject's symptoms was esophageal spasm caused by rapid eating prior to the infusion and/or anxiousness. This subject completed follow-up.

Table: TEAEs resulting in study agent discontinuation

Treatment group	Subject ID	MedDRA Preferred Term	Study day	Severity	Relationship to study agent
Placebo – double dose (but received single dose)	US001-002	Skin infection	12	Moderate	Not related
Raxibacumab – single dose	US002-011	Dyspnea	0	Moderate	Probably not related

Deaths
There was 1 death (Subject US001-002) in the placebo double-dose group, which was not related to study agent. A narrative of this event is provided below:

Subject US001-002 was a 45-year-old white female in the placebo double-dose group, and she received the infusions of study drug per protocol on Day 0 and Day 14. No relevant medical history or concomitant medications were reported. The subject was involved in a motorcycle accident that occurred 25 days after the 2nd placebo dose (Day 39), and died as a result of injuries sustained in the accident. The investigator indicated that the injuries sustained in the accident (life-threatening) were not related to study agent.

SAEs
There were 2 SAEs, 1 in the placebo double-dose group and 1 in the raxibacumab double-dose group as shown in the table below.

Table: Treatment emergent SAEs

Treatment group	Subject ID	Age/Sex/Race	MedDRA Preferred Term	AE start -	AE duration	Outcome	Relationship to study	Action taken with

				stud y day			agent	study agent
Placebo – double dose	US0 01-002	45/F/ W	Injury	39	1	Fatal	Not related	None
Raxibac umab-double dose	US0 01-017	53/F/ W	Cholecysti tis	24	3	Recovere d/resolved	Possibly related	None

Subject US001-017, a 52-year-old white female in the raxibacumab double-dose group, developed upper abdominal pain, nausea, and low grade fever 10 days after her second raxibacumab dose (Day 24). The subject was hospitalized and underwent a cholecystectomy on Day 26; the post-operative diagnosis was confirmed to be cholecystitis. Dosing was completed and no action was taken with regard to raxibacumab. The investigator indicated that the cholecystitis (severe) was possibly related to raxibacumab, and most likely caused by progression of concurrent disease as the subject had multiple risk factors for cholelithiasis including female gender, age, obesity (body weight of 111.5 kg), elevated serum glucose and elevated triglycerides

Subgroup Analysis
The Applicant also performed TEAE analysis stratified by age (< 65 years of age and ≥age 65) and found no difference in the incidence or type of AEs when analyzed by this factor. Please refer to the original study report for details.

Laboratory

Hematology
There were no hematology laboratory values above Grade 2 for subjects in double-dose groups. Hematology laboratory values above Grade 2 included Grade 3 leukocytosis (1 subject each in the placebo and raxibacumab single-dose groups), Grade 4 neutropenia (1 subject in the raxibacumab single-dose group), and Grade 3 PT (2 subjects in the raxibacumab single-dose group).

There were no 2-grade shifts in hematology laboratory parameters for subjects in double-dose groups. Incidences of 2-grade hematology laboratory shifts were comparable between raxibacumab-treated subjects and placebo-treated subjects. Transient incidences of 2-grade worsening in leukocyte count were observed in 1 placebo single-dose subject and 2 raxibacumab single-dose subjects. One of the leukocytosis events in a raxibacumab single-dose subject was recorded as a mild AE (not related, resolved). All events were transient and resolved. Two subjects in the raxibacumab single-dose group had isolated incidences of 2-grade worsening for PT.

Subjects with Grade 3 or Higher Hematology Laboratory Values

The following subjects had a Grade 3 or higher (and a 2-grade shift) hematology laboratory value:

Subject US004-036 (placebo single-dose group) had Grade 1 (11.47 x 103/μL) WBC count at baseline, which shifted to Grade 3 (20.53 x 103/μL) on Day 1. WBCs returned to Grade 0 by Day 14. No action was taken with regard to placebo. This event was recorded as a Grade 3 AE (not related, resolved). No other AEs were reported. The subject reported concomitant treatment for ear pain and presumptive Bell's palsy with prednisone. The investigator determined that the leukocytosis was probably not related to raxibacumab, and most likely caused by the prednisone.

Subject US001-069 (raxibacumab single-dose group) had a Grade 1 (11.44 x 103/μL) WBC count at baseline, and a transient shift to Grade 3 WBC count (22.17 x 103/μL) on Day 2. This event was recorded as a mild AE (not related, resolved). The subject also had AEs of moderate abdominal pain on Day 3 (not related, resolved), which lasted for 3 days, and mild malaise on Day 4 (possibly related to study agent, resolved), which lasted for 1 day. The investigator indicated that the elevated WBC count was probably not related to raxibacumab and that the most likely cause of the event was a laboratory error.

Subject US006-029 (raxibacumab single-dose group) had a Grade 0 (4.46 x 103/μL) ANC at baseline, which shifted to Grade 4 (0.41 x 103/μL, concurrent Grade 1 total WBC count of 3.01 x 103/μL,) on Day 28. The subject was asymptomatic and had no relevant clinical findings. Anti-neutrophil antibody testing done on samples obtained at screening, Day 28 and Day 34 were all negative. The ANC returned to Grade 0 (1.7 x 103/μL) at an unscheduled visit on Day 33. This event was recorded as a mild AE (possibly related to raxibacumab, resolved). No other AEs were reported.

Subject US006-030 (raxibacumab single-dose group) had a Grade 0 PT (10.3 seconds) at baseline, and an isolated shift to Grade 3 (25.7 seconds) PT on Day 28. All prior assessments of coagulation studies (PT, PTT, and international normalized ratio [INR]) were within reference range. The subject was asymptomatic. Upon repeat testing 6 days later, the value normalized. The investigator indicated that the elevated PT was probably not related to raxibacumab, and that the most likely cause of the event was a laboratory error. This event was recorded as a mild AE (not related, resolved). No other AEs were reported.

Subject US003-001 (raxibacumab single-dose group) had a Grade 0 PT (11.7 seconds) at baseline, and an isolated shift to Grade 3 (23.9 seconds) on Day 28. Concurrent APTT was Grade 1 (47 seconds). All prior coagulation studies (PT, PTT, and INR) for this subject had been within normal limits. The subject was asymptomatic. On repeat testing 3 days later, the results normalized. This event

was recorded as a Grade 3 (severe) AE, which was not related to study agent, and which resolved. The investigator indicated that the most likely cause of the event was an error in specimen collection, processing, or analysis.

Liver and Kidney Function

There was one raxibacumab subject with a grade 3 laboratory value for liver parameters (ALT) with no grades ≥2 for any other parameters (AST, GGT, ALP or bilirubin). Subject US004-057 (raxibacumab single-dose group) had a Grade 0 ALT of 23 IU/L at baseline, which shifted to Grade 3 (304 IU/L) on Day 56. Other notable laboratory abnormalities for this subject were Grade 1 AST (97 IU/L) and Grade 1 GGT (124 IU/L) also on Day 56. The subject reported symptoms consistent with an upper respiratory tract infection and reported taking over-the-counter medications. ALT of 146 IU/L (Grade 2) and AST of 90 IU/L (Grade 1) were noted upon repeat laboratory assessment on Day 68. On Day 75, ALT decreased to 57 IU/L (Grade 1), and AST decreased to 34 IU/L (Grade 0). No changes were noted in measurements of bilirubin or coagulation studies (PT, PTT). The investigator indicated that the elevated ALT was probably not related to raxibacumab, and that the most likely cause of the event was exposure to other substance. AEs for this subject included myalgia, headache, and sinus congestion (all mild, not related, and resolved).

Electrolytes

There were no subjects with electrolyte values above Grade 2. There were no 2-grade shifts in electrolytes for subjects in double-dose groups. Six subjects in the raxibacumab single-dose group (1 hypercalcemia and 5 hypophosphatemia) and 1 subject in the placebo single-dose group had a transient shift to Grade 2 electrolytes; all were asymptomatic and no interventions were given.

Other laboratory

With respect to raxibacumab treated subjects, there were no Grade 3 laboratory tests with the exception of one subject with Grade 3 amylase. Most cases were few reports (less than 5%), and those that were reported at higher frequency, such as Grade 1 hyperglycemia (18.4%) were similar in frequency to the placebo group (placebo hyperglycemia 18.8%). In the Phase 1 study PAM-NH-01 which used the M10 formulation, isolated Grade 2 hypoglycemia was observed in 6 subjects in the IV dose cohorts (1 subject each in the 1 mg/kg [Day 42], 3 mg/kg [Day 28], and 20 mg/kg [Day 14] groups and 3 subjects in the 10 mg/kg group [Day 1, Day 2, and Day 7]). None of these subjects were noted to be symptomatic. The rates of hypoglycemia in the placebo groups vs. the raxibacumab groups were: Grade 1 placebo 6.3%, raxibacumab 5.4%, Grade 2 placebo 2.5%, raxibacumab 2.1% and no cases of Grades 3 or 4 in either group.

The only exception of a laboratory test which was reported in higher frequency in the raxibacumab group compared to placebo was amylase level, which was reported at the following rates as shown in the table below:

Table: Summary of worst amylase grades in Study HGS1021-C1063

Amylase grade	Placebo single dose n=74 (%)	Placebo double dose n=6 (%)	All placebo n=80 (%)	Raxibacumab single dose n=217 (%)	Raxibacumab double dose n=23 (%)	All raxibacumab subjects n=240 (%)
0	65 (87.8)	6 (100)	71 (88.8)	185 (85.6)	20 (87.0)	205 (85.8)
1	5 (6.8)	-	5 (6.3)	25 (11.6)	2 (8.7)	27 (11.3)
2	4 (5.4)	-	4 (5.0)	5 (2.3)	1 (4.3)	6 (2.5)
3	-	-	-	1 (0.5)	-	1 (0.4)
4	-	-	-	-	-	-

The narrative for the 1 subject with Grade 3 amylase is provided below.

Subject US003-002 (raxibacumab single-dose group) had a Grade 2 amylase level of 159 U/L at screening and a Grade 3 level of 208 U/L on Day 14. The screening value was considered not clinically significant by the investigator. However, the site was not aware of the grading of this value and did enroll the subject; the event was documented as a protocol violation. The subject was asymptomatic. Repeat assessment on Day 0 was within normal limits (amylase level of 69 U/L). All other relevant laboratory values were within normal limits and the subject was again asymptomatic. On repeat testing, lipase and amylase were within normal limits (lipase 55 U/L, amylase 83 U/L); amylase fractionation was also obtained and found to be within normal limits with no evidence of macroamylasemia. The subject underwent an abdominal ultrasound revealing steatohepatitis, with no stones or wall thickening in the gallbladder, no pericholecystic fluid, and normal pancreas. The findings were determined to be consistent with nonalcoholic steatohepatitis (NASH) and considered by the investigator to be not clinically significant. No action was taken with regard to raxibacumab. The investigator indicated that the elevated amylase (severe) was probably not related to raxibacumab. The investigator indicated that there was no clear cause of the asymptomatic recurring elevation in amylase, but that it likely pre-dated exposure to raxibacumab. This event was recorded as a Grade 3 AE (resolved).

Similarly, shifts in laboratory tests of at least 2 grades occurred at similar frequency between placebo and raxibacumab groups.

Vital signs
Three subjects (US005-023, US005-024, and US005-047) in the raxibacumab single-dose group had an AE of mild hypertension, which were judged to be not related to raxibacumab.

Immunogenicity
No subjects developed an anti-raxibacumab antibody response during the study.

Pharmacokinetics

Please refer to the Clinical Pharmacology review for the PK portion of this study.

Safety Summary

Subjects treated with raxibacumab had comparable incidences of AEs, SAEs, or severe AEs compared to placebo treated subjects. Subjects in the raxibacumab double-dose group had a similar incidence of AEs, and SAEs compared to subjects in the raxibacumab single-dose group. Headache (8.3% vs. 10%) and upper respiratory tract infection (5.4% vs. 5.0%) were the most frequently reported AEs in raxibacumab-treated subjects and placebo-treated subjects respectively.

Two SAEs were reported in this study. One case was a subject in the placebo double-dose group who died due to injuries sustained in a motor vehicle accident (not related to study agent), and the other was a Grade 3 event of cholecystitis in the raxibacumab double-dose group. The cholecystitis resolved and was coded as possibly related to raxibacumab as the cholecystitis was most likely caused by progression of concurrent disease. Two subjects, 1 in the placebo double-dose group (skin infection) and 1 in the raxibacumab single-dose group (dyspnea) discontinued treatment due to moderate AEs which resolved.

The incidence of rash was similar among raxibacumab-treated subjects (2.5%) and placebo-treated subjects (2.5%). Of the 8 incidences of rash, 4 were found to be related to study drug (3 in the raxibacumab single dose group and 1 in the placebo double dose group) and all were transient. Two subjects had a rash that was ongoing at the end of the study (1 subject in the raxibacumab single dose group and 1 subject in the placebo double dose group); both ongoing rashes were not related to study drug.

Laboratory abnormalities of Grade 3 or higher were infrequently reported in this study. Seven of the 320 subjects in this study (2.2%) had a Grade 3 or higher laboratory abnormality; 6/217 (2.8%) from the raxibacumab single-dose group, and 1/74 (1.4%) from the placebo single-dose group. Hematology laboratory abnormalities above Grade 2 included Grade 3 leukocytosis (1 subject each in the placebo and raxibacumab single-dose groups, Grade 4 neutropenia (1 subject in the raxibacumab single-dose group), and Grade 3 PT (2 subjects in the raxibacumab single-dose group). Chemistry laboratory abnormalities above Grade 2 included Grade 3 ALT and Grade 3 amylase (1 subject each in the raxibacumab single-dose group). All values returned to baseline or to Grade 1 (ALT) prior to completion of study and the events resolved without intervention.

Incidences of 2-grade shifts in hematology, liver parameters, electrolytes, other chemistries, and urinalysis were comparable between raxibacumab-treated subjects and placebo-treated subjects. Incidences of 2-grade shifts in laboratory values were not higher among subjects in double-dose groups as compared with single dose groups. The majority of 2-grade shifts were transient, not associated with AEs of significance, and required no intervention.

Lastly, there were no subjects who developed an anti-raxibacumab antibody response during the study.

9.5 Individual Study Report

Study Report HGS1021-C1069: An Open-Label Study to Evaluate the Immunogenicity and Safety of Raxibacumab (Human Monoclonal Antibody to *B. anthracis* Protective Antigen) Administered in Healthy Subjects

Study Phase: 2/3

Indication: Therapeutic treatment of inhalational anthrax

First Subject Enrolled: 08 January 2008
Last Subject Last Visit: 08 May 2008

Investigators and Study Centers: 2 Centers (US)

Study Sponsor: Human Genome Sciences, Inc., 14200 Shady Grove Road Rockville, MD 20850

Primary Objective
To evaluate immunogenicity in subjects receiving 2 doses of raxibacumab with a delay prior to the 2nd dose.

Secondary Objectives
• To evaluate safety in subjects receiving 2 doses of raxibacumab with a delay prior to the 2nd dose.
• To determine serum raxibacumab PK in subjects receiving 2 doses of raxibacumab with a delay prior to the 2nd dose.

Study Design
This was a single-group, open-label study to evaluate the immunogenicity and safety of raxibacumab in healthy adult male and female subjects. Subjects who had received raxibacumab ≥4 months prior to this study in Study HGS1021-C1064 were eligible for enrollment. The longest half-life for the terminal elimination phase for any subject administered an IV raxibacumab dose in the Phase 1 study PAM-NH-01 was 26.4 days. This justified selection of a 4-month washout period between raxibacumab doses in this study, as this ensured that more than 95% of the 1st dose would be eliminated before the 2nd dose was administered.

In Study HGS1021-C1064, 3 treatment groups were evaluated:

Group 1: 15 doses of PO ciprofloxacin (500 mg every 12 hours) from Day 0 to Day 7. On Day 5, prior to the 1st daily ciprofloxacin dose, the subjects were also to receive a single dose of raxibacumab (40 mg/kg) IV.

Group 2: a single dose of raxibacumab (40 mg/kg) IV on Day 0.

Group 3: One dose of IV ciprofloxacin (400 mg) on Day 0 immediately followed by IV raxibacumab (40 mg/kg). A 2nd dose of IV ciprofloxacin (400 mg) was to be administered on Day 0, 12 hours after the 1st infusion was completed. Subjects in Group 3 were to continue with PO ciprofloxacin (500 mg Q12h) on Days 1 to 7 for a total of 13 doses.

For the current study, a maximum of 25 subjects (to include at least 3 evaluable female subjects) were to receive a 2nd dose of raxibacumab 40 mg/kg IV ≥4 months following their 1st dose. Subjects were to remain in the clinical unit from Day 0 until Day 1 and were followed for 70 days after receiving their 2nd dose of raxibacumab.

Inclusion Criteria

• Enrolled and treated with raxibacumab in another HGS protocol ≥4 months ago.
• Male or female ≥18 and ≤64 years of age.
• Laboratory values that were Grade 0 by the modified Division of Microbiology and Infectious Diseases (DMID) toxicity tables. Subjects with laboratory values that were Grade 1 and were not considered clinically significant by the principal investigator may have been enrolled following consultation with the medical monitor.
• A female subject was eligible to enter the study if she was:
• Not pregnant or nursing.
• Post menopausal, had a hysterectomy, or documentation of sterility.
• Of child-bearing potential (i.e., woman with an intact uterus and ovaries and no documentation of oviductal or uterine dysfunction that would cause sterility). These women must have had a negative blood pregnancy test at screening and on Day -1 prior to dosing and agree to 1 of the following:
• Complete abstinence from intercourse from the date of screening through the duration of follow-up.
• Consistent and correct use of 1 of the following medically accepted methods of birth control, in addition to a male partner who correctly used a condom or was sterile prior to the female subject's entry into the study and was the sole sexual partner for the female subject from the date of screening through the duration of follow-up:
• implants of levonorgestrel.
• injectable progesterone.
• any intrauterine device (IUD) with a documented failure rate of less than 1% per year.
• oral contraceptives (either combined or progesterone only).
• double barrier method: condom, cervical cap or diaphragm with spermicidal agent.
• transdermal contraceptive patch.

• All males who were not sterile must have agreed to either abstain from intercourse or consistently and correctly use a condom while their female partner agreed to use 1 of the appropriate medically accepted methods of birth control listed above from the date of screening through the duration of follow-up.
• Have the ability to understand the requirements of the study, provide written informed consent (including consent for the use and disclosure of research-related health information), comply with the study protocol procedures, and agree to return for the required study visits.

Exclusion Criteria
• History or clinical evidence of significant, acute, or chronic diseases (i.e., cardiovascular, pulmonary, gastrointestinal, hepatic, renal, neurological, or infectious diseases), which could have confounded the results of the study or put the subject at undue risk.
• Prior immunization with AVA, prior treatment with investigational anthrax therapies (other than raxibacumab ≥4 months ago), prior treatment for anthrax exposure, or a confirmed anthrax infection.
• History of Type I hypersensitivity reaction to food or drugs, IV contrast dye, or history of urticaria.
• Previous hypersensitivity to raxibacumab.
• Previous serious or Grade 3 or greater raxibacumab-related AE.
• Drug or alcohol addiction within the last 12 months. Subjects who had a documented addiction-free period of at least 12 months and in the clinical judgment of the investigator were not at risk for relapse may have been enrolled in the study.
• Evidence of active or suspected malignancy or history of malignancy within the last 5 years (with the exception of adequately treated basal cell carcinoma of the skin or in situ carcinoma of the cervix).
• Participation in any other clinical trials of an investigational compound (other than raxibacumab ≥4 months prior) within 60 days of initiating study agent or refusal to refrain from participation during this study.

Treatments Administered
Eligible subjects were to receive a 2nd dose of raxibacumab by IV infusion equal to that of their previous dose ≥4 months following the 1st dose (all subjects were treated with a 40 mg/kg raxibacumab dose). All subjects were to be treated with 50 mg oral (PO) diphenhydramine up to 60 minutes prior to infusion of raxibacumab.

Schedule of Study Procedures

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Procedure	Footnotes	Screening Day -28 to -1	Day 0	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56	End of Study (Day 70)
Informed Consent		X										
Inclusion-Exclusion Criteria		X										
Medical History-Demographics		X										
Vital Signs	1	X	X	X			X					X
Weight	2	X	X									
Physical Examination	2	X	X	X			X					X
Hematology and Clinical Chem	2,3	X	X	X	X	X	X	X	X	X	X	X
Urinalysis	2,3	X	X		X							X
Pregnancy Test	4	X	X									X
Study Agent Administration	5		X									
Immunogenicity	6	X	X						X		X	X
Pharmacokinetics (PK)	7		X	X	X	X	X	X	X	X	X	
Telemetric Monitoring	8		X									
Record Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X
Adverse Experience Monitoring			X	X	X	X	X	X	X	X	X	X

1 In house period for all subjects from Day 0 until Day 1 (24 hours following administration of raxibacumab).

2 Vital signs were performed supine, following 5 minutes of rest. On the day of dosing, vital signs were evaluated predose, 30 minutes postdose, and every 4 hours postdose for 24 hours.

3 Performed prior to raxibacumab dosing.

4 At Screening: hematology, chemistry and prior to dosing on Day 0.

5 Serum pregnancy test (if required) and confirmatory urine test prior to dosing on Day 0. Serum pregnancy test only on Day 70.

6 Subjects received a single dose of raxibacumab (40 mg/kg) IV on Day 0.

7 Immunogenicity testing on Day 0 was performed prior to infusion. Subjects with a positive anti-raxibacumab antibody titer following raxibacumab administration were to have a serum sample collected approximately 6 months after the dose for follow-up immunogenicity assessment.

8 PK sampling times for raxibacumab: Prior to raxibacumab dosing (Day 0), 5 minutes, and 6 hours after completion of the infusion, and at 1, 3, 7, 14, 21, 28, 42, and 56 days after the raxibacumab dose.

9 Telemetric monitoring for all subjects on Day 0: prior to dosing with raxibacumab, during infusion, and for 2 hours after the completion of the raxibacumab infusion.

Immunogenicity testing and Assay Methods

Immunogenicity testing was performed to determine if raxibacumab induced an anti-raxibacumab immune response. Subjects with a positive anti-raxibacumab antibody response anytime following the 2nd dose were to have a serum sample collected approximately 6 months after the last dose for a follow-up immunogenicity assessment.

Testing comprised 2 assays (screening and confirmatory). The screening assay (direct binding) was an electrochemiluminescence (ECL)-based bridging assay, with rabbit polyclonal antibody used as a positive control. Samples above the assay cut point were considered positive. Samples identified as positive in the screening assay were to be confirmed positive in a confirmatory assay. Samples must have demonstrated a significant percent drop in the confirmatory inhibition of binding assay to be considered positive. The inhibition of binding confirmatory assay was performed identically to the direct binding screening assay with the exception that the samples were tested in parallel with excess unlabeled raxibacumab. The assay limit of detection was 62.5 ng/mL.

Adverse Events

An AE was defined as any unfavorable or unintended sign, symptom, or disease that is temporally associated with the use of a study agent but is not necessarily caused by the study agent. This included worsening of pre-existing conditions. All AEs and serious AEs (SAEs) that were identified from study agent administration on Day 0 through end of study (Day 70) were recorded on the AE CRF. The record of each AE on the CRF included AE description or diagnosis, start date, end date, whether the AE was ongoing, whether any medication was taken, seriousness, severity, action taken with study agent, relationship to study agent (causality), and outcome.

Severity Grading
The investigator evaluated all AEs with respect to severity using the modified DMID Toxicity Tables. For AEs that were not included in the modified DMID Toxicity Tables, the following guidelines applied:

Mild - causing no limitation of usual activities
Moderate - causing some limitation of usual activities
Severe - causing inability to carry out usual activities
Life Threatening* - potentially life threatening or disabling

Serious Adverse Events
An SAE was defined as any AE resulting in any of the following outcomes:
• death.
• was life threatening (i.e., an immediate threat to life).
• inpatient hospitalization.
• prolongation of an existing hospitalization.
• persistent or significant disability/incapacity.
• congenital anomaly/birth defect.
• was medically important.

Follow-up of AEs and SAEs
AEs that occurred from the start of study agent administration on Day 0 through End of Study (Day 70) were followed until final outcome was known or until the end of the study if the event was ongoing. AEs that had not resolved by the end of the study were recorded as ongoing. SAEs that had not resolved by the end of the study were to be followed until final outcome was known. If it was not possible to obtain a final outcome for an SAE (e.g., the subject was lost to follow-up), the reason that a final outcome could not be obtained was to be documented by the investigator.

Clinical Laboratory Tests
The following laboratory tests were performed.

Hematology/Coagulation Panel	Clinical Chemistry Panel-liver function
Total white blood cell (WBC) count with differential:	Alkaline phosphatase
Neutrophils	Alanine transaminase (ALT, SGPT)
Bands	Aspartate transaminase (AST, SGOT)
Lymphocytes	Bilirubin (total)
Monocytes	Gamma glutamyl transpeptidase (GGT)
Eosinophils	Lactate dehydrogenase (LDH)
Basophils	
Hematocrit	**Clinical Chemistry Panel-electrolytes**
Hemoglobin	Bicarbonate
Platelet count	Calcium
Red blood cell (RBC) count	Chloride
Prothrombin time (PT)	Phosphorus
Partial thromboplastin time (PTT)	Potassium
	Sodium
Urinalysis Panel	Magnesium
Glucose	
Ketones	**Clinical Chemistry Panel-other**
Protein	Albumin to globulin (A/G) ratio
Occult blood	Albumin
Color	Amylase
Clarity	Blood urea nitrogen (BUN)
pH	Complement 3 (C3)
Specific gravity	Cholesterol (total)
Reflex microscopic examination	Creatinine
	Glucose
Other	Protein (total)
Serum pregnancy	Uric acid
(women of childbearing potential only)	Creatine kinase MB fraction (CK-MB)
	Creatine phosphokinase (CK, total and isoenzyme)
	T4 (free and total)
	Thyroid-stimulating hormone (TSH)

Vital Signs, Physical Examination and Telemetry

Vital signs (body temperature, systolic and diastolic blood pressure, and heart rate) were to be assessed at screening, and on Days 0, 1, 14, and 70 (End of Study). On Day 0 (day of raxibacumab dosing) vital signs were to be assessed predose, 30 minutes postdose, and every 4 hours postdose for 24 hours.

Physical Examination

Physical examination was to be performed at screening, prior to raxibacumab dosing on Day 0, Day 1, Day 14, and Day 70 (End of Study). Any new or worsening findings were to be reported as AEs on the AE CRF.

Telemetric monitoring was to be performed for all subjects prior to raxibacumab infusion, during infusion, and for 2 hours after the completion of the raxibacumab infusion on Day 0.

Protocol Amendments

Changes Resulting from Amendment 01 (16 October 2007)

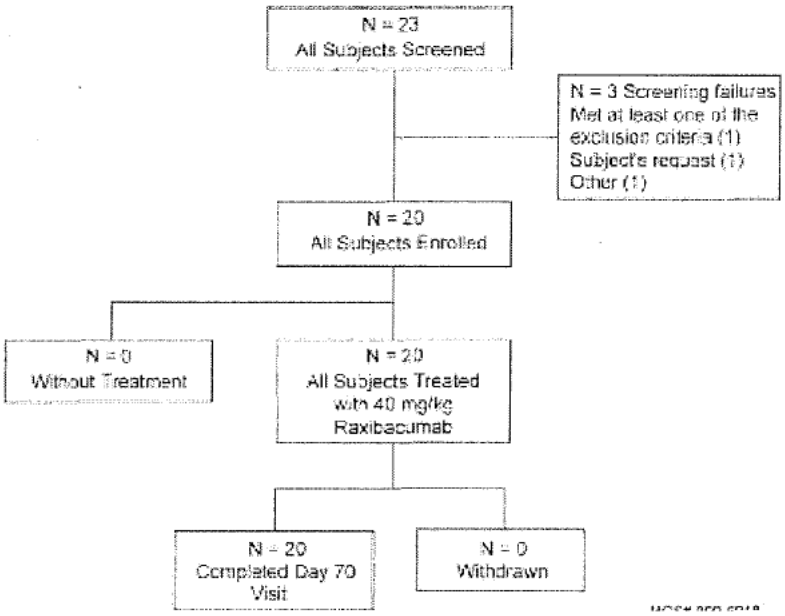
• A requirement for in-house observation for 24 hours following dosing and telemetric monitoring during study agent infusion was added at the request of the FDA.

• Wording for the inclusion criterion that subjects were to have been previously treated with raxibacumab in the last 4-6 months was changed to specify that the

subjects were to have been previously treated with raxibacumab at least 4 months prior to the planned dose for this study.
• A period of at least 4 months or greater was sufficient to ensure that more than 95% of the 1st raxibacumab dose had been eliminated. Inclusion of subjects who were dosed ≥4 months ago would allow subjects from previous HGS protocols to be included.
• Wording was changed to specify that "subjects will receive raxibacumab at a dose equal to that of the 1st dose rather than specifying 40 mg/kg raxibacumab for a 2nd dose."
• This change was to allow potential inclusion of subjects who might have received other raxibacumab doses in HGS protocols.
• Blood alcohol and urine drug tests were removed from the study schedule.
• Exposure to alcohol or drugs was not expected to affect the results of this trial.
• Procedures to mitigate anticipated and observed infusion reactions were added at the request of FDA.
• The following text was added: "If an infusion reaction occurs, the infusion should be slowed or interrupted and treatment administered based on the severity of the reactions. Subjects will be strongly encouraged to notify medical staff immediately if they develop any sign of infusion reaction."
• The post raxibacumab infusion follow-up period was changed from 90 days to 70 days.
• New immunogenicity qualification experiments demonstrated that in the presence of the predicted concentration of raxibacumab on Day 70 post infusion (16.6 μg/mL), the assay was able to reliably detect less than 1.0 μg/mL of anti-drug antibody. In the presence of the predicted concentration of raxibacumab on Day 90 post infusion (5.7 μg/mL) the assay was able to reliably detect less than 0.5 μg/mL of anti-drug antibody. As the difference in sensitivity was minor, these findings supported moving the last time point from Day 90 to Day 70.
• New information on study agent background and data management was added, including:
• Preliminary results of a Good Laboratory Practices (GLP) rabbit embryo-fetal toxicity study, in which no adverse effects of raxibacumab on embryo/fetal viability and growth and no maternal toxicity occurred.
• Safety and PK results obtained in a study of healthy volunteers co-administered raxibacumab and ciprofloxacin (HGS1021-C1064).
• Additional details on how CRFs were completed, reviewed by the investigator, retrieved by the sponsor, and processed by the sponsor's Data Management department were added.

Subject Disposition
A total of 23 subjects were screened to provide 20 subjects for the study. The 3 screening failures were due to failure to meet entry criteria (1), subject request (1), and other (1) (subject was screened outside of screening window), respectively. All 20 subjects were treated with 40 mg/kg IV raxibacumab per protocol, and all subjects completed the study.

Figure: Summary of subject disposition (from Applicant's submission)



All subjects in the study had previously received a complete raxibacumab dose of 40 mg/kg at least 4 months prior in Study HGS1021-C1064, and all subjects received a complete raxibacumab dose of 40 mg/kg in the current study. The mean time between raxibacumab doses between these 2 studies was 7.6 months with a range of 6 to 9 months, median 8 months.

Demographics

Demographics are summarized in the table below. There were a similar number of male and female subjects in this study (12 males and 8 females). The majority of subjects in this study were white (13/20, 65%) with African Americans 7/20, (35%) and subjects of Hispanic origin 4/20 (20%) completing the racial demographic. The age range was 23 to 61 years.

Table: Summary of demographics and baseline characteristics (from Applicant's submission)

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	Raxibacumab (40 mg/kg) N = 20
Sex	
Male	12 (60.0 %)
Female	8 (40.0 %)
Race	
White	13 (65.0 %)
Asian	-
Black or African American	7 (35.0 %)
American Indian or Alaska Native	-
Native Hawaiian or Other Pacific Islander	-
Not Listed	-
Black/African Amer. & Nat. Hawaiian/Other Pacific Islander	-
Hispanic or Latino origin	4 (20.0 %)
Age (years)	
Mean ± SD	40.8 ± 13.5
Median	38.5
Range	(23, 61)
Weight (kg)	
Mean ± SD	60.1 ± 14.9
Median	51.9
Range	(56.1, 100.5)

Immunogenicity Results

None of the subjects in study HGS1021-C1064 developed an anti-raxibacumab antibody response, and none of the subjects in this study had a positive anti-raxibacumab antibody response following raxibacumab re-administration.

Treatment-Emergent Adverse Events (TEAEs)

Forty percent (8/20) of subjects in this study reported at least 1 AE. There were no subjects with AEs considered to be at least possibly related to raxibacumab. In addition, there were no subjects with SAEs or severe AEs. Three subjects (15%) had Grade 2 AEs.

The table below shows TEAEs by MedDRA System Organ Class (SOC).

Table: Subjects with TEAEs by SOC

MedDRA system organ class	Raxibacumab n=20 (%)
Nervous system disorders	3 (15)
Gastrointestinal disorders	1 (5)
General disorders and administration site conditions	1 (5)
Infections and infestations	1 (5)
Injury, poisoning and procedural complications	1 (5)
Musculoskeletal and connective tissue disorders	1 (5)
Skin and subcutaneous tissue disorders	1 (5)

Subjects with TEAEs by MedDRA preferred Term (PT), ordered by total frequency is shown in the table below, with additional details in the following table. The most common AE observed was headache (3/20, 15%). There were

no severe AEs in this study. Three subjects had a Grade 2 AE, 1 with abdominal pain, 1 with joint stiffness, and 1 with headache. All of the Grade 2 AEs resolved after 1 to 3 days and none were considered related to raxibacumab.

There were no AEs ongoing at the end of the study.

Table: Subjects with TEAEs by Preferred Term

MedDRA system organ class	MedDRA Preferred Term	Raxibacumab n=20 (%)
Nervous system disorders	Headache	3 (15)
Gastrointestinal disorders	Abdominal pain	1 (5)
General disorders and administration site conditions	Pain	1 (5)
Infections and infestations	Upper respiratory tract infection	1 (5)
Injury, poisoning and procedural complications	Contusion	1 (5)
Musculoskeletal and connective tissue disorders	Joint stiffness	1 (5)
Skin and subcutaneous tissue disorders	Dry skin	1 (5)

Table: TEAEs in Study HGS1021-C1069

Subject ID	Age/ Sex/ Race	MedDRA system organ class	MedDRA Preferred Term	Study day of AE start	Duration	Outcome	Severity	Relationship to raxibacumab	Action taken with study agent
US001-001	52/M/W	Infections and infestations	Upper respiratory tract infection	39	14	Recovered	Mild	Probably not related	None
US002-001	50/F/W	Nervous system disorders	Headache	14	1	Recovered	Mild	Not related	None
US002-003	39/F/B	Nervous system disorders	Headache	57	1	Recovered	Mild	Not related	None
US002-008	28/F/B	Injury, poisoning and procedural complications	Contusion	14	11	Recovered	Mild	Not related	None
US002-009	29/M/W	General disorders and administration site conditions	Pain	8	2	Recovered	Mild	Probably not related	None

US002-011	48/F/B	Gastrointestinal disorders	Abdominal pain	41	3	Recovered	Moderate	Not related	None
US002-012	58/M/W	Nervous system disorders	Headache	6	1	Recovered	Moderate	Not related	None
US002-013	61/F/W	Musculoskeletal and connective tissue disorders	Joint stiffness	18	1	Recovered	Moderate	Probably not related	None
-	-	Skin and subcutaneous tissue disorders	Dry skin	1		Recovered	Mild	Not related	None

Discontinuations due to adverse events
There were no treatment discontinuations due to AEs.

Deaths
There were no deaths in this study.

Serious Adverse Events
There were no SAEs in this study.

Clinical Laboratory Tests

Hematology
Two subjects had Grade 2 hematology values. Subject US002-007 had fluctuating prothrombin time (PT) and partial thromboplastin time (PTT) values. This subject had a Grade 2 PT at screening and Grade 1 upon rescreening 1 week later. The subject's PT shifted to Grade 2 on Day 7, decreased to Grade 0 by Day 14, increased again to Grades 3 and 4 (27.5 seconds) on Days 42 and Day 56 and further shifted to Grade 4 (37.9 seconds) on Day 70. PT Grades of 2 and 3 were observed at 2 unscheduled assessments done after the end of the study period (103 days after raxibacumab dosing). PT had returned to Grade 0 at a post-study, unscheduled assessment performed 112 days after raxibacumab infusion. This subject also had a Grade 0 PTT (32 seconds) at baseline, which shifted to Grade 2 on Day 70. The PTT had returned to baseline at the unscheduled assessment conducted 112 days after raxibacumab infusion. The subject remained asymptomatic throughout this period and had no complaints of bleeding or easy bruising, and no other clinically significant lab abnormalities. No AEs were reported. The additional laboratory assessments for this subject were conducted to verify the abnormal results, and thus did not trigger the recording of the abnormal laboratory values as AEs at the discretion of the investigator as the subject was without clinical symptoms. No relevant medical history was reported for this subject. The subject moved overseas and was not available for further testing. Relationship to study agent was judged to be probably not related.

Subject US002-013 had a normal white blood cell count at baseline, and an isolated Grade 2 lymphopenia on Day 70. All other assessments for this subject were Grade 0. No concurrent AEs were reported.

Liver and Kidney Function
One subject had a Grade 2 laboratory test for liver parameters. Subject US002-016 had a Grade 0 (32 IU/L) ALT at baseline, and an isolated shift to Grade 2 (147 IU/L) ALT on Day 28. AST was also mildly elevated to Grade 1 (58 IU/L) on Day 28. There were no other clinically significant laboratory abnormalities obtained on this date, and no AEs were reported. All other ALT and AST assessments both prior to Day 28 and subsequent to this time point for this subject were Grade 0

Electrolytes
There were no electrolyte grades of 2 or higher.

Other Laboratory Tests
The following subjects had Grade 2 values for other chemistries:

Subject US002-005 had a Grade 1 (127 U/L) amylase at baseline, which shifted to Grade 2 (153 U/L) on Day 70. The subject was asymptomatic and no intervention was given.

Subject US002-018 had a Grade 0 (80 mg/dL) glucose at baseline and an isolated shift to Grade 2 (161 mg/dL) on Day 28. This subject also had a Grade 1 (59 mg/dL) hypoglycemia assessment on Day 70. The subject was asymptomatic and no AEs were reported.

Two subjects in the raxibacumab/ciprofloxacin group had isolated elevated total CK values (greater than 2.5 fold ULN). Subject US002-003 had elevated CK at baseline on Day 0 (527 U/L) and on Day 3 (445 U/L). Subject US002-009 had normal CK at baseline (101 U/L) and an elevation on Day 7 (1353 U/L). Liver enzymes were normal and there were no associated AEs of note.

There were no toxicity grades above 2 for other chemistries.

Vital Signs and Physical Exam
No AEs related to vital signs were reported and no clinically significant reductions in hemodynamic parameters (heart rate, blood pressure) were observed during the study. There were no clinically significant abnormalities in physical exam or telemetry assessments.

Safety Summary
Safety findings were limited in this study. There were no deaths, SAEs, AEs of severe intensity, or subjects who discontinued raxibacumab treatment due to AEs. All AEs were transient, mild to moderate in severity, and resolved. The

relationship of these AEs to raxibacumab was deemed to be not related or probably not related in all instances.

With respect to immunogenicity, no subjects in this study developed an anti-raxibacumab antibody response.

With respect to laboratory tests, there were a small number of transient shifts to Grade 2 or higher clinical laboratory values in this study with no consistent pattern of change, and there were no temporally associated AEs or symptoms reported. There were few changes in hematology and liver and kidney laboratory parameters.

9.6 Individual Study Report

REVIEW OF THE ANIMAL STUDY REPORT 789-G923702

BB-IND: 11069
Report Date: 03/26/2009
Products: Raxibacumab (Human monoclonal antibody to *B. anthracis* protective antigen) (bulk drug product manufactured by Human Genome Sciences, Inc. Rockville, MD, 20850; final drug product manufactured and labeled by [redacted] (b) (4), [redacted] (b) (4))
Ciprofloxacin (Bayer Health Care Pharmaceuticals, Inc., Wayne, NJ, 07470
Sponsor: Human Genome Sciences, Inc., Rockville, MD, 20850
Study Location: Battelle Biomedical Research Center (BBRC)
State Route 142
West Jefferson, OH 43162
Reference Lab: Human Genome Sciences, Inc.
(PCR for *B. anthracis,* 14200 Shady Grove Rd
PA ECL) Rockville, MD 20850
PK/PK Kinetics Analyses: [redacted] (b) (4)
Ciprofloxacin Assay: [redacted] (b) (4) (b) (4)
Telemetry Surgery: Battelle Memorial Institute, BBRC
Columbus OH 43201
Animal Supply: [redacted] (b) (4)
Submission Title: Evaluation of the Efficacy of Raxibacumab in Combination with Ciprofloxacin for Therapeutic Treatment in the Cynomolgus Monkey Inhalation Anthrax Model (Battelle Study 789-G923702)

Evaluation of the Efficacy of Raxibacumab in Combination with Ciprofloxacin for Therapeutic Treatment in the Cynomolgus Monkey Inhalation Anthrax Model

STUDY DIRECTOR: Daniel Sanford, Ph.D.

STUDY ASSOCIATE DIRECTOR: Jason M. Mott, DVM, Ph.D.

STUDY CENTER: Battelle Biomedical Research Center (BBRC)

STUDY PERIOD: February 18, 2008 to March 6, 2009

REPORT DATE: March 26, 2009

STUDY PROCEDURE: Study was conducted under GLP

OBJECTIVES:
Primary Objective:
To evaluate the efficacy of raxibacumab when administered as a therapeutic agent in
combination with ciprofloxacin against lethality due to inhalation exposure of *B. anthracis* in cynomolgus monkeys

Additional Objectives:
To determine the pharmacokinetics (PK) of intragastric (IG) ciprofloxacin doses and of an intravenous (IV) raxibacumab dose when co-administered, as well as the kinetics of *B. anthracis* protective antigen (PA), in monkeys with inhalation anthrax

METHODOLOGY: Parallel-group, double-blind, randomized, placebo-controlled study to evaluate the efficacy of raxibacumab in combination with ciprofloxacin as a therapeutic treatment in cynomolgus monkeys with inhalation anthrax

NUMBER OF ANIMALS: 40 naïve cynomolgus monkeys were randomized into 2 separate treatment groups of 14 animals and a placebo group of 12 monkeys (each 50% male and 50% female). Three animals were held as replacements but were not used.

STUDY DRUG: Treatment with study agent was triggered upon detection of serum PA via the screening assay or at 54 hours post-challenge. Ciprofloxacin (75 mg) or control material (sterile water for injection, WFI) was administered IG for 6 doses: initially once the criterion for treatment was met and then every (Q) 12 hours (± 1 hour) for 5 doses. In addition, a single IV bolus dose of raxibacumab (40 mg/kg) or raxibacumab buffer (1 mL/kg) was administered immediately after administration of the first ciprofloxacin dose. Within 5 minutes prior to treatment each monkey received a single 1 mg/kg intramuscular (IM) dose of diphenhydramine to parallel the treatment paradigm for humans in Phase 2/3 studies.

Raxibacumab is a recombinant, fully human IgG1λ mAb that binds PA with high affinity and inhibits its biological activity. Raxibacumab is expressed in the NS0 mouse myeloma cell line, secreted into culture media, and purified by a series of chromatography and filtration steps. Raxibacumab was supplied by HGS as a

ready-to-use sterile liquid formulation (50 mg/mL) in a formulation buffer of 0.13 mg/mL citric acid, 2.8 mg/mL
sodium citrate, 10 mg/mL sucrose, 18 mg/mL glycine, 0.2 mg/mL polysorbate 80, pH 6.5. Storage was at 2-8°C in the dark. During dosage periods the bottles were to be wrapped to keep in the dark and stored on ice. A certificate of analysis and stability data was provided by the sponsor. Raxibacumab from lot 71044 was used.

Ciprofloxacin (Cipro® IV, 10 mg/mL) was obtained from a commercial source (Bayer Health Care Pharmaceuticals, Inc., Wayne, NJ). Records of the ciprofloxacin lot number, expiration date, and storage conditions were retained in the study record. A ciprofloxacin dose equivalent to a 500 mg human dose was used in this study. The ciprofloxacin dose was calculated using the relative body surface area of the rhesus monkey, with body surface area estimated from body weight. Taking a midpoint in the body weight of cynomolgus monkeys, a dose of 75 mg was calculated using a similar approach. Ciprofloxacin Batch 5400PLX was used.

Placebo for raxibacumab was raxibacumab buffer, administered at 1 mL/kg. Placebo from Lot 71043 was used. The placebo for ciprofloxacin was sterile WFI (Lot 710375F [redacted] (b) (4)), and was administered IG.

All monkeys received a single IM dose of diphenhydramine (1 mg/mL) within 5 minutes prior to study agent administration.

DURATION OF TREATMENT: A single dose of raxibacumab or raxibacumab buffer was given on Day 0. Ciprofloxacin or sterile WFI was given every 12 hours for a total of 6 doses.

STUDY DESIGN: This was a parallel-group, double-blind, randomized, placebo-controlled GLP study to evaluate the efficacy of raxibacumab in combination with ciprofloxacin as a therapeutic treatment in the cynomolgus monkey inhalation anthrax model. PA kinetics and raxibacumab and ciprofloxacin PK were also examined.

Forty (50% male, 50% female) juvenile (< 5 years of age) cynomolgus macaques (*Macaca fascicularis*) weighing between 2.0 to 6.0 kg at randomization were randomized into 3 treatment groups (see Table 1 below), and randomly assigned to 1 of 3 aerosol challenge days and a challenge order.

Group	Number of NHPs	Ciprofloxacin/Control	Raxibacumab/Control	Treatment Point
Group 1 (Control)	12	Sterile water IG	Raxibacumab buffer IV (1mL/kg)	Individual treatment times were
Group 2	14	75 mg IG every 12h for	Raxibacumab buffer IV	

(Ciprofloxacin)		6 doses	(1mL/kg)	based on serum PA levels as detected by ECL
Group 3 (Ciprofloxacin/ raxibacumab)	12	75 mg IG every 12h for 6 doses	Raxibacumab IV 40mg/kg after the first ciprofloxacin dose	

Table 1. Treatment Groups

On Day 0, monkeys were aerosol challenged with a targeted 200 x LD50 [1.24 x 107 spores] dose of *B. anthracis* spores (Ames strain) aerosolized by a Collison nebulizer and delivered via a head-only inhalation exposure chamber. Treatment with study agent was triggered upon detection of serum PA via the ECL screening assay or at 54 hours post-challenge. Ciprofloxacin (75 mg) or control material (sterile water for injection, WFI) was administered IG for 6 doses: initially once the criterion for treatment was met and then every (Q) 12 hours (± 1 hour) for 5 doses. In addition, a single IV bolus dose of raxibacumab (40 mg/kg) or raxibacumab buffer (1 mL/kg) was administered immediately after administration of the first ciprofloxacin dose. Within 5 minutes prior to treatment each monkey received a single 1 mg/kg intramuscular (IM) dose of diphenhydramine to parallel the treatment paradigm for humans in Phase 2/3 studies. Staff members administering study agents were blinded to study agent preparation to preclude introduction of bias into the study following treatment.

MO comment: *The raxibacumab treatment in the monkeys was administered as a bolus injection. In human studies, raxibacumab is to be administered by infusion over 2.5 hours. This difference in administration times needs to be taken into account in matching the animal and human PK parameters, especially related to Cmax.*

Monkeys were observed every 6 hours beginning 24 hours post median challenge time and ending 192 hours (8 days) post median challenge time for clinical signs including, but not limited to, anorexia, lethargy, respiratory distress, moribundity, activity (recumbent, weak, or unresponsive), seizures, and other abnormal clinical observations, after which they were observed twice daily, or more often if there was any sign of distress. Monkeys surviving the post-challenge could be returned to the sponsor (if required) or donated following observation for an additional 60 days under BL-2 laboratory conditions for recrudescence. Gross necropsy was performed on all monkeys that were found dead or were euthanized. Histopathology was to be performed, as deemed necessary by a board-certified veterinary pathologist, to confirm death or illness due to anthrax. Blood samples were collected on the days specified in Table 5-3 for hematology and CRP, as well as to measure bacteremia by culture, serum PA levels, anti-PA and TNA levels, serum raxibacumab levels and ciprofloxacin levels.

Inclusion criteria:

- Male and female juvenile (< 5 years of age) naïve Chinese colony bred cynomolgus monkeys by [redacted] (b) (4).
- Weight 2.0 to 6.0 kg at randomization.
- Free of malformations and clinical signs of disease.
- Monkeys were to be tested and verified negative for tuberculosis and screened within 30 days prior to receipt at Battelle and found to be negative for Simian Immunodeficiency Virus, Simian T-Lymphotrophic Virus-1 (STLV-1), and *Cercopithecine herpesvirus* 1 (Herpes B virus). All monkeys were required to be tested negative for Simian Retrovirus (SRV1 and SRV2) by polymerase chain reaction (PCR).

Exclusion criteria:

- Malformations.
- Clinical signs and symptoms of disease.
- Any monkeys outside the specified weight range.
- Any monkey with any one of the infections listed above.

Euthanasia Criteria:

Any of the following criteria prompted immediate euthanasia:

- Presence of any seizure denoting primary central nervous system disease.
- Respiratory distress, dyspnea, or forced abdominal respirations.
- Unresponsive to touch or external stimuli.
- Moribundity.

Euthanasia was warranted when all the clinical signs below were observed:

- Recumbence and weakness.
- ≥20% body weight loss.
- Body temperature below 97°F; indicative of shock.
- Total anorexia with duration longer than 48 hours.

All monkeys that were judged to be moribund by a highly trained life sciences technician, Battelle veterinarian, or by the study director were euthanized immediately. Euthanasia was accomplished by anesthetizing with Telazol (1-6 mg/kg) IM, collecting blood if possible for tests, and immediate euthanasia using an overdose of a barbiturate that is administered IV or by intracardiac injection or other American Veterinary Medical Association (AVMA) approved method of euthanasia.

Aerosol Challenge:

On Day 0, monkeys were anesthetized with Telazol (1-6 mg/kg, IM) and placed into a plethysmography chamber and a Class III cabinet system. Monkeys were aerosol-challenged with a targeted 200 x LD50 [1.24 x 107 spores] dose of *B. anthracis* spores (Ames strain) aerosolized by a Collison nebulizer and delivered via a head-only inhalation exposure chamber.

Randomization of Monkeys:

Forty-three naïve cynomolgus macaques were randomized into 1 group of 12 animals,

2 groups of 14 animals (all 3 groups having 50% female, 50% male), and 1 group of 3 replacement animals (all male), and then into 3 aerosol challenge sets of animals. The 3 sets of monkeys were aerosol challenged on 3 staggered challenge days (Day 0). Randomizations were performed with SAS® statistical software.

Treatments and Study Groups:
Cynomolgus monkeys were randomized to one of three aerosol challenge days and a challenge order per day. On day 0, monkeys were challenged with a targeted aerosol dose of 200 x LD_{50} spores of *B. anthracis* (Ames strain). For individual monkeys, treatment was to be administered immediately following detection of serum PA via the PA screening assay. Animals that did not have a positive serum PA screening assay result by 54 hours post challenge were to be treated with their intended treatment (because additional screening was not possible due to blood collection limits). Within 5 minutes prior to treatment each monkey was to receive a single 1 mg/kg IM dose of diphenhydramine to reflect the proposed treatment paradigm in humans.

Blinding:

Staff administering raxibacumab and raxibacumab buffer and/or conducting assessments of monkeys post challenge were to be blinded to their treatment assignments. Pathologists and the technicians performing the bacteremia cultures were also blinded to treatment group assignment. BBRC personnel not involved with the treatment or assessment of monkeys were to aseptically dilute raxibacumab with raxibacumab buffer to assure that the volume of raxibacumab and raxibacumab buffer were equal. A BBRC staff member who was not associated with the conduct of the study was to randomly assign vial identifications to each of the 3 treatment groups. Dilutions of study raxibacumab and raxibacumab buffer were to be sealed in sterile dosing vials labeled X, Y, or Z (only 1 vial identification was to be used for the dilutions of study agent; the other 2 were to be used for the control material). Dilutions of study agent and transfer of control material were to be performed no earlier than 3 days prior to challenge and were to be stored at 2-8°C in the dark prior to dosing. Vial assignments were to be maintained by Battelle's Quality Assurance Unit until 32 days following the 3rd challenge day.

Clinical Observations:
The study duration was 28 days, with an additional post-Day 28 observation period of
60 days. Monkeys were to be observed every 6 hours beginning 24 hours post median
challenge time and ending 192 hours (8 days) post median challenge time for clinical signs including, but not limited to, anorexia, lethargy, respiratory distress, moribundity, activity (recumbent, weak, or unresponsive), seizures, and other

abnormal clinical observations. Should any animals have been documented as having respiratory distress or as being unresponsive during the 8 days following challenge, more frequent observations may have been performed and telemetry data may have been reviewed to determine if body temperature or activity levels were decreasing. Any additional observations were to be documented at the time of the observation. If at the final clinical observation on Day 8 no animal was exhibiting unresponsiveness or respiratory distress, observations performed every 6 hours were to cease and animals were to be observed twice daily thereafter. In addition, appetite was to be monitored twice daily during this observation period. During the quarantine and prechallenge period and from Day 9 through Day 32 post-exposure, clinical observations were to be recorded twice daily. Observed signs of illness or live/found dead/euthanized animals were to be recorded at the time of the observation. Any animal judged by a trained animal technician or study veterinarian to be moribund (in consultation with the study director) may have undergone, if possible, a terminal blood draw collected in ethylenediaminetetraacetic acid (EDTA) and serum separator (SST) tubes for bacteremia, serum PA analysis, and CRP.

Necropsy and Histopathology:
Gross necropsy was to be performed on all monkeys that were found dead or were euthanized. Sections of target tissues, including but not limited to brain/meninges, lungs, liver, spleen, kidney, and mediastinal lymph nodes as well as all gross lesions, were to be preserved in 10% neutral buffered formalin. Histopathology was to be performed, as deemed necessary by a board-certified veterinary pathologist, to confirm death or illness due to anthrax. Representative samples of spleen, liver, and mediastinal lymph node were to be collected during gross necropsy, homogenized, and plated on blood agar plates for qualitative assessment of bacterial presence in the target tissues. The study pathologist was to be blinded to the treatment group assignment until all necropsy and histopathology study activities were completed. In order to confirm a cause of death or moribundity, tissues from all animals that died on study were processed to approximately 5-micron sections for routine hematoxylin and eosin staining and were examined microscopically by a board-certified veterinary pathologist. Diagnoses were entered into the PATH/TOX SYSTEM (Xybion Medical Systems Corporation) data management system for data recording and analysis. Due to the study blinding requirement, treatment groups were randomly relabeled X, Y and Z, and the pathologist had no knowledge of the associated treatment for any animal. Photos of relevant microscopic lesions were obtained and included in electronic form in the study file.

MO comment: *Histopathology analysis was performed on all animals that died, in response to the FDA request in amendment 4 (November 10, 2008) of the protocol.*

Blood Collection and Testing:
Blood samples were to be taken from a femoral artery or vein, saphenous vein, or other appropriate vein on the days specified in Table 2 below. All post challenge pretreatment blood samples were to be collected relative to the median challenge time at the timepoints stated (± 1 hour). Median challenge time was to be calculated from the end time of the 1st and last animals challenged within a single challenge day. Blood collections were to be scheduled based on their post challenge or posttreatment times, as indicated. All posttreatment blood samples were to be collected relative to the end time of each animal's raxibacumab (or raxibacumab buffer) treatment. Exact blood draw times were to be documented. The number of blood samples collected may have been reduced if it was determined by the study director in consultation with a Battelle veterinarian that additional collections would have significantly compromised the animal's health. Should this have occurred, documentation was to be included in the study file, and analyses limited to those samples collected.

Time Point	Tube Type/ Blood Volume	Bacteremia (Culture)	Serum PA (ECL screening assay)	Serum Shipped to HGS for Raxibacumab, PA (ECL), Anti-PA and/or TNA Analyses[5]	Serum for Ciprofloxacin PK assay	CBC/ CRP
Study Day -7	EDTA ~1.5 mL SST[1] ~3.0 mL	X	X	X	X	X
24 hours post challenge followed by every 6 hours up to treatment (or up to 54 hours post challenge)	EDTA ~0.5 mL SST ~2.0 mL	X	X	X	-	-
Just prior to treatment	EDTA ~1.5 mL SST ~1.0 mL	X	-	X	-	X
5 minutes posttreatment	SST ~1.0 mL	-	-	X	-	-
1.5 hours posttreatment	SST[2] ~1.0 mL	-	-	-	X	-
12 hours posttreatment (just prior to 2nd Cipro treatment)	SST[2] ~1.0 mL	-	-	-	X	-
24 hours posttreatment (just prior to 3rd Cipro treatment)	EDTA ~1.5 mL SST[2] ~2.0 mL	X	-	X	X	X
36 hours posttreatment (just prior to 4th Cipro treatment)	SST[2] ~1.0 mL	-	-	-	X	-
48 hours posttreatment (just prior to 5th Cipro treatment)	EDTA ~1.5 mL SST[2] ~2.0 mL	X	-	X	X	X
60 hours posttreatment(just prior to 6th Cipro treatment)	SST[2] ~1.0 mL	-	-	-	X	-
72 hours posttreatment	EDTA ~1.5 mL SST[2] ~2.0 mL	X	-	X	X	X
5 days posttreatment	EDTA ~0.5 mL SST ~1.0 mL	X	-	X	-	-
8 days post challenge	EDTA ~1.5 mL SST ~1.0 mL	X	-	X	-	X
14 days post challenge	EDTA ~1.5 mL SST ~1.0 mL	X	-	X	-	X
21 days post challenge	EDTA ~1.5 mL SST ~1.0 mL	X	-	X	-	X
28 days post challenge	EDTA ~1.5 mL SST ~1.0 mL	X	-	X	-	X
Terminal	EDTA ~1.5 mL SST[2] ~2.0 mL	X	-	X	X	CRP only

Table 2. Blood Collection and Assay Schedule

(Adapted from HGS' BLA submission)

Post challenge pretreatment bleed timepoints were to be relative to a median challenge time for a challenge cohort. Post-treatment bleed times were to be calculated from the time each animal's IV treatment ended. Blood samples were to occur within ± 60 minutes of the calculated time, except for the first 2 post-treatment blood samples, which were to occur within 5 and 15 minutes of the calculated time, respectively. The Day 8, Day 14, Day 21, and Day 28 post challenge blood samplings were to be

relative to the day of challenge. Body temperatures and activity were to be monitored at least hourly beginning 10-14 days prior to challenge, and until the end of the in-life animal phase (32 days post challenge). Body temperatures were to be recorded via TA-D70 telemetry transmitters (DSI, St. Paul, MN) after surgical implantation, which was conducted during quarantine.

[1] Blood specimens for anti-PA antibodies and TNA were to be collected from all monkeys at 7 days prior to spore challenge as well as 28 days after spore challenge. The blood specimens were allowed to clot and were then centrifuged. The serum was harvested, filtered, cultured for presence of *B. anthracis*, and stored at ≤-70ºC prior to shipment to HGS for assay. If sufficient volume for the 14 and 21 days post spore challenge specimens remained, those specimens may also have been analyzed for anti-PA antibody and TNA.

[2] Processing to serum was to be initiated within 60 minutes of collection.

Assays:

For assessment of bacteremia by culture, approximately 40 μL of whole blood or tissue homogenate (prepared in 2 mL of phosphate buffered saline) was to be collected in EDTA tubes, streaked on blood agar plates, and incubated at approximately 37°C for a minimum of 48 hours per SOP BBRC.X-096. Following incubation, macroscopic observations were to be performed to assess the presence or absence of bacterial colonies. Colonies were to be assessed for hemolysis type, size, color, and morphology. If γ-hemolytic, white colonies, 4-10 mm in diameter with a rough appearance and irregular edges were present, the sample was to be documented as positive for *B. anthracis*.

For levofloxacin and ciprofloxacin MICs, serial dilutions of levofloxacin and ciprofloxacin were to be prepared in sterile water and added to a volume of sterile media in microplates for a final concentration of 0.0156 to 32 μg/mL. A dilution of the *B. anthracis* inoculum was to be prepared in sterile broth and used to inoculate the microplates. The microplates were to be incubated at approximately 37°C for approximately 16 to 20 hours. Macroscopic observations were to be performed to assess the level of growth (turbidity) and determine the MIC of the antibiotics. The MIC was recorded as the lowest concentration of drug resulting in no visible growth.

Hematology evaluation was performed with the Advia® 120. Hematology slides were to be prepared and reviewed when necessary as determined by a highly-trained hematology technician or the Study Director. Hematology parameters that were monitored may have included but was not limited to: WBC, differential leukocyte count, RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelets, MPV, and neutrophil to lymphocyte number ratio. CRP analysis was to be performed on residual plasma collected from each whole blood sample after processing, with no repeat analysis. CRP analysis was also to be conducted on terminal blood samples collected in EDTA tubes if the plasma could be isolated.

CRP analysis was a non-validated assay run according to the manufacturer's instructions.

Serum PA screening assay was an ECL based assay. Undiluted serum samples in triplicate wells were to be incubated in the presence of rabbit anti-PA-biotin and rabbit anti-PA-SULFOTAG for 2 hours at 37^0C in a streptavidin-coated assay plate. The limit of detection was established at 1.0 ng/mL based on the qualification experiments. Total PA (free + raxibacumab-bound) concentrations in serum samples were determined using an ECL-based bridging assay (SOP CLI-2996). Diluted serum samples in duplicate wells were combined with monkey polyclonal antibody (pAb) anti-PA-biotin (capture) and monkey pAb anti-PA-SULFO-TAG (detector) and allowed to equilibrate in a streptavidin-coated assay plate. Following equilibration, plates were washed and read for ECL counts. The concentration of PA in serum samples and controls was interpolated from a reference standard curve. The assay was required to have met system suitability criteria to be valid. The lower limit of quantitation (LLOQ) was 0.65 ng/mL of PA in 100% monkey serum.

Anti-PA antibody concentrations in serum samples were to be determined using an ELISA. Anti-PA antibody was to be captured on wild-type PA coated plates and the concentration was interpolated from a reference standard curve. The lower limit of quantification was < 600 ng/mL of anti-PA antibodies in 100% monkey serum.

TNA titers were to be determined using a cell killing assay in J744A.1 macrophages. Inhibition of PA binding is correlated with increased cell viability and is used to determine the relative titer of serum samples compared with a known positive control. The lower limit of quantification was < 52 titer in 100% monkey serum.

Serum samples were analyzed for raxibacumab using an ECL-based assay where raxibacumab in serum binds to the biotinylated-PA on a streptavidin-coated plate and is detected by the addition of rabbit anti-raxibacumab antibody followed by goat anti-rabbit antibody labeled with MSD SULFOTAG. The concentration was interpolated from a reference standard curve. The lower limit of quantification was 940 ng/mL in 100% monkey serum.

Serum samples were analyzed for ciprofloxacin at CSU, using a high performance liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) assay. The analyte ciprofloxacin and its internal standard enrofloxacin were extracted from a 100 μL aliquot of monkey serum via solid phase extraction. The extracted samples were injected into a liquid chromatograph equipped with an Agilent Zorbax, 3.5 μm, SB-C8, 4.6 x 75 mm column. The mobile phase was 50% acetonitrile; 50% 10 mM ammonium acetate + formic acid, pH 2.5. The detection method used was mass spectrometry/mass spectrometry. Peak areas of the *m/z* 332.1 → 288.3 ion transition of ciprofloxacin

were measured against the *m/z* 360.2 → 316.2 ion transition of the internal standard. The calibration range for the assay was from 10 to 5000 ng/mL.

MO comment: *Please refer to Microbiology Review for assessment of the assay methods that were employed in this study. The most critical assay in this study was the screening PA, as a positive PA screen was the trigger for treatment. Bacteremia as defined by culture is considered the gold standard. The PA screen, the quantitative PA test, the anti-PA antibody test, and the TNA assay are all exploratory tests being used in this study. It will be important to validate the performance characteristics of these studies.*

Statistical Methods:
The sponsor stated that this study design provided approximately 85% power at a 5% overall significance level to detect an absolute improvement of 56% or more survival at Day 28 post challenge in the ciprofloxacin/raxibacumab combination group. The sample size calculation assumed an 8.3% (1 of 12) survival at Day 28 in the placebo group and a 64.3% (9 of 14) survival at Day 28 in the ciprofloxacin /raxibacumab combination group.

Analyses of the primary and secondary efficacy endpoints was to be performed on the intention-to-treat (ITT) population defined as all animals that were randomized and challenged with *B. anthracis*. The ITT analysis was to be based on the treatment group planned at randomization rather than the actual treatment the animals received. Animals that were spore-challenged, but died before receiving placebo or active treatment, were to be included in this population as treatment failures. All statistical tests were to be 2-sided and performed at a significance level of 0.05.

The modified ITT (MITT) population was defined as a subset of the ITT population that included all animals that received a study agent. The MITT analysis was to be based on the planned treatment group rather than the actual treatment received. The as-treated population was defined as the set of all animals that received a study agent with the assignment to treatment group that was based on the actual treatment administered to the subjects. The as-treated analysis was to be based on the actual treatment that the animals received.

MO comment: *Please refer to Statistical review by Lan Zeng, Ph. D. In communication with the Sponsor, FDA indicated that the per protocol population (those that were bacteremic at the time of treatment) would be the study population of interest for efficacy analysis.*

Endpoints:
Primary Efficacy Endpoint:
The primary efficacy endpoint was survival at Day 28 in the ITT population. Animals that were spore-challenged, but died before receiving placebo or any

active treatment, were included in this population as treatment failures. The primary analysis of the primary efficacy endpoint was to compare the percent of animals alive at study Day 28 post challenge between the placebo group (Group 1) and the ciprofloxacin/raxibacumab group (Group 3). The comparison was performed using the 2-sided Fisher's exact test.

As an exploratory analysis of the primary efficacy endpoint, the point estimate with a
95% confidence interval (CI) was presented for the difference in the survival at Day 28 between the ciprofloxacin group (Group 2) and the ciprofloxacin/raxibacumab combination group (Group 3).

As a sensitivity analysis of the primary efficacy endpoint, the percent of animals alive at study Day 28 post challenge between the placebo group (Group 1) and the ciprofloxacin/raxibacumab combination group (Group 3) was to be compared via the 2 sided Fisher's exact test in the as-treated population, the modified ITT population, and the population of all animals that were randomized, including those that were randomized but not challenged with *B. anthracis.* The same sensitivity analyses was to be performed for the point estimate and 95% CI of the difference in the survival at Day 28 between the ciprofloxacin group (Group 2) and the ciprofloxacin/raxibacumab combination group (Group 3). The percent of animals alive at Day 28 post challenge between the placebo group (Group 1) and the ciprofloxacin/raxibacumab combination group (Group 3) was to be compared in (but not limited to) the following subgroups via 2-sided Fisher's exact test if the sample size was large enough: Monkeys that were confirmed to be toxemic at the time of treatment initiation, monkeys that were confirmed to be bacteremic at the time of treatment initiation, monkeys that were confirmed to be toxemic and bacteremic at the time of treatment initiation, and monkeys that were confirmed to be toxemic and/or bacteremic at the time of treatment initiation. In addition, the point estimate of the difference in the survival at Day 28 between the ciprofloxacin group (Group 2) and the ciprofloxacin/raxibacumab combination group (Group 3) was to be presented with a 95% CI in each of the above subgroups. Subgroup analyses were not to be subject to multiple comparison adjustment.

MO comment: *This study report has been submitted in support of the licensing application for the treatment of inhalational anthrax in humans under the Animal Rule so it is important to ensure that the study population in nonhuman primates is sufficiently reflective of the potential human population with anthrax disease. Therefore, the efficacy assessments in this study will focus on the outcome in the per protocol population of animals that were bacteremic at the time of treatment. The outcomes in the ITT and the modified ITT populations should trend in the same direction as the outcomes in the per protocol population.*

Secondary Efficacy Endpoint:

The secondary efficacy endpoint was survival time from spore challenge to death or to the end of the study period. Analyses of the secondary efficacy endpoint were to be performed on the ITT population. For animals that were alive at the end of the 28-day post challenge period, survival times were to be censored on the date of study completion. The log-rank test was to be used to compare the animals' survival time between the placebo group (Group 1) and each of the treatment groups. The survival time was also to be compared between the ciprofloxacin group (Group 2) and the ciprofloxacin/raxibacumab combination group (Group 3) using the same method. The analysis of the secondary efficacy endpoint was not to be subject to multiple comparison adjustment.

Exploratory analyses were to be conducted to explore the associations between monkeys' survival at Day 28 with the inhaled dose exposure to *B. anthracis* spores, time to onset of symptoms, time to detectable serum PA levels, and time to onset of bacteremia. Additional analyses were to be conducted to explore the immune response generated following anthrax spore challenge by comparing the seroconversion from baseline in anti-PA antibodies and TNA among surviving monkeys in each group. If data were available, additional subgroup analysis of the primary efficacy endpoint were to be conducted for monkeys confirmed to be anti-PA antibody negative at baseline.

Statistical Methods for Ciprofloxacin and Raxibacumab PK, and Serum PA Kinetics

Serum ciprofloxacin concentration-time data, serum raxibacumab concentration-time data, serum PA concentration-time data, individual ciprofloxacin PK parameters, individual raxibacumab PK parameters, individual PA kinetic parameters, serum anti-PA antibody concentrations, and serum TNA titers were summarized using descriptive statistics (number of observations [N], mean, standard deviation [SD], standard error [SE], coefficient of variation [CV%], minimum, median, maximum, geometric mean, and 95% CI). Unpaired t-tests were used to compare ciprofloxacin Cmax,1 and Cmax, n between Groups 2 and 3. For animals that survived, an unpaired t-test was used to compare PA t1/2 between Group 2 and Group 3. Unpaired t-tests were used to compare serum anti-PA antibody or TNA concentrations at corresponding collection times between Group 2 and Group 3. All comparisons were carried out at the $\alpha = 0.05$ level of significance.

MO comment: *Please refer to the Clinical Pharmacology Review for PK analysis.*

PROTOCOL CHANGES:

The original protocol was dated February 18, 2008. Four amendments were issued. Amendment 1 was issued prior to challenge, amendment 2 was issued

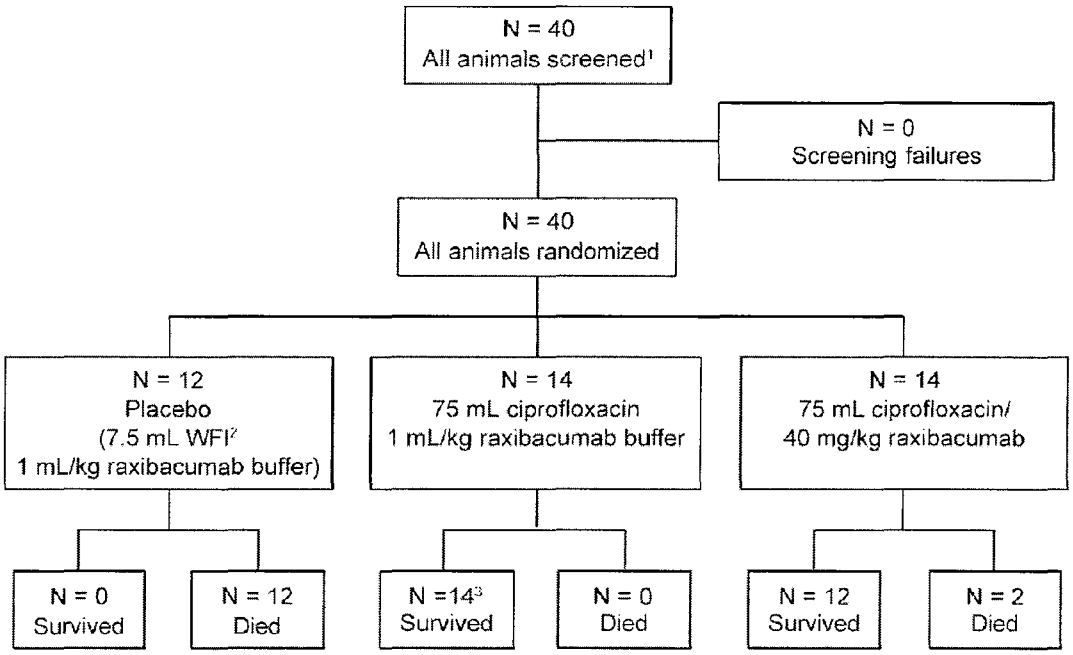
post-challenge, and amendments 3 and 4 were issued after the in-life phase of the study.

- Amendment 1 – March 17, 2008
    - The credentials for the Safety and Health Officer for the study were incorrect in the original protocol and were corrected.
    - The dose of sterile WFI used for the ciprofloxacin control was changed from 2 mL/kg to 7.5 mL in the 1st footnote of the study design table. This was done to ensure that all groups received comparable treatments.
    - The blood collection schedule (Table 2) was updated to specify that blood collected in SST tubes for ciprofloxacin analysis would not be chilled or placed on ice packs after collection. The requirement was changed as such because the flow properties of the gel barriers in the SST tubes used is temperature-dependent; therefore gel flow could be impeded if these tubes were chilled or placed on ice packs. In addition, this amendment specified that a single 2 mL SST tube may be used to collect blood at the required timepoints rather than two 1 mL tubes.
    - The protocol was amended to specify that all serum samples to be shipped to HGS for analysis of raxibacumab, PA, anti-PA and/or TNA would be divided into 2 aliquots of approximately the same volume prior to shipment. This was done to reduce the number of freeze/thaw cycles for backup samples, thus reducing the chance that sample integrity might be compromised. The wording in Section 6.11 (Ciprofloxacin [PK] assay) of the protocol was also adjusted to reflect this change.
    - Per FDA request, a statement was added to the end of Section 6.14 (Necropsy and Histopathology) to specify that the study pathologist was to be blinded to the treatment group assignment until all necropsy and histopathology study activities had been completed.
    - The Statistical Methods section of the protocol was updated to include all of the statistical methods included in the analytical plan submitted by HGS to FDA.
- Amendment 2 – March 25, 2008
    - Wording in Section 8.4 (Dose Confirmation) was changed to specify that Cipro® IV, 10 mg/mL was to be used on study rather than ciprofloxacin oral solution (which was not commercially available).
    - Section 6.9 (Raxibacumab Assay) was changed to clarify that raxibacumab PK analysis would not be performed on all samples collected as indicated in Table 2; the "X" in the table indicates that a serum sample was to be collected, processed, and shipped to HGS for testing.

- Per FDA request, a statement was added to the end of Section 6.6 (Bacteremia [Culture]) indicating that the technicians preparing the bacteremia cultures would be blinded to the treatment assignments for the duration of the study.

- Amendment 3 – July 3, 2008
  - Section 6.2 (Aerosol Challenge) was corrected to specify that the targeted inhaled *B anthracis* dose of 200 x LD50 was 1.24 x 107 spores rather than 2.47 x 107 spores per animal as was incorrectly stated in the original protocol.
  - Section 6.15 (Challenge Survivors) was corrected to specify that monkeys surviving the 30-day post-treatment period will receive exit physical examinations and TB tests prior to leaving the BL-3 facility, and that blood samples will be collected for use in assay development.
  - Section 11.0 (Biosafety Considerations) was corrected to clarify that the entire 60-day post-study observation period would not be conducted under BL-2 conditions.
- Amendment 4 – November 10, 200
  - Section 3.0 (Location of Testing Facilities) was corrected to specify that PK and kinetic analyses were conducted at FSC.
  - Section 6.14 (Necropsy and Histopathology) was corrected to specify that histopathology will be performed on all animals that died on study.

STUDY 789-G923702 RESULTS
Disposition of Animals



[1]Forty-three (43) animals (40 for study, 3 extra animals for replacement if necessary) were screened.
[2]Water for injection (control for ciprofloxacin).
[3]One animal – C30988 in the Ciprofloxacin group died (survival time = 36.17 days) during the 60-day additional observational period after the 28-day study period.

HGS# 000-6948

(Adapted from HGS' BLA submission)
Figure 1. Summary of Animal Disposition

***MO comment**: This figure represents the disposition of the sponsor's ITT population. The review for efficacy will focus on the analysis of animals that were bacteremic prior to treatment.*

Demographic and Baseline Characteristics

	Placebo N = 12	Ciprofloxacin N = 14	Ciprofloxacin/ Raxibacumab N = 14	All Groups N = 40	P-value[1]
Sex	-	-	-	-	1.0000
n	12	14	14	40	-
Male	6 (50.0%)	7 (50.0%)	7 (50.0%)	20 (50.0%)	-
Female	6 (50.0%)	7 (50.0%)	7 (50.0%)	20 (50.0%)	-
Prechallenge weight (kg)	-	-	-	-	0.7103
n	12	14	14	40	-
Mean ± SD	3.4 ± 0.8	3.6 ± 1.2	3.3 ± 0.7	3.4 ± 0.9	-
Median	3.2	3.2	3.1	3.1	-
Range	(2.6, 4.8)	(2.6, 6.5)	(2.5, 5.3)	(2.5, 6.5)	-
Age at randomization (yrs)	-	-	-	-	0.8595
n	12	14	14	40	-
Mean ± SD	4.2 ± 0.3	4.2 ± 0.6	4.3 ± 0.5	4.2 ± 0.5	-
Median	4.1	4.2	4.1	4.1	-
Range	(3.6, 4.7)	(2.9, 5.0)	(3.3, 5.1)	(2.9, 5.1)	-
Anti-PA antibody positive at baseline[2]	-	1/14 (7.1%)	2/12 (16.7%)	3/26 (11.5%)	0.5800
TNA positive at baseline[2]	-	0/14 (0.0%)	0/12 (0.0%)	0/26 (0.0%)	-

[1] P-value for comparison of the 3 groups, based on Fisher's exact test for categorical variables, and ANOVA for continuous variables.

[2] Data on anti-PA antibody and TNA was available for survivors only.

(Adapted from HGS' BLA submission)

Table 3. Summary of demographics and baseline characteristics for the ITT population

One monkey in the ciprofloxacin group (C24792) had a weight of 6.4 kg at randomization. One animal in the ciprofloxacin group and two animals in ciprofloxacin/raxibacumab group were anti-PA antibody positive at baseline. All surviving animals were TNA negative at baseline.

***MO comment**: Demographics and baseline characteristics were similar among the treatment groups. Three animals were positive for anti-PA antibodies at baseline. Of note, baseline antibody status was recorded only for animals who survived, so no results were available for any of the placebo animals.*

Extent of Anthrax Exposure

Anthrax Exposure Level by Challenge Group

The mean exposure to *B. anthracis* in the ITT population was 275.7±87.0 X LD_{50}. The means for each of the treatment groups are presented in Table 4. There was no statistically different exposure among the three treatment groups.

	Placebo N = 12	Ciprofloxacin N = 14	Ciprofloxacin/ Raxibacumab N = 14	All Groups N = 40	P-value[1]
Anthrax exposure (LD_{50})	-	-	-	-	0.0688
n	12	14	14	40	-
Mean ± SD	228.0 ± 58.0	290.8 ± 97.5	301.5 ± 85.5	275.7 ± 87.0	-
Median	212.5	254.0	321.8	248.7	-
Range	(166.1, 382.0)	(168.0, 450.0)	(146.0, 409.5)	(146.0, 450.0)	-

[1] P-value based on 1-way ANOVA for comparison among all treatment groups.

(Adapted from HGS' BLA submission)

Table 4. Anthrax Spore Exposure by Treatment Group

***MO comment**: The mean spore challenge dose was slightly lower in the placebo group than in the two treatment groups, but there were no statistically significant differences in the three groups. In addition, there were no survivors in the placebo group. In general, the spore exposures in this study were higher than those used in the previous cynomolgus monkey efficacy study.*

Therapeutic Trigger Symptoms and Timing of Treatment Initiation
Table 5 below summarizes the signs and symptoms in the ITT population including the time to treatment and the documentation of toxemia as defined by quantitative ECL and bacteremia as determined by culture.

	Placebo N = 12	Ciprofloxacin N = 14	Ciprofloxacin/ Raxibacumab N = 14	All Groups N = 40	P-value[1]
Time to treatment[2] (hours)	-	-	-	-	0.5684
n	12	14	14	40	-
Mean ± SD	41.5 ± 6.4	38.4 ± 8.2	38.2 ± 6.3	39.3 ± 7.0	-
Median	40.9	35.4	39.7	39.7	-
Range	33.9-58.1	28.7-56.3	28.8-47.8	28.7-58.1	-
Trigger event	-	-	-	-	0.7487
n	12	14	14	40	-
Detectable PA	11 (91.7%)	13 (92.9%)	14 (100.0%)	38 (95.0%)	-
PA not detected	1 (8.3%)	1 (7.1%)	-	2 (5.0%)	-
Toxemia[3] at or before treatment initiation	11 (91.7%)	13 (92.9%)	14 (100.0%)	38 (95.0%)	0.7487
Toxemia at the time of treatment initiation	11 (91.7%)	13 (92.9%)	14 (100.0%)	38 (95.0%)	0.7487
Toxemia at any time	12 (100.0%)	14 (100.0%)	14 (100.0%)	40 (100.0%)	-
Bacteremia[3] at or before treatment initiation	10 (83.3%)	13 (92.9%)	13 (92.9%)	36 (90.0%)	0.6654
Bacteremia at the time of treatment initiation	10 (83.3%)	13 (92.9%)	13 (92.9%)	36 (90.0%)	0.6654
No Bacteremia at the time of treatment initiation	2 (16.7%)	1 (7.1%)	1 (7.1%)	4 (10.0%)	0.6654
Bacteremia at any time	12 (100.0%)	13 (92.9%)	13 (92.9%)	38 (95.0%)	1.0000
Toxemia and bacteremia at or before treatment initiation	10 (83.3%)	12 (85.7%)	13 (92.9%)	35 (87.5%)	0.8490
Toxemia and bacteremia at the time of treatment initiation	10 (83.3%)	12 (85.7%)	13 (92.9%)	35 (87.5%)	0.8490
Toxemia and bacteremia at any time	12 (100.0%)	13 (92.9%)	13 (92.9%)	38 (95.0%)	1.0000
Toxemia and/or bacteremia at or before treatment initiation	11 (91.7%)	14 (100.0%)	14 (100.0%)	39 (97.5%)	0.3000
Toxemia and/or bacteremia at the time of treatment initiation	11 (91.7%)	14 (100.0%)	14 (100.0%)	39 (97.5%)	0.3000
Toxemia and/or bacteremia at any time	12 (100.0%)	14 (100.0%)	14 (100.0%)	40 (100.0%)	-
Bacteremia without toxemia at or before treatment initiation	0 (0.0%)	1 (7.1%)	0 (0.0%)	1 (2.5%)	1.0000

1 P-value for comparison of the 3 groups, based on log-rank test for time to treatment and 2-sided Fisher's exact test for other parameters.

2 Time to treatment was calculated relative to the start of challenge.

3 Throughout this table toxemia was determined by quantitative ECL and bacteremia was determined by culture. Only post challenge bacteremia and toxemia were counted.

(Adapted from HGS' BLA submission)

Table 5. Signs and Symptoms in the ITT Population

***MO comment**: For the FDA efficacy analysis, bacteremia was defined as blood culture positivity. Toxemia as detected by screening PA ECL, and toxemia as*

detected by quantitative PA ECL are definitions that utilize tests that require qualification. Data have been submitted for qualification of these tests and they are being reviewed. As noted above, there were 4 animals (2 in the placebo group, 1 in the ciprofloxacin group, and 1 in the ciprofloxacin/raxibacumab group) that were not bacteremic prior to treatment. The FDA analysis for efficacy was based on the outcome of the 36 animals that were bacteremic as defined by culture positivity at the time of treatment. For discussion of the individual animal triggers, see the comment below the efficacy analysis.

Primary Efficacy Analysis

The sponsor analyzed survival at day 28 in the ITT population (see Table 9). Statistically significant survival was demonstrated compared to placebo for both the ciprofloxacin and the ciprofloxacin/raxibacumab groups. There was no statistically significant difference in survival for the two treatment groups.

Treatment	N	Number (%) of Survivors	Difference in % Survivors (95% CI)[1]	P-value[2]
Placebo	12	0 (0.00%)	-	-
Ciprofloxacin	14	14 (100.0%)	100.00 (73.03, 100.00)	< 0.0001
Ciprofloxacin/ Raxibacumab	14	12 (85.71%)	85.71 (54.97, 98.22)	< 0.0001

[1] Difference in % survivors between each active treatment vs placebo with exact 95% CI.

[2] P-value based on 2-sided Fisher's exact test for the comparison vs placebo group.

Difference of % survivors (95% CI) is -14.29 (-42.81, 11.88) between the 2 active treatment groups (ciprofloxacin/raxibacumab versus ciprofloxacin groups). P-value = 0.4815 based on Fisher's exact test for the comparison of the 2 active treatment groups.

(Adapted from HGS' BLA submission)

Table 6. Sponsor's Analysis of the Survival in the ITT Population

All randomized monkeys were challenged with *B. anthracis* spores and received study agent according to their planned treatment group with the exception of placebo animal C25576 who had evidence of exposure to ciprofloxacin based on the PK data. This animal remained in the placebo group because it was thought that it received placebo throughout the majority of the ciprofloxacin dosing schedule.

All of the animals in the ciprofloxacin group survived the 28 day evaluation period. One animal (C30988) died on day 36 from left leg arterial thrombosis with inflammation and fibrosis. No anthrax bacteria were found in any of his tissues at necropsy. Two animals were non-survivors in the ciprofloxacin/raxibacumab group. One animal died of pneumonia related to a potential gavage error. The other non-survivor tested negative for bacteremia at the time of death but had evidence of meningitis and CNS hemorrhage on necropsy.

***MO comment**: For the FDA efficacy analysis, bacteremia was defined as blood culture positivity. The results of the FDA statistical analysis compared with the Applicant's ITT analysis was performed by Dr. Lan Zeng are presented in Table 7.*

Table 7. Comparison of Sponsor and FDA Efficacy Analysis

Population	Treatment	N	No. of Survivors (%)	P-value*	95% CI of treatment-placebo**	95% CI of Raxi/Cipro-Cipro**
ITT	Placebo	12	0 (0.0%)			
	Ciprofloxacin	14	14 (100.0)	<0.0001	(73.0, 100)	
	Ciprofloxacin/raxibacumab	14	12 (85.7)	<0.0001	(55.0, 98.2)	(-42.8, 11.9)
FDA Primary	Placebo	10	0 (0.0%)			
	Ciprofloxacin	13	13 (100%)	<0.0001	(68.9, 100)	
	Ciprofloxacin/raxibacumab	13	11 (84.6%)	<0.0001	(50.2, 98.1)	(-45.4, 11.4)

The following graphic (Figure 2) shows the disposition of the animals based on the FDA primary analysis:

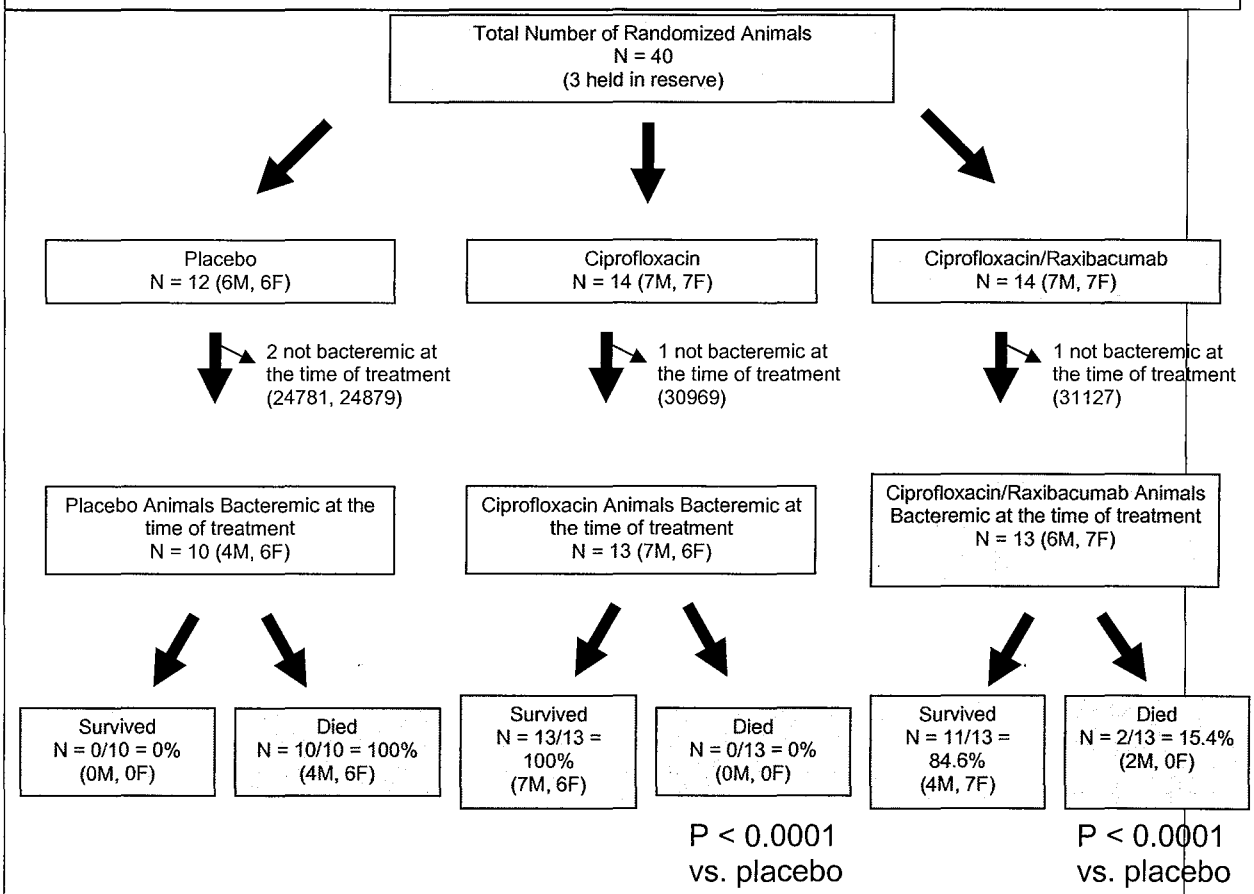


Figure 2. Animal disposition based on FDA analysis

Each of the animals was treated on the basis of a positive PA screening test or after 54 hours following challenge. The disposition of each individual animal with

respect to bacteremia by culture, bacteremia by PCR, and qualitative PA is shown in Figure 3.

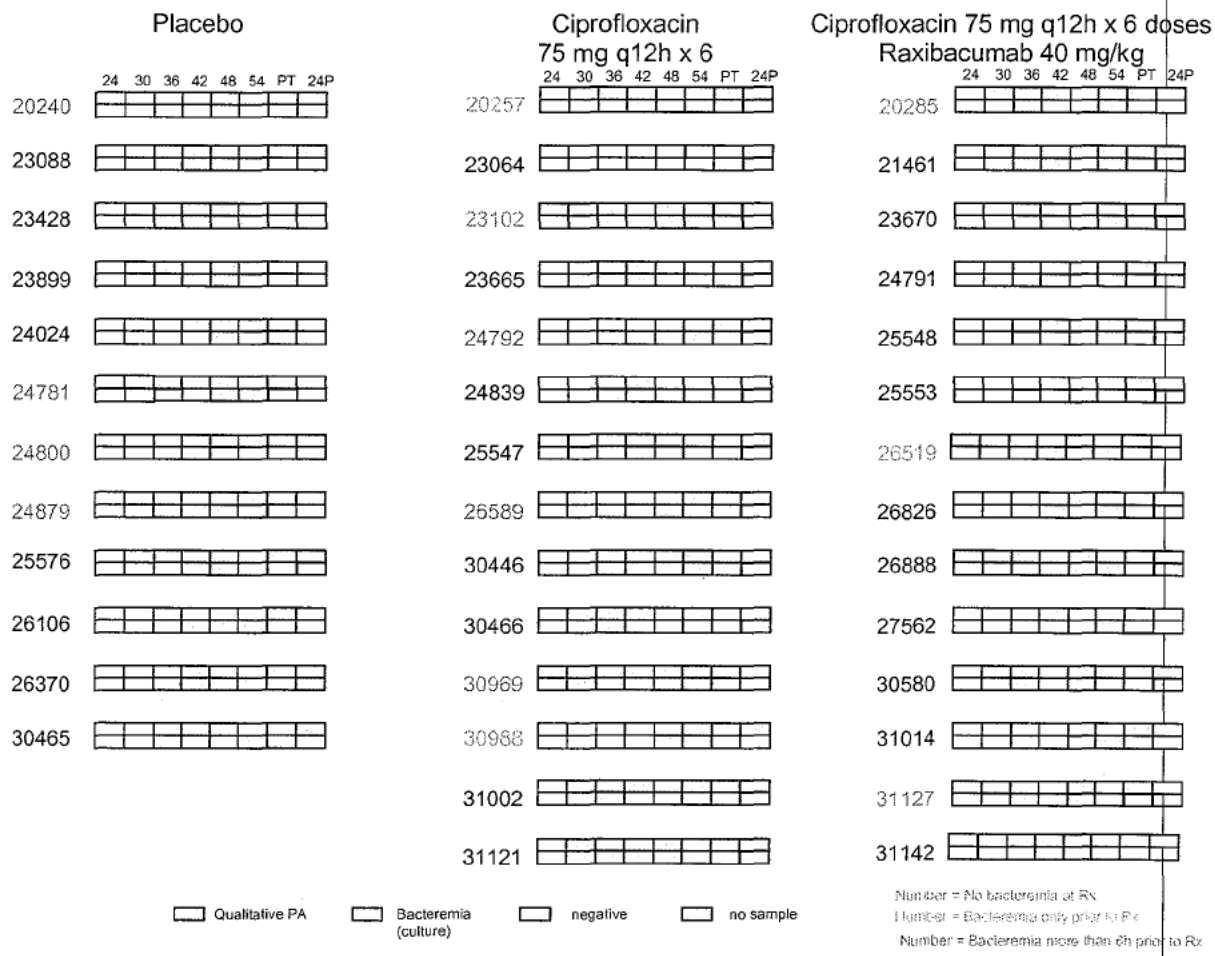


Figure 3. Individual animal disposition with respect to PA and culture

In figure 3, animals with red numbers were not bacteremic at the time of treatment (N=4). Animals with green numbers were bacteremic only at the blood draw just prior to treatment (N=5). Animals with blue numbers were bacteremic for more than 6 hours prior to treatment (N=4).

One animal was noted to have PK values for ciprofloxacin even though it was a placebo animal. Because the values were low at 72 hours, it was assumed that this animal only received a single dose of ciprofloxacin because the levels decreased by 72 hours. Of interest, that animal survived 128.5 hours which was the 4th longest survivor in the placebo group. In the placebo treated group, only

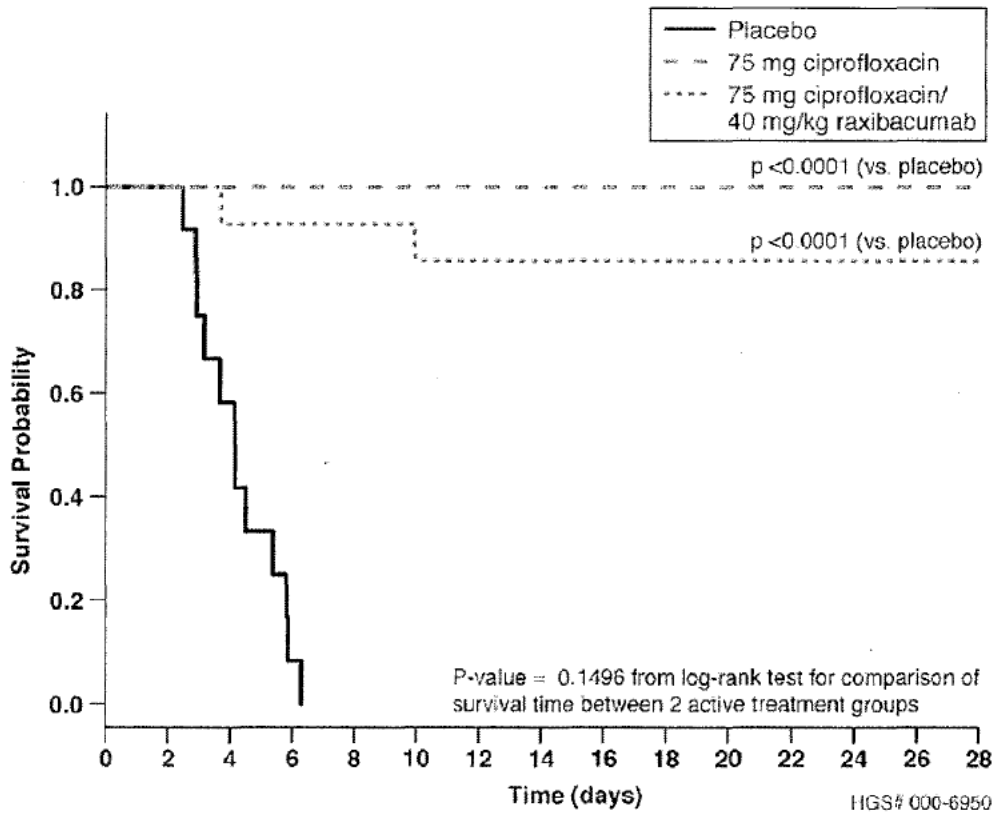
one animal had evidence of moderate meningitis and hemorrhage. That animal was the one that received the dose of ciprofloxacin.

The time to death statistics for the sponsor's ITT population and the FDA primary population are presented in Table 8, as prepared by Dr. Lan Zeng.

Population	*Treatment*	*N*	*No. of deaths (%)*	*Median (days)* *	*Range (days)*	*P-value**	*P-value***
Sponsor's ITT	Placebo	12	12 (100%)	4.2	(2.5, 6.3)		
	Ciprofloxacin	14	0 (0.0%)	-	(-, -)	<0.0001	
	Raxi/Cipro	14	2 (14.3%)	-	(3.7, -)	<0.0001	0.150
FDA primary	Placebo	10	10 (100%)	3.9	(2.5, 6.3)		
	Ciprofloxacin	13	0 (0.0%)	-	(-, -)	<0.0001	
	Raxi/Cipro	13	2 (15.4%)	-	(3.7, -)	<0.0001	0.149

*P-values are based on two-sided Fisher's exact test for comparisons between each active group and placebo.
**P-values are based on two-sided Log-rank test for comparisons between the Levo and Raxi/Levo groups.
Table 8. Time to Death

Secondary Efficacy Results
The secondary efficacy endpoint as defined by the sponsor was survival time defined as the time from spore challenge to death during the 28-day post challenge period in the ITT population. The Kaplan-Meier survival curves for the ITT population are shown in Figure 4.



(Adapted from HGS' BLA submission)

Figure 4. Survival Time in the ITT Population

MO Comment: *Since the FDA primary analysis only included those animals that were bacteremic at the time of treatment, Figure 5 shows the FDA primary Kaplan-Meier survival curves, as per Dr. Lan Zeng.*

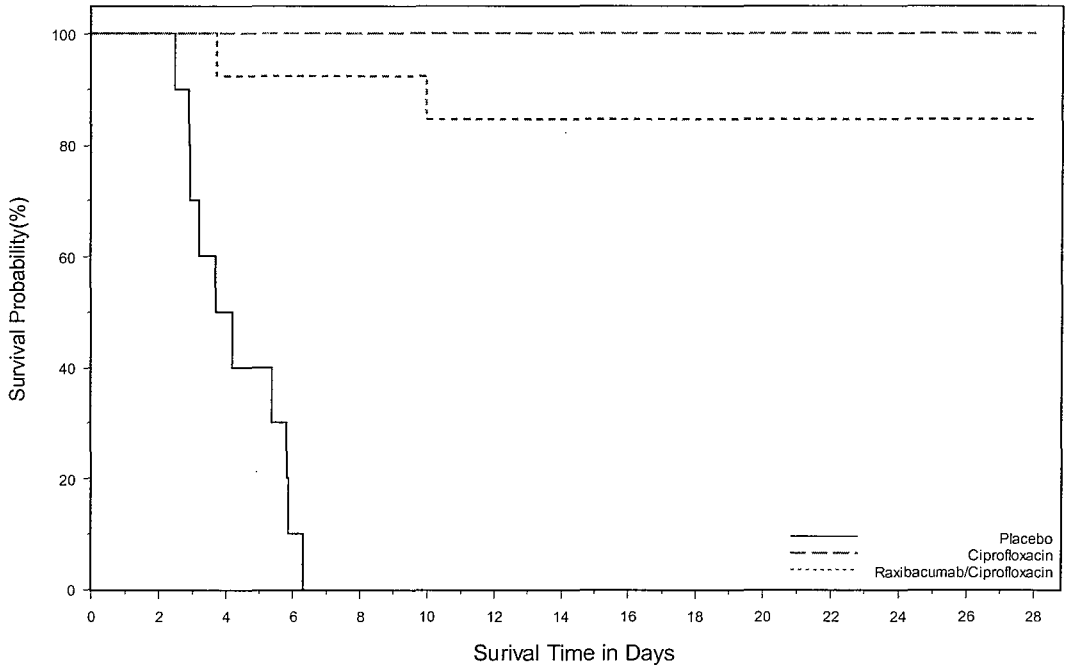


Figure 5. Survival Time in the FDA Primary Population

Other Parameters Measured:
The majority of animals exhibited clinical signs consistent with anthrax following the spore challenge. The most common clinical features were lethargy, not eating, and respiratory abnormalities.

The MIC results confirmed that bacteria cultured from the blood of challenged animals were
inhibited by similar concentrations of levofloxacin and ciprofloxacin as was the challenge
material.

Body temperature was monitored in all animals. An elevation in body temperature or loss of diurnal rhythm was apparent at 1 to 4 days post-challenge. The trends observed suggest that the animals were presenting non-specific signs of illness similar to results documented during previous animal therapeutic studies. Body weights were not collected after day 0.

CRP levels were relatively consistent prior to challenge and prior to treatment. For most of the animals, CRP levels increased and peaked between 24-72 hours post-treatment. A majority of the monkeys that survived showed low CRP levels

at 14 days post challenge. Placebo-treated monkeys (all non-survivors) had elevations in CRP prior to death.

Positive blood cultures were documented in all the placebo animals at some point post-challenge (2 were not bacteremic at the time of treatment). One animal in each of the two treatment groups was not bacteremic at the time of treatment. Those animals were never bacteremic and they both survived.

Anti-PA antibodies were collected pre-dose and at 21 and 28 days following treatment for surviving animals. The data are presented in Table 8. There were no statistically significant differences in mean serum anti-PA antibody concentrations between the 2 treatment groups, indicating that co-administration of raxibacumab with ciprofloxacin did not affect the formation of anti-PA antibodies by the surviving monkeys, relative to ciprofloxacin alone.

		Serum Anti-PA Antibody Concentration (µg/mL)[1]		
		Predose	21 Days Postchallenge	28 Days Postchallenge
Ciprofloxacin Alone	N	14	14	14
	Mean ± SD	0.068 ± 0.253	65.447 ± 37.687	89.126 ± 48.833
Ciprofloxacin Plus Raxibacumab	N	12	12	12
	Mean ± SD	0.295 ± 0.837	72.506 ± 33.779	100.899 ± 83.862

(Adapted from HGS' BLA submission)

Table 8. Serum Anti-PA Antibody Concentrations in Surviving Animals

Serum TNA titers were drawn for all surviving animals predose and at days 21 and 28 for all surviving animals, and the data are presented in Table 9. The mean serum TNA titers for the ciprofloxacin alone dose group were significantly higher (about 2-fold) than those for the ciprofloxacin plus raxibacumab dose group. It should be noted that these differences were not reflected in altered survival between the 2 groups, suggesting that the TNA response in monkeys administered both ciprofloxacin and raxibacumab was adequate for survival.

		Serum TNA Titer[1]		
		Predose	21 Days Postchallenge	28 Days Postchallenge
Ciprofloxacin Alone	N	14	14	14
	Mean ± SD	0 ± 0	6369 ± 5288	7266 ± 4864
Ciprofloxacin Plus Raxibacumab	N	12	12	12
	Mean ± SD	0 ± 0	3020 ± 1802	3400 ± 1869

(Adapted from HGS' BLA submission)

Table 9. Serum TNA Titers in Surviving Animals

Serum PA concentrations were not measurable in the specimen collected about 7 days prior to the spore challenge for three monkeys. The exceptions were C31014 (ciprofloxacin/raxibacumab treatment group, at 3.76 ng/mL) and C23088 and C25576 in the control group, with prechallenge concentrations of 0.94 and 3.39 ng/mL, respectively. According to the sponsor, PA values rose sharply just prior to 40 hours post challenge. Among some placebo-treated non-survivors, PA values exhibited a brief plateau during the period from 40 to 80 hours post challenge, which was generally followed by a 2nd rise in PA levels that lasted until death. There was no plateau among ciprofloxacin/raxibacumab-treated non-survivors, and PA levels declined through time of death; for 1 of these animals (C31142) the decline was followed by a rise in PA just prior to death. Among ciprofloxacin and ciprofloxacin/raxibacumab-treated survivors, the 2nd rise in PA did not occur; rather, the PA declined to undetectable levels for the majority of monkeys by the end of the study.

***MO comment**: While there were some increases in temperature noted in the monkeys, this was not used as a trigger for treatment in the monkeys in this study. While some trends in CRP data were noted, this was not used as a trigger for treatment or a predictive factor in this study.*

There was no statistically significant difference between anti-PA levels in animals treated with ciprofloxacin alone or with the ciprofloxacin/raxibacumab combination. It is interesting to note that the anti-PA levels were higher in the primary efficacy trial compared with the values seen in this combination trial (775µg/mL in the primary efficacy trial raxibacumab group vs. 100µg/mL in animals that received raxibacumab in the combination trial).

Serum TNA titers were higher in the ciprofloxacin only treated animals. Of note, TNA titers in the animals that received raxibacumab in this combination study were markedly lower (3400) than levels seen with the same dose of raxibacumab in the pivotal trial (14,827).

Please see the Microbiology review for a discussion of the PA assays. PA values appear to demonstrate inter-animal variability. It is not clear why 3 animals had detectable levels of PA prior to challenge.

Safety Results

Limited safety information was provided during this study. Gross pathology and histopathology were available on those animals that died.

The sponsor notes that although there was a general trend of increasing WBC counts following challenge in all animals, a more rapid rise in WBC was generally noted in the placebo group and a comparatively stable pattern among active

treatment group survivors. Following treatment, WBC counts continued to rise through Day 8 post challenge. Elevated counts were observed in all groups post-treatment. Surviving animals demonstrated normalization of their WBC by 2-3 weeks post challenge.

The sponsor noted that following treatment, platelet levels dropped consistently in placebo-treated monkeys until death. Monkeys in both active treatment groups had a decrease in platelet counts through 72 hours post-treatment followed by increases by 8 days post challenge.

The sponsor noted that RBC levels for the vast majority of monkeys decreased following spore challenge. RBCs in placebo-treated monkeys trended negative until their death, while in the majority of monkeys in both active treatment groups, the RBC counts stabilized and trended positive through the end of the study.

Profiles for neutrophil and lymphocyte counts were similar to those seen for the WBCs.

MO comment: *The hematology data are most suggestive of a non-specific process associated with the acute anthrax infection in these animals, as was seen in the pivotal efficacy trial in the cynomolgus monkeys. Of note, in this study, there was no increase in the number of basophils seen after challenge, as had been seen in the pivotal efficacy trial in the cynomolgus monkeys.*

Necropsy and Histopathology:

In brief, gross lesions at necropsy consistent with anthrax included adrenal gland discoloration (indicating the presence of bacteria); brain/meningeal red-stained accumulation, discoloration or foci (hemorrhage and inflammation); abdominal and/or thoracic cavity fluid (effusion); enlargement of axillary, bronchial, mandibular and/or mediastinal lymph nodes (edema, fibrin exudation, and hemorrhage); and skin or thymic fluid (edema). Gross lesions unrelated to anthrax were liver foci in C24781 in the placebo group (metazoan parasites), lung discoloration in C24791 in the ciprofloxacin/raxibacumab group (pneumonia related to gavage error), and a tan lesion in the inguinal skin of C30988 in the ciprofloxacin group (chronic cellulitis and myositis). Pathology results for C24791 (ciprofloxacin/raxibacumab group) was consistent with fibrinosuppurative bronchopneumonia related to gavage error, as no *B. anthracis* organisms were evident in any tissue according to the sponsor. The large tan lesion in the left inguinal region of animal C30988 (ciprofloxacin group), which led to euthanasia, was consistent with chronic inflammation in the subcutis and muscle with thrombosis of regional arteries. It is possible that arterial thrombosis was the primary lesion, with inflammation, tissue degeneration and fibrosis occurring secondary to hypoxia and trauma to the area by the animal. While the initial insult cannot be determined, this lesion is not likely related to anthrax, as no anthrax bacteria were present in any tissue.

Microscopic findings consistent with anthrax were present in 13/15 animals examined histologically. Lesions typical of anthrax in this study included brain hemorrhage and meningitis; tubular degeneration in the kidney; liver necrosis and sinusoidal leukocytosis; edema, fibrin exudation, hemorrhage, neutrophilic inflammation, and lymphocyte necrosis affecting the spleen, thymus, lung and multiple lymph nodes; and the presence of large rodshaped bacteria consistent with *B. anthracis* in many of the organs evaluated histologically.

The only antibiotic-treated monkey to die with lesions of anthrax was animal C31142 (ciprofloxacin/raxibacumab group). This animal had the most severe brain lesions of all animals evaluated histologically but had fewer lesions and bacteria in other organs. Notably, time to death for this animal was 10 days, which was more than the time to death for any of the placebo-treated animals with brain lesions.

***MO comment**: The histopathology observed in the animals that died in this study is consistent with anthrax. Animal C24791 had evidence of death due to bronchopneumonia from a gavage error. The animal was not bacteremic at the time of death but the mediastinal lymph node showed 1-5 colonies on primary streak but contamination was noted. The bronchial lymph node was enlarged and mottled. No bacteria were noted in any tissues on histopathology.*

In the 12 placebo treated animals, 8 had no gross and no to level 1 bacteria with an average time to death of 99.1 hours. Two of the placebo animals had meningeal congestion and level 1 bacteria with an average survival time of 103.6 hours. One of the placebo animals had discolored meninges, hemorrhage, and level 2 bacteria with a survival time of 100 hours, and one placebo animal had hemorrhagic meningitis with level 3 bacteria, hemorrhage and meningitis and a survival time of 128.5 hours. In summary, 11 of the 12 placebo animals had a low level of pathology (as defined in the pivotal efficacy trial in cynomolgus monkeys) and one animal had a high level of pathology. It is interesting to note that the animal with the high level of pathology inadvertently received a dose of ciprofloxacin.

Only one treated animal in the ciprofloxacin/raxibacumab group died of anthrax disease. That animal had evidence of hemorrhagic meningitis that affected the entire brain with level 3 bacteria, level 1 hemorrhage, and level 3 meningitis. That animal survived 238.7 hours.

There was no report of any neurologic sequelae in any of the surviving animals, but it is not known how they were evaluated.

STUDY 789-G923702 CONCLUSIONS

This was a parallel-group, double-blind, randomized, placebo-controlled GLP study in 40 healthy male and female cynomolgus monkeys to evaluate the efficacy of

raxibacumab in combination with ciprofloxacin as a therapeutic treatment in cynomolgus monkeys with inhalation anthrax

Efficacy conclusions:

- Both the ciprofloxacin treated group and the ciprofloxacin/raxibacumab treated group showed statistically significant superiority over placebo in both the sponsor's ITT analysis and the FDA primary analysis.
- For the FDA primary analysis the survival rate in the placebo group was 0%, the survival rate in the ciprofloxacin treated group was 100% and the survival rate in the ciprofloxacin/raxibacumab treated group was 84.6 percent. The treated groups were superior to placebo ($p<0.0001$).
- There was no differences in survival rates between the two treated groups.

Safety conclusions:

- Safety data were limited in the study to findings on gross necropsy, histopathology, and serial CBCs.
- In the placebo animals, 11 animals had evidence of low CNS pathology while 1 animal had high CNS pathology. That animal inadvertently was exposed to ciprofloxacin. Only one treated animal died of anthrax disease and that animal had evidence of hemorrhagic meningitis that affected the entire brain. No surviving animals were sacrificed.
- Based on the available analyses, the pathophysiologic basis for the increased CNS inflammation seen in the pivotal efficacy trial in cynomolgus monkeys is not clear. The number of animals analyzed is small. While it is reassuring that no gross neurologic findings were seen in the surviving animals, and no brain abnormalities were seen in toxicologic studies, a potential interaction between anthrax and raxibacumab contributing to CNS inflammation and hemorrhage cannot be ruled out. It will be important to examine the histopathology in animals that die in any subsequent studies and it would be reassuring to examine the brains of those animals that survive in order to establish normal brain histology in survivors. . If it is not possible to sacrifice survivors, then more formal neurologic assessments including imaging studies should be performed in subsequent studies.

9.7 Individual Study Report

CLINICAL REVIEW OF THE ANIMAL STUDY REPORT 724-G005829 and the HISTOPATHOLOGY STUDY PROTOCOLS 901-G005829 (Battelle) and 866-001 (EPL)

BB-IND:	11069
Report Date:	04/26/2008
Product:	Raxibacumab (Human monoclonal antibody to *B. anthracis* protective antigen)
Sponsor:	Human Genome Sciences, Inc.
Study Location:	Battelle Biomedical Research Center (BBRC) State Route 142 West Jefferson, OH 43162
Independent Pathology Analysis:	Experimental Pathology Laboratories, Inc. Herndon, VA
Reference Lab: (PCR for *B. anthracis,* PA ECL)	Human Genome Sciences, Inc. 14200 Shady Grove Rd Rockville, MD 20850
Drug Manufacture:	(b) (4)
Telemetry Surgery:	Battelle Memorial Institute Columbus OH 43201
Animal Supply:	(b) (4)
Submission Title:	Evaluation of Raxibacumab Efficacy as Therapeutic Treatment Against Inhalation Anthrax in the Cynomolgus Macaque (Battelle Study 724-G005829)

Nonclinical Study Report 724-G005829: Evaluation of Raxibacumab Efficacy as Therapeutic Treatment Against Inhalation Anthrax in the Cynomolgus Macaque

PRINICIPAL INVESTIGATOR: Jason M. Mott, DVM, Ph.D.

STUDY CENTER: Battelle Biomedical Research Center (BBRC)

STUDY PERIOD: September 15, 2007 to January 11, 2008

REPORT DATE: April 26, 2008

STUDY PROCEDURE: Study was conducted under GLP

OBJECTIVES:
Primary Objective:
To evaluate the efficacy of raxibacumab when administered as a therapeutic treatment against lethality due to inhalation exposure to *B. anthracis* in cynomolgus monkeys

Additional Objectives:
To determine the PK of raxibacumab following a single IV raxibacumab dose and the kinetics of *B. anthracis* PA in monkeys with inhalation anthrax

METHODOLOGY: Parallel-group, double-blind, randomized, placebo controlled GLP study in healthy male and female cynomolgus monkeys to evaluate the therapeutic efficacy of a single IV dose of raxibacumab in anthrax spore inhalation-challenged monkeys experiencing symptoms of inhalation anthrax

NUMBER OF ANIMALS: 40 naïve cynomolgus monkeys were randomized into 2 separate treatment groups of 14 animals and a placebo group of 12 monkeys (each 50% male and 50% female). Two animals were held as replacements but were not used.

STUDY DRUG: Upon detection of serum PA via the screening assay, IV raxibacumab was given as a single bolus infusion at either a 20 or 40 mg/kg dose.

Raxibacumab, is a recombinant, fully human $IgG_1\lambda$ mAb that binds *B. anthracis* PA with high affinity and inhibits its biological activity. Raxibacumab is expressed in the NS0 mouse myeloma cell line and secreted into culture media, from which it is purified by a series of chromatography and filtration steps.

Raxibacumab was supplied by the sponsor as a sterile liquid formulation (50 mg/mL) in a formulation buffer consisting of 0.13 mg/mL citric acid, 2.8 mg/mL sodium citrate, 10 mg/mL sucrose, 18 mg/mL glycine, 0.2mg/mL polysorbate 80, pH 6.5. Storage was at 2-8^{0}C.

Raxibacumab used for the two treatment groups came from lot #71044, manufactured on October 13, 2006. The placebo buffer came from lot #71043, manufactured on October 4, 2006.

MO comment: *There was no documentation of the actual product used for this study (M10 vs. M11). Subsequent to the study report submission, HGS confirmed that M11 was the product used for this study.*

All monkeys received a single IM dose of diphenhydramine (1 mg/mL) within 5 minutes prior to study agent administration.

DURATION OF TREATMENT: Raxibacumab or placebo was to be administered IV immediately to each individual animal following detection of serum PA via the PA screening assay. Within 5 minutes prior to treatment each monkey was to receive a single 1 mg/kg IM dose of diphenhydramine.

TREATMENT COMPLIANCE: Study drug infusion information, including dosage and volume prescribed, and volume administered, was recorded.

STUDY DESIGN: This was a parallel-group, double-blind, randomized, placebo-controlled GLP study in healthy male and female cynomolgus monkeys to evaluate the therapeutic efficacy of a single IV dose of raxibacumab in anthrax spore inhalation-challenged monkeys experiencing symptoms of inhalation anthrax. Forty naïve cynomolgus monkeys were to be randomized into 3 separate groups, two groups of 14 monkeys and 1 group of 12 monkeys (each group 50% male, 50% female).

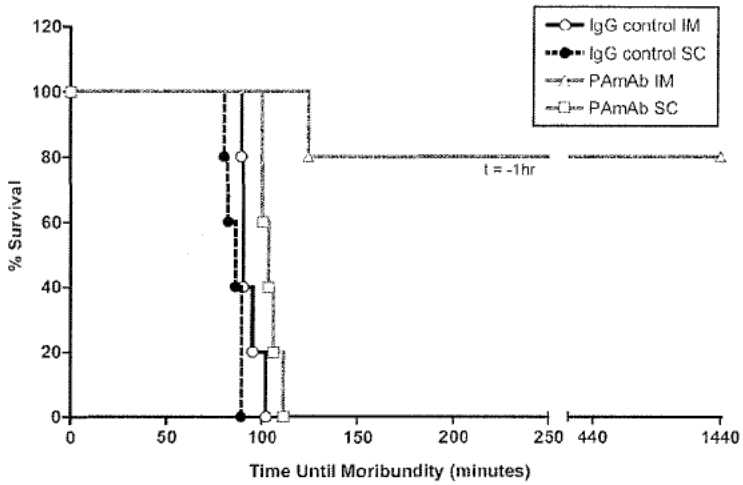
Monkeys were to be randomized to 1 of 3 aerosol challenge days and randomized to a challenge order each day. On Study Day 0, monkeys were to be challenged with a targeted 200 x lethal dose (LD_{50}) (1.24×10^7 spores) of *B. anthracis* spores (Ames strain) by a Collison nebulizer and delivered using a head-only inhalation exposure chamber. When an animal exhibited a positive serum PA level via the screening assay or at 54 hours post challenge, a single bolus IV injection of either 40 or 20 mg/kg of raxibacumab, or 1 mL/kg of raxibacumab buffer (dependent upon that animal's treatment assignment) was to be administered. Just prior to treatment intervention (within 5 minutes), monkeys were administered a single 1 mg/kg dose of diphenhydramine (or equivalent) intramuscularly (IM).

BACKGROUND:
Raxibacumab is a fully human monoclonal antibody (mAb) antitoxin that specifically binds protective antigen (PA), preventing the association of PA with the anthrax toxin receptor. The indication currently being sought is the therapeutic treatment of inhalation anthrax. It is hypothesized that raxibacumab could provide benefit following exposure to bioengineered, antibiotic-resistant strains *B. anthracis*.

Based on the *in vitro* inhibition of PA-mediated cytotoxicity by raxibacumab, HGS performed a number of *in vivo* studies in animal models of anthrax-induced lethality. These studies included pre- and post-exposure prophylaxis studies in Fisher 344 rats injected with lethal toxin, and anthrax spore inhalation studies in New Zealand white rabbits and cynomolgus monkeys.

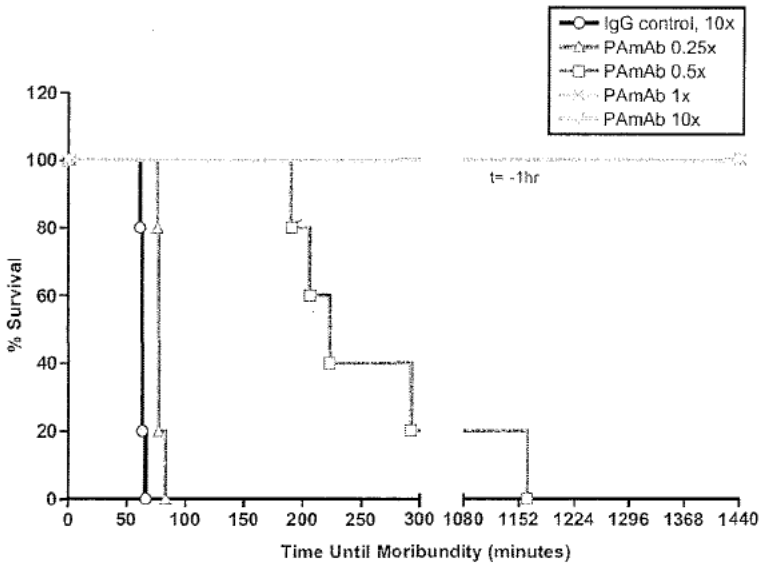
Rat Studies:
In the rat model, raxibacumab provided protection against lethality when administered by subcutaneous, IM, or IV routes. A single administration protected the rats for a duration of three weeks. The continuous infusion lethal toxin rat model showed that treatment of symptomatic rats with raxibacumab as

late as 12 hours after initiation of lethal toxin resulted in improved hemodynamic effects and survival.



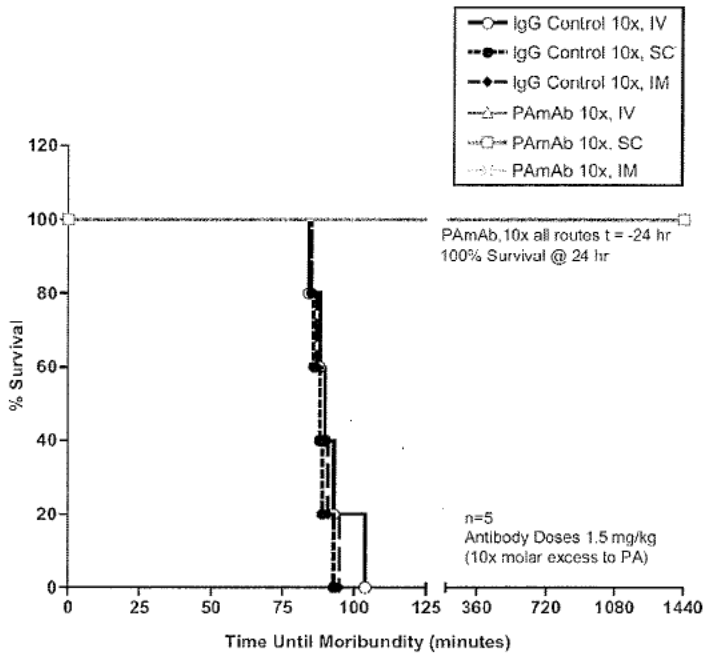
- Monoclonal administered 60 minutes before toxin
- Monoclonal dose of 1.44 mg/kg
- None of the SC animals

Effect of raxibacumab on survival of Fisher 344 rats challenged with lethal toxin, SC or IM administration of antibody at t = -60 minutes
(Adapted from HGS' BLA submission)



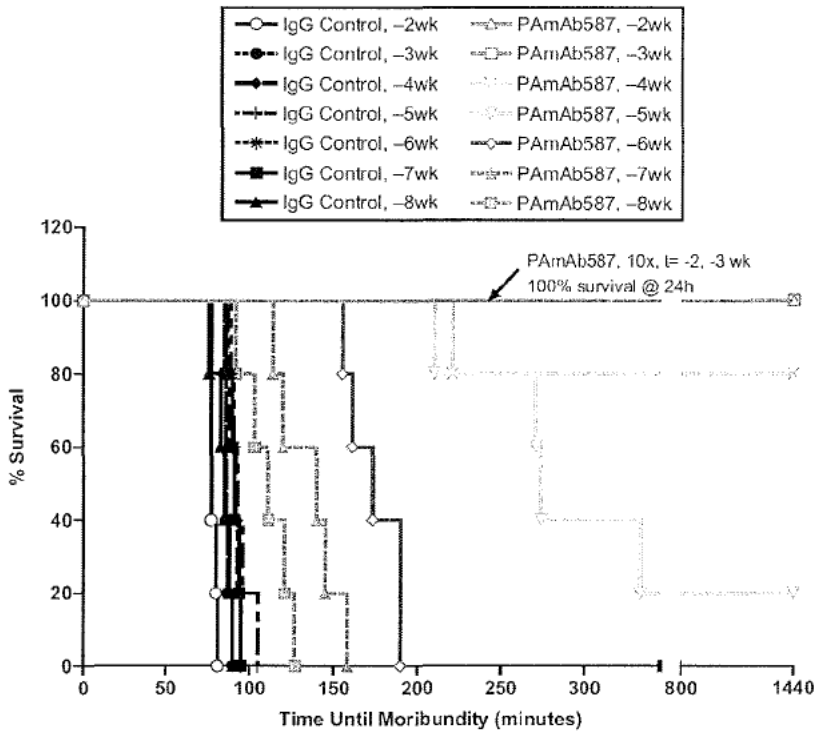
- Monoclonal administered 60 minutes before toxin
- Monoclonal dose 0.039-1.5 mg/kg
- 100% survival with IV doses of 0.15 and 1.5 mg/kg

Effect of raxibacumab on survival of Fisher 344 rats challenged with lethal toxin, IV administration of antibody at t = -60 minutes
(Adapted from HGS' BLA submission)



- Monoclonal given SC, IM, or IV 24 hours prior to toxin
- Monoclonal dose of 1.5

Effect of raxibacumab on survival of Fisher 344 rats challenged with lethal toxin; IV, SC or IM administration of antibody at t = -24 hours
(Adapted from HGS' BLA submission)



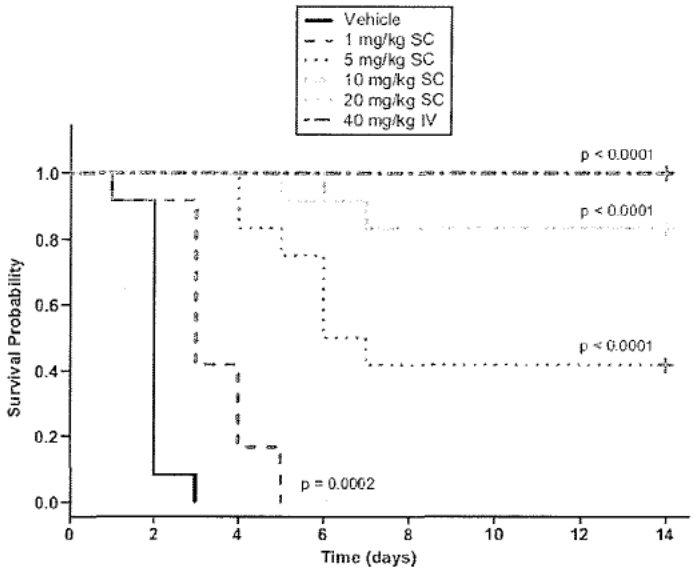
- Monoclonal given IV up to 8 weeks prior to toxin
- Monoclonal dose 0.9 mg/kg
- 100% survival if monoclonal given 2 or 3

Effect of raxibacumab on survival of Fisher 344 rats challenged with lethal toxin, IV administration of antibody at t = -2 to -8 weeks

(Adapted from HGS' BLA submission)

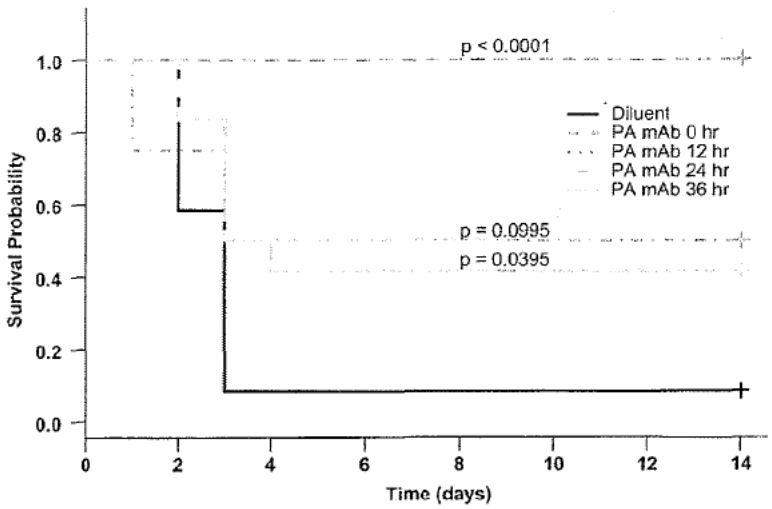
Rabbit Studies:

In the NZW rabbits, 100% survival was seen when 40 mg/kg of raxibacumab was given within 12 hours of challenge. When raxibacumab was given prior to challenge, there was a dose response with 40 mg/kg producing 100% survival. Survival decreased when raxibacumab was given 24 and 36 hours following aerosol challenge with only 33% survival in the 40 mg/kg group when given at 24 hours post challenge and 0% survival when the monoclonal (20 mg/kg) was given at 36 hours after challenge. In a treatment study, when the treatment was initiated when the animals had persistent fever or PA positivity, 5/18 in the 20 mg/kg group and 8/18 in the 40 mg/kg group survived.



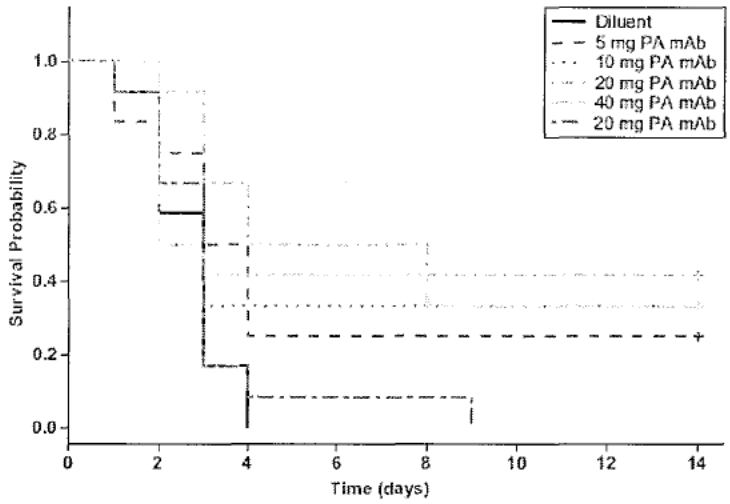
- Target challenge dose of 100 LD_{50} but mean exposure was 202 LD_{50}
- Monoclonal administered SC at 1, 5, 10, 20 mg/kg two days prior to challenge
- Monoclonal administered IV at 40 mg/kg immediately after spore challenge

Rabbit Survival Curves
(Adapted from HGS' BLA submission)



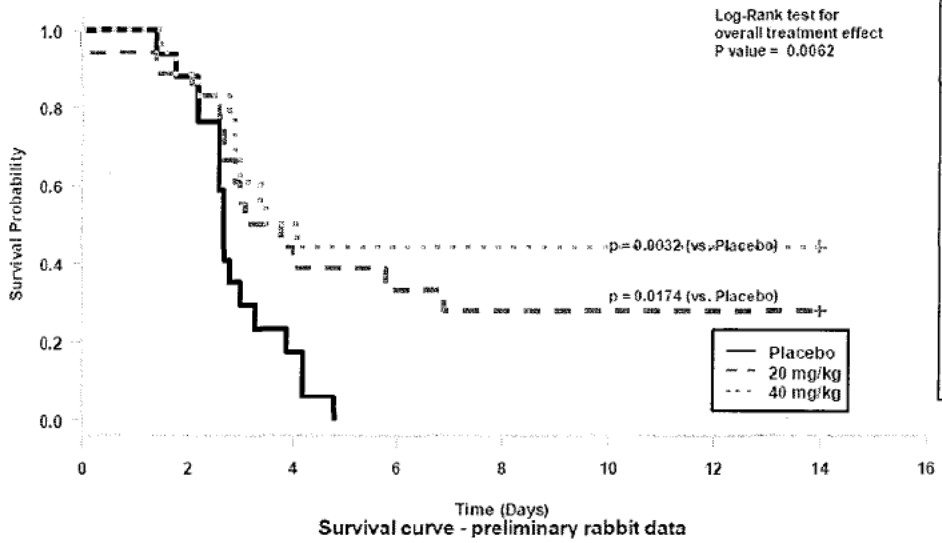
- NZW rabbits treated with 40 mg/kg monoclonal IV (60 animals)
- Mean challenge 103 LD_{50}
- 100% survival when monoclonal given at the time of challenge or at 12 hours post challenge
- 50% survival if monoclonal given 24 hours after challenge
- 42% survival if monoclonal given 36 hours after

Survival curves of the rabbits in 14-day inhalation study
(Adapted from HGS' BLA submission)



- 72 NZW rabbits
- Mean aerosol exposure 128 LD_{50}
- Monoclonal 5, 10, 20, and 40 mg/kg IV at 24 hours post challenge
- Monoclonal 20 mg/kg IV at 36 hours post challenge
- 0% survival in vehicle
- For animals treated 24 h post challenge, 25% survival for 5mg/kg, 33% survival for 10mg/kg, 42% survival for

Rabbit Survival Curves
(Adapted from HGS' BLA submission)

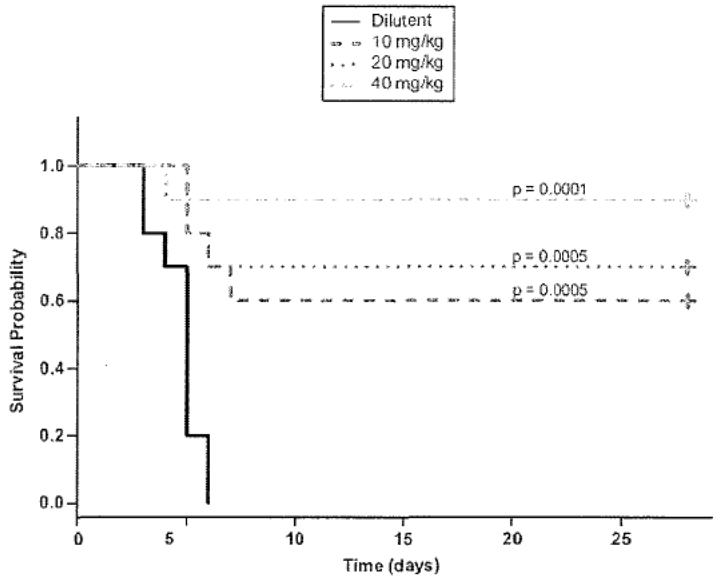


- 54 NZW rabbits with a targeted exposure of 200 LD_{50}
- Treatment based on detectable serum PA or persistently elevated temperature
- Monoclonal given IV at 20 or 40 mg/kg
- Preliminary data showed 0% survival in

Rabbit survival curves for therapeutic treatment in symptomatic rabbits
(Adapted from HGS' BLA submission)

NHP Studies:
A pre-exposure prophylaxis spore challenge study was done in cynomolgus monkeys. Raxibacumab at doses of 10, 20 and 40 mg/kg were given 2 days prior to aerosol challenge. Survival was 0%, 60%, 70% and 90% in the placebo, 10 mg/kg, 20 mg/kg, and 40 mg/kg doses respectively. Median survival time was longer in all raxibacumab treated groups. Those animals that survived were re-

challenged (Study 374-N006090) approximately 11 months after the first study. All control animals (6) died while the 21 animals that were re-challenged all survived.



- 40 cynomolgus NHP
- Mean spore challenge was 186 LD_{50}
- Monoclonal 10, 20, and 40 mg/kg SC given 2 days prior to challenge
- Survival evaluated at day 28
- 0% survival in placebo group, 60% survival in the 10 mg/kg group, 70% survival in the 20 mg/kg group, and 90% survival in the 40 mg/kg group

Survival Curves for Cynomolgus Monkeys
(Adapted from HGS' BLA submission)

A natural history study was done in the cynomolgus monkey (study # 685-G005762). Eight animals were studied. They received a mean aerosol challenge of 260 x LD_{50}. Six of eight monkeys died and neither survival or survival time were correlated with challenge dose. The median time to develop bacteremia by culture was 36 hours. The earliest time to death was 85 hours and the latest time to death of those who died was 156 hours. Survival time was correlated with time of PA detection indicating that PA was a predictor of ensuing death. In monkeys and rabbits, the serum PA concentrations rise, plateau, and rise again in those who die but the timing and the magnitude of these changes differ between the species.

MO comment: *The information in this background section has been culled from submitted study reports and annual reports. There has been no official review of these studies and these summary data may need to be revised after such review has occurred.*

STUDY 724-G005829 PROTOCOL

Design:
This was a parallel-group, double-blind, randomized, placebo-controlled GLP study in healthy male and female cynomolgus monkeys to evaluate the therapeutic efficacy of a single IV dose of raxibacumab in anthrax spore inhalation challenged monkeys experiencing the symptoms of inhalation anthrax.

Inclusion criteria:
- Male and female juvenile (< 5 years of age) naïve Chinese colony bred cynomolgus monkeys by [redacted] (b) (4).
- Weight 2.5 to 4.5 kg at randomization.
- Free of malformations and clinical signs of disease.
- Monkeys were to be tested and verified negative for tuberculosis and screened within 30 days prior to receipt at Battelle and found to be negative for Simian Immunodeficiency Virus, Simian T-Lymphotrophic Virus-1 (STLV-1), and *Cercopithecine herpesvirus* 1 (Herpes B virus). All monkeys were required to be tested negative for Simian Retrovirus (SRV1 and SRV2), anti-PA reactive antibodies, and circulating PA prior to randomization.

Exclusion criteria:
- Malformations.
- Clinical signs and symptoms of disease.
- Any monkeys outside the specified weight range.
- Any monkey with any one of the infections listed above.

Euthanasia Criteria:
Any of the following criteria prompted immediate euthanasia:
- Presence of any seizure denoting primary central nervous system disease.
- Respiratory distress, dyspnea, or forced abdominal respirations.
- Unresponsive to touch or external stimuli.
- Moribundity.

Euthanasia was warranted when all the clinical signs below were observed:
- Recumbence and weakness.
- Progressive state of depression.
- ≥20% body weight loss.
- Body temperature below 97°F; indicative of shock.
- Total anorexia with duration longer than 48 hours.

MO comment: *In amendment 1 of the protocol, the FDA requested more frequent observations of the monkeys. Without this recommended increase in observation frequency, it is likely that more animals would have been found*

dead. Observations were required every 6 hours starting 24 hours after challenge to minimize the chance that animals would be found dead. On inspection of the records, there are no clear violations of the euthanasia criteria. Despite these criteria, 11 of the 24 animals who died were found dead.

Aerosol Challenge:
Monkeys were randomized by sex to study group and randomized to one of three challenge days. The monkeys were then assigned a random challenge order within the challenge day using random numbers generated by the SAS® RANUNI function. Animals were challenged via the inhalation route with *B. anthracis* (Ames strain). On the day of challenge, monkeys were anesthetized with Telazol and placed into a plethysmography chamber within a Class III cabinet system where they were challenged with a targeted dose of 200 x LD_{50} using a Collison nebulizer and delivered via a head-only inhalation exposure chamber.

Randomization of Monkeys:
A tattoo was used to identify each animal. Following telemetry transmitter implantation, the monkeys were allowed to recover until approximately 10-14 days prior to challenge when they were transferred into the BL-3 facility. Monkeys were identified by individual cage cards that stated tattoo number, study number, study director, and sex of the monkey. Forty-two cynomolgus monkeys were available for the study (20 females and 22 males) and 40 (20 females and 20 males) were randomly selected to be placed on study utilizing random numbers generated by the SAS® RANUNI function. The two replacement animals were not used. The 40 monkeys were to be randomized by sex to one group of 12 animals, and 2 groups of 14 animals (all three groups with equal numbers of males and females) and aerosol challenge day utilizing the SAS® PLAN procedure. The monkeys were assigned a randomized challenge order within aerosol challenge day utilizing random numbers generated by the SAS® RANUNI function.

Treatments and Study Groups:
Cynomolgus monkeys were randomized to one of three aerosol challenge days and a challenge order per day. On day 0, monkeys were challenged with a targeted aerosol dose of 200 x LD_{50} spores of *B. anthracis* (Ames strain). When an animal had a positive serum PA level via screening assay or at 54 hours post-challenge, a single bolus IV injection of 40 mg/kg raxibacumab, 20 mg/kg raxibacumab, or 1 mL/kg raxibacumab buffer was administered. Just prior to treatment intervention, all monkeys were administered a single 1 mg/kg IM dose of diphenhydramine. Treatment groups are defined in Table 1.

	Number of Monkeys Planned	Dose	Route/Frequency	Treatment Point
Group 1	14	40 mg/kg raxibacumab (1 mL/kg)	IV/single dose	Individual treatment times based on serum PA levels
Group 2	14	20 mg/kg raxibacumab (1 mL/kg)	IV/single dose	
Group 3 (placebo)	12	Raxibacumab buffer (1mL/kg)	IV/single dose	

(Adapted from HGS' BLA submission)
Table 1 Treatment Group Designation

Blinding:
Staff administering study agent to and/or conducting assessments of study monkeys post challenge were to be blinded to their treatment assignments in order to preclude the introduction of bias into the study. Dilutions of the study agents were to be sealed in sterile dosing vials labeled X, Y, or Z. Dilutions were to be performed no earlier than 3 days prior to challenge and were stored at 2-8^{0}C prior to dosing. Documentation of vial content was to be controlled by Battelle's Quality Assurance Unit until 32 days post-challenge of the final challenge cohort. All animals were to receive 1mL/kg of study agent to maintain blinding of treatment assignment.

Study Agent Administration:
Raxibacumab or placebo were to be administered IV via a single bolus injection upon detection of positive serum PA via screening assay.

MO comment: *The treatment in the monkeys was administered as a bolus injection. In human studies, raxibacumab is to be administered by infusion over 2.5 hours. This difference in administration times needs to be taken into account in matching the animal and human PK parameters, especially related to Cmax.*

Clinical Observations:
All monkeys were to be observed every 6 hours beginning 24 hours post median challenge time and ending 192 hours (8 days) post median challenge time. Animals were observed for clinical signs including, but not limited to, anorexia, lethargy, respiratory distress, moribundity, activity (recumbent, weak, or unresponsive), seizures, and other clinical observations (noted by the observer). Appetite was monitored twice a day during this period. In the pre-challenge period and from days 9 through 28 post-challenge, animals were observed twice daily. In order to limit the number of animals that were found dead, animals documented as having respiratory distress or being unresponsive may have had

more frequent observations performed and telemetry data reviewed to determine if temperature or activity levels were decreasing.

MO comment: *It is important to note that despite the observations every 6 hours, 11 of the 24 animals who died were found dead. Respiratory distress was infrequently seen in the animals (labored respirations in 2 and respiratory distress in 1). There is no evidence of more frequent monitoring in any of the animals documented in the clinical observations data. One animal was recorded as being normal in the 6 hours prior to being found dead.*

Body temperatures and activity levels were to be monitored at least hourly beginning 10 to 14 days prior to challenge, and until the end of the in-life animal phase (32 days post challenge). Body temperatures were to be recorded via TA-D70 telemetry transmitters (b) (4) after surgical implantation.

MO comment: *Isolated temperature spikes into the high 40's and low 50's were noted for all of the monkeys in the study. HGS postulates that there is the possibility that transmitter frequencies from different animals may have interacted with each other due to the proximity of the monkeys to each other in the cages. The manufacturer suggests that the receivers may be reading the interaction signals and this may result in an additive effect and abnormally high readings can be observed. It is not clear how significant a problem this interference might be. Since temperatures were not used as a trigger for treatment in this study, there was no significance of these spikes in this study.*

Necropsy and Histopathology:
Gross necropsies were to be performed on all monkeys that were found dead or were euthanized. Sections of target tissues including but not limited to brain/meninges, lungs, liver, spleen, kidney and mediastinal lymph nodes as well as all gross lesions were to be preserved in 10% neutral buffered formalin. To confirm death or illness due to anthrax, histopathology was to be performed, as deemed necessary, by a board certified veterinary pathologist. A representative sample of spleen, liver, and mediastinal lymph node was to be homogenized and plated on blood agar plates for qualitative assessment of *B. anthracis*.

Following the analysis of the initial necropsy data that included histopathology analysis of only the two animals that did not have gross evidence of anthrax disease, the FDA requested that histopathology analysis be performed on all tissues in all animals that died. The reading of the tissues from study 724-G005829 was performed under GLP as Protocol 901-G005829. The draft report of this study was submitted as serial 0088 dated October 31, 2008. The study was read in a blinded manner with the study pathologist only having access to pre-study animal numbers and gross observations during initial microscopic interpretation. Study blinding was removed after all of the slides were read by

the study pathologist but prior to peer review. The FDA also asked for independent pathology review of the slides. The independent evaluation of the pathology slides was completed by Experimental Pathology Laboratories, Inc. (EPL). The draft report of their results was submitted as serial 0093 on December 23, 2008. At EPL, all the slides were read in a blinded manner and then peer reviewed.

Blood Collection and Testing:

Blood volumes were taken from a femoral artery or vein, saphenous vein, or other appropriate vein on the days specified in Table 2. Blood volumes were within the guidelines established by the Battelle IACUC. All post-challenge pre-treatment blood samples were to be collected relative to the median challenge time at the time points stated ± 1 hour. Median challenge time was to be calculated from the challenge end time of the first and last monkeys challenged within a single challenge day. All post-treatment blood collections were to be collected relative to the end time of each animal's IV treatment.

Time Point	Blood Tube type/Approximate Blood volume	Bacteremia (Culture)	Bacteremia (PCR: DNA shipped to HGS)	Serum PA level (via screening ECL assay)	Serum PA level (via quantitative ECL; serum shipped to HGS)	CBC/ CRP	PK (serum shipped to HGS)	Anti-PA and TNA[2]
Study day -3	EDTA ~1.5 mL + SST ~1.0 mL	X	X	X	X	X	X	X
24 h post-challenge followed by every 6 h up to treatment[3] (or up to 54 h post-challenge)	EDTA ~1.5 mL + SST ~1.0 mL	X	X	X	X	X	-	-
IV treatment time (pre)	EDTA ~1.5 mL + SST ~1.0 mL	X	X	-	X	X	X	-
5 minutes after IV treatment	SST ~1.0 mL	-	-	-	X	-	X	-
12h after IV treatment	EDTA ~0.5 mL + SST ~1.0 mL	X	-	-	X	-	X	-
24h after IV treatment	EDTA ~1.5 mL + SST ~1.0 mL	X	-	-	X	X	X	-
3 days after IV treatment	EDTA ~0.5 mL + SST ~1.0 mL	X	-	-	X	-	X	-
5 days after IV treatment[3]	EDTA ~1.5 mL + SST ~1.0 mL	X	-	-	X	X	X	-
8 days after IV treatment[3]	EDTA ~0.5 mL + SST ~1.0 mL	X	-	-	X	-	X	-
14 days after IV treatment[3]	EDTA ~1.5 mL + SST ~1.0 mL	X	-	-	X	X	X	-
28 days after IV treatment[3]	EDTA ~0.5 mL + SST ~1.0 mL	X	-	-	X	-	X	X
Terminal	EDTA ~2.0 mL + SST 1.0 mL	X	X[4]	-	X	CRP only	X	-

1 Post-challenge pre-treatment bleed time points are relative to a median challenge time for a challenge cohort and were to be calculated from the time each animal's aerosol challenge exposure ended. Post-treatment bleed times were to be calculated from the time each individual animal's IV treatment ended. Blood sampling was to occur within ± 60 minutes of the calculated time, except for the first 2 post-treatment blood samples, which were to occur within 5 and 15 minutes of the calculated time, respectively, and the 3, 5, and 8 day post-treatment samples which were to occur within ± 3 hours relative to the previous blood collection time. Day 14 and Day 28 post-treatment samples were to be relative to the day of treatment.

2 Baseline TNA was tested for survivors only.

3 Treatment was to be administered when a positive PA level was detected in serum via the ECL screening assay. Following treatment, blood collections were to be based on the post-treatment blood collection schedule.

4 From the terminal sample, ~1.0 mL whole blood was to be stored at ≤ -70°C for possible DNA processing upon sponsor's request.

(Adapted from HGS' BLA submission)

Table 2. Blood Collection and Assay Schedule

Assays:

Bacteremia by blood culture was performed at Battelle according to their SOP. Macroscopic observations were performed and colonies were assessed for hemolysis, type, size, color, and morphology. If γ-hemolytic, white colonies of 4 to 10 mm in diameter with a rough appearance and irregular edges were present, the sample was documented as positive for *B. anthracis* bacteremia.

For bacteremia by PCR, genomic DNA was isolated using a QIAGEN DNeasy Kit. Samples were shipped to HGS where PCR amplification was conducted using *B. anthracis* specific primers according to HGS SOPs. The PCR procedure consisted of 3 assays: the 18s PCR assay, the pagA PCR assay, and the chromosomal PCR assay. The 18s assay tests the quality and concentration of genomic DNA, the pagA assay tests for the presence of the *B. anthracis* pX01 DNA plasmid, and the chromosomal assay tests for the presence of *B. anthracis* chromosomal DNA.

MICs of levofloxacin and ciprofloxacin for *B. anthracis* challenge material, on positive cultures pre-IV treatment, and on positive cultures from terminal blood samples were to be determined.

Hematology evaluation was performed with the Advia® 120. Hematology slides were to be prepared and reviewed when necessary as determined by a highly-trained hematology technician or the Study Director. Hematology parameters that were monitored were WBC, RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelets, MPV, % and numbers of neutrophils, % and numbers of lymphocytes, % and numbers of monocytes, % and numbers of eosinophils, % and numbers of basophils, neutrophil to lymphocyte % ratio, and neutrophil to lymphocyte number ratio. CRP analysis was to be performed on residual plasma. The CRP analysis was a non-validated assay.

Serum PA screening assays were performed at Battelle. Undiluted serum samples in triplicate wells were to be incubated in the presence of rabbit anti-PA-biotin and rabbit anti-PA-SULFOTAG for 2 hours at 37^0C in a streptavidin-coated assay plate. The limit of detection was established at 1.0 ng/mL based on the qualification experiments. Blood was shipped to HGS for the PA quantitative assay. Total PA (free and raxibacumab-bound) concentrations in serum samples were to be determined using an ECL-based bridging assay using rabbit polyclonal antibody anti-PA-biotin and rabbit pAb anti-PA-SULFO-TAG. The concentrations were interpolated from a reference standard curve. The lower limit of quantification was 0.65 ng/mL.

Anti-PA antibody concentrations in serum samples were to be determined using an ELISA. Anti-PA antibody was to be captured on wild-type PA coated plates and the concentration was interpolated from a reference standard curve. The lower limit of quantification was < 600 ng/mL.

TNA titers were to be determined using a cell killing assay in J744A macrophages. Inhibition of PA binding is correlated with increased cell viability. The lower limit of quantification was < 52 titer.

Serum samples were to be analyzed for raxibacumab using an ECL-based assay where raxibacumab in serum binds to the biotinylated-PA on a streptavidin-coated plate and is detected by the addition of goat anti-human antibody labeled with MSD SULFOTAG. The concentration was interpolated from a reference standard curve. The lower limit of quantification was 940 ng/mL.

MO comment: *Please refer to Microbiology Review for assessment of the assay methods that were employed in this study. The most critical assay in this study was the screening PA, as a positive PA screen was the trigger for treatment. Bacteremia as defined by culture is considered the gold standard. The PA screen, the quantitative PA test, the bacteremia by PCR, the anti-PA antibody test, and the TNA assay are all exploratory tests being used in this study. It will be important to validate the performance characteristics of these studies.*

Statistical Methods:
The study design provided 74% power at a 5% significance level to detect an absolute improvement of approximately 49% or more in the 40 mg/kg raxibacumab group assuming an 8.3% survival in the placebo group and a 57.1% survival in the 40 mg/kg raxibacumab group. The primary analysis was performed on the intention-to-treat (ITT) population. All statistical tests were 2-sided and performed at a significance level of 0.05, unless otherwise specified. The primary efficacy analysis was subject to multiple comparison adjustment using a step-down sequential testing procedure. The ITT population was defined as all monkeys that were randomized and challenged with anthrax spores. The modified ITT population was defined as a subset of the ITT population that included all animals that received a study agent based on the planned treatment. The as-treated population was defined as the set of all animals that received a study agent based on the actual treatment.

MO comment: *Please refer to Statistical review by Hongling Zhou, Ph. D. In communication with the Sponsor, FDA indicated that the per protocol population (those that were bacteremic at the time of treatment) would be the study population of interest for efficacy analysis.*

Endpoints:

Primary Efficacy Endpoint:
The primary efficacy variable was survival at day 28, defined as the percent of monkeys alive on day 28 in the ITT population. The primary efficacy analysis was performed using a 2-sided likelihood test and was subject to multiple comparison adjustment using a step-down sequential testing procedure:

- First the 40 mg/kg raxibacumab group was compared with placebo. If this was statistically significant then the 20 mg/kg raxibacumab group was compared with placebo for statistical significance.

Sensitivity analyses were to be performed for the as-treated population, the modified ITT population, and the group of all animals that were randomized including any that were randomized but not challenged with spores.

Pre-specified subgroups included monkeys that were confirmed to be bacteremic at the time of treatment and monkeys that were not bacteremic at the time of treatment. If sample size was large enough, subgroups to be evaluated included monkeys toxemic at the time of treatment, monkeys that were bacteremic and toxemic at the time of treatment, and monkeys that were bacteremic and/or toxemic at the time of treatment. These subgroups were identified for events at the time of treatment, events at or before the time of treatment and events at any time. In the subgroup analysis, bacteremic was defined as animals with bacteremia as indicated by culture or PCR assay, and toxemic was defined as animals with toxemia as indicated by positive PA by quantitative ECL assay.

MO comment: *This pivotal study report has been submitted for licensing application for the treatment of inhalational anthrax in humans under the Animal Rule so it is important to ensure that the study population in nonhuman primates is sufficiently reflective of the potential human population with anthrax disease. Therefore, the efficacy assessments in this study will focus on the outcome in the per protocol population of animals that were bacteremic at the time of treatment. The outcomes in the ITT and the modified ITT populations should trend in the same direction as the outcomes in the per protocol population.*

Secondary Efficacy Endpoint:
The secondary efficacy endpoint was survival time, defined as the time from spore challenge to death during the 28 day study period. The Cochran-Armitage test was used to examine the dose response trend in 28-day survival for doses ranging from placebo to 40 mg/kg raxibacumab. The association between survival, inhaled spore dose, time to detectable serum PA and time to onset of bacteremia was to be explored.

Raxibacumab PK
PK analyses of raxibacumab concentration-time profiles for all raxibacumab-dosed monkeys were conducted using population analysis techniques with NONMEM software. Serum raxibacumab concentration-time data, serum PA concentration-time data, individual raxibacumab PK parameters, individual PA

kinetic parameters, serum anti-PA antibody concentrations, and serum TNA titers were summarized using descriptive statistics.

MO comment: *Please refer to the Clinical Pharmacology Review for PK analysis.*

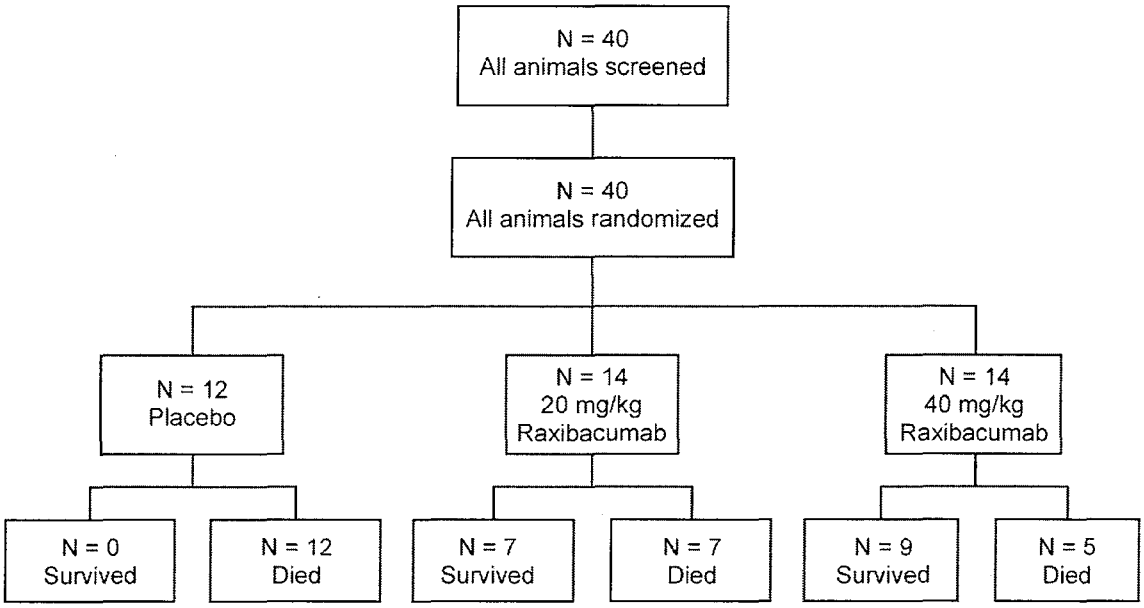
PROTOCOL CHANGES:

The original protocol was dated July 20, 2007. Four amendments were issued. Amendment 1 was issued prior to randomization, amendment 2 was issued prior to spore challenge, and amendments 3 and 4 consisted of editorial changes.

- Amendment 1 – August 23, 2007
  - Frequency of clinical observations was increased to every 8 hours during the 8 days following challenge if animals were having respiratory distress or being unresponsive in order to minimize the potential of an animal being found dead. It was also noted that more frequent observations may have induced stress on the other animals.
  - In addition to anti-PA antibody analysis, TNA analysis was to be performed on serum samples on day -7 and 30 days post treatment for surviving monkeys.
  - Additional detail was included to describe the procedure of blinding Battelle personnel to ensure that the Study Director and all the technicians assessing the animals were adequately blinded to study group assignment.
- Amendment 2 – August 30, 2007
  - An aliquot of test material, control material stock, and dilutions of the test material made for each challenge day were to be shipped to HGS for dose confirmation analysis.
  - The day -7 and 30 day post treatment blood collections were deemed unnecessary for the anti-PA antibody quantitative ECL and TNA analysis and were replaced with blood collections on day -3 and 28 days post treatment.
- Amendment 3 – March 25, 2008
  - A description of the secondary efficacy analysis of survival time over 28 days was added to the Analytical and Statistical Plan. This analysis was pre-specified in the Analytical Plan and similar language was added to the protocol for consistency.
- Amendment 4 – April 7, 2008
  - In the Study Design, the heading for quantitative serum PA level incorrectly referred to ELISA and was changed to quantitative ECL assay. Mention of ELISA in subsection 6.8 was also corrected.

- In section 6.2, the targeted spore dose was incorrectly stated to be 2.47 x 10^7 spores and was corrected to 1.24 x 10^7 corresponding to a targeted 200 x LD_{50}.

STUDY 724-G005829 RESULTS
Disposition of Animals



(Adapted from HGS' BLA submission)
Figure 1. Disposition of the ITT population

***MO comment**: This figure represents the disposition of the ITT population. The review for efficacy will focus on the analysis of animals that were bacteremic prior to treatment.*

Demographic and Baseline Characteristics

	Placebo N = 12	20 mg/kg N = 14	40 mg/kg N = 14	All Active N = 28	All Groups N = 40	P-Value[1]
Sex						1.0000
n	12	14	14	28	40	
Male	6 (50.0%)	7 (50.0%)	7 (50.0%)	14 (50.0%)	20 (50.0%)	
Female	6 (50.0%)	7 (50.0%)	7 (50.0%)	14 (50.0%)	20 (50.0%)	
Weight (kg)						0.6794
n	12	14	14	28	40	
Mean ± SD	3.7 ± 0.8	3.4 ± 0.9	3.5 ± 0.7	3.5 ± 0.8	3.5 ± 0.8	
Median	3.6	3.3	3.6	3.5	3.5	
Range	(2.6, 4.8)	(2.3, 5.1)	(2.3, 4.4)	(2.3, 5.1)	(2.3, 5.1)	
Age at randomization (years)						0.2799
n	12	14	14	28	40	
Mean ± SD	3.8 ± 0.4	3.5 ± 0.5	3.7 ± 0.4	3.6 ± 0.4	3.7 ± 0.4	
Median	3.9	3.6	3.8	3.8	3.8	
Range	(2.9, 4.5)	(2.7, 4.3)	(3.0, 4.3)	(2.7, 4.3)	(2.7, 4.5)	

[1] P-value for comparison of the 3 groups, based on Fisher's exact test for categorical variables, and ANOVA for continuous variables.

(Adapted from HGS' BLA submission)

Table 3. Summary of demographics and baseline characteristics for the ITT population

Three monkeys in the placebo group (C24784, C24853, and C24858) weighed more than the protocol-specified range of 2.5 to 4.5 kg. Two monkeys in the 20 mg/kg raxibacumab group (24755, and 24840) weighed more than the specified range and one monkey (21466) weighed less than the specified range. In the 40 mg/kg raxibacumab group, one monkey (21474) weighed less than the specified range.

***MO comment**: Demographics and baseline characteristics were similar among the treatment groups.*

Extent of Anthrax Exposure

Anthrax Exposure Level by Challenge Group

The mean exposure to *B. anthracis* in the ITT population was 184.0 $\pm$ 46.8 x LD_{50} and is summarized in Table 4. Monkeys in the 40 mg/kg raxibacumab group received a lower mean inhaled dose of spores (157 x LD_{50}) compared to the placebo group (198 x LD_{50}) and the 20 mg/kg raxibacumab group (199 x LD_{50}). This finding prompted a sensitivity analysis.

Three monkeys in the 40 mg/kg raxibacumab group (C23640, C23655, and C24823) were challenged with a dose of spores less than the lowest challenge dose in the placebo group. Excluding those animals did not change the survival benefit (Table 5). The Cox model which included all 40 monkeys showed that, adjusting for the dose of spores, the survival benefit remained statistically significant in the treated groups compared to the placebo group.

	Placebo N = 12	20 mg/kg N = 14	40 mg/kg N = 14	All Groups N = 40	P-Value[1]
Anthrax exposure (x LD_{50})					0.0255
n	12	14	14	40	
Mean ± SD	197.6 ± 43.5	199.0 ± 52.4	157.2 ± 32.4	184.0 ± 46.8	
Median	207.9	208.5	161.6	175.0	
Range	121.0-277.0	130.0-301.0	106.9-218.0	106.9-301.0	

[1] P-value based on 1-way ANOVA for comparison among all treatment groups.

(Adapted from HGS' BLA submission)

Table 4. Anthrax spore exposure by treatment group

Treatment	N	Number(%) of Survivors	P-Value[1]
Control	12	0 (0.0%)	-
20 mg/kg raxibacumab	14	7 (50.0%)	0.0064
40 mg/kg raxibacumab	11	8 (72.7%)	0.0003

[1] P-value based on 2-sided Fisher's exact test for the comparison vs the placebo group.

(Adapted from HGS' BLA submission)

Table 5. Survival at day 28 excluding animals with anthrax exposure < 120 x LD_{50}

***MO comment**: The spore challenge dose was lower in the 40 mg/kg raxibacumab group than in the other two treatment groups. Dr. Hongling Zhou, statistical reviewer, analyzed the spore challenge doses and confirmed the results in Table 4. There are statistically significant differences in mean spore exposure levels among the three treatment groups (F test, p=0.0255). The p-values (t-test) for further pair-wise comparisons are 0.9413 for the 20 mg/kg raxibacumab group vs. placebo, 0.0123 for the 40 mg/kg raxibacumab group vs. placebo, and 0.0176 for the 20 mg/kg raxibacumab vs. 40 mg/kg raxibacumab groups. The difference in inhaled spores between the 40 mg/kg raxibacumab group and the placebo group is statistically significant even after the conservative Bonferroni multiplicity adjustment. Further examination of the three animals that received the lowest challenge dose revealed that C23640 received the lowest dose at 106.9 x LD_{50} and died, C24823 received 112 x LD_{50} and survived, and C23655 received 116 x LD_{50} and died.*

Dr. Zhou confirmed the sensitivity analysis regarding the exclusion of the three animals with spore exposure less than 120 x LD_{50}. Dr. Zhou performed additional sensitivity analyses excluding animals with exposure less than 150 x LD_{50}. For animals that had exposures >150 x LD_{50}, there were 11 animals in the placebo group, 10 in the 20 mg/kg raxibacumab group, and 9 in the 40 mg/kg raxibacumab group. The fisher's exact test comparing survival rates between the 20 mg/kg raxibacumab group and placebo returned a p-value of 0.0039, and between the 40 mg/kg raxibacumab group and placebo returned a p-value of 0.0005. Statistically significant differences in survival were seen when animals receiving lower spore exposures were excluded.

The monkeys were randomized to be challenged with spores on 1 of three days (9/18/07, 9/25/07, and 10/2/07). The following table prepared by Dr. Hongling Zhou summarizes the exposure by challenge day.

Table 6. Anthrax exposure by challenge date

	9/18/07 N = 14	9/25/07 N = 13	10/2/07 N =13	P-value
mean	157.9	192.6	203.4	0.0257
SD	45.4	43.6	41.4	
Range	112.0-277.0	106.9-256.0	140.2-301.0	

There are statistically significant differences in mean anthrax exposure on the three challenge days (F test, p=0.0257) with animals on 9/18/07 receiving a lower mean challenge dose of spores. The p-values (t test) for pair-wise comparisons are 0.0544 for 9/18/07 vs. 9/25/07, 0.0119 for 9/18/07 vs. 10/2/07, and 0.5226 for 9/25/07 vs. 10/2/07. Dr. Zhou found this difference to be statistically significant even after the conservative Bonferroni multiplicity adjustment.

Survival rates were then examined based on challenge day (Table 7). The survival rate for animals challenged on 9/18/07 was higher than the overall survival rate in the 20 mg/kg raxibacumab group and was slightly lower than the overall survival rate in the 40 mg/kg raxibacumab group. Both raxibacumab treated groups had lower survival rates for the animals challenged on 9/25/07 than the overall survival rates. It is interesting to note that 5/5 animals in the 40 mg/kg raxibacumab group who were challenged on 10/2/07 survived. There does not appear to be a relationship between challenge day, challenge dose, and survival.

Table 7. Survival rate (%) by challenge day

Challenge Date	**Placebo**	**20 mg/kg**	**40 mg/kg**
9/18/07	0/4 (0)	3/5 (60)	3/5 (60)
9/25/07	0/4 (0)	2/5 (40)	1/4 (25)
10/2/07	0/4 (0)	2/4 (50)	5/5 (100)

In preliminary observations, there was a suggestion that on 9/25/08, there might be a survival difference based on challenge order. Dr. Zhou examined the relationship between survival and the challenge order on 9/25/08. All three monkeys that survived were challenged on or after 11:41 AM. None of the 8 animals who were challenged prior to 11:41 AM survived. The amount of anthrax exposure was slightly lower on average for those who were challenged before 11:41 AM (186.1 x LD_{50} before and 202.9 x LD_{50} after). The numbers of animals in the subgroups was not sufficient to identify a statistically significant trend of survival for treatment group with respect to challenge order. It is interesting to note that on 9/25/07, there were no early survivors bringing into question whether the spores were hardier during the early part of the challenge. However, no similar findings were seen on the other two challenge days.

Dr. Zhou also explored the relationship between anthrax exposure level and the probability of survival. The mean dose of spores in those animals that died was compared to the mean dose in those who survived. The 16 animals who survived had a lower mean spore exposure (179 $\pm$ 37.4 x LD_{50}) than those who died (187 $\pm$ 52.7 x LD_{50}). In the 20 mg/kg raxibacumab group those who survived had an exposure level of 194.8 $\pm$ 42.1 x LD_{50} and those that died had an exposure level of 203.2 $\pm$ 64.3 x LD_{50}. For the 40 mg/kg raxibacumab group the mean exposure level was higher for those who survived (167.5 $\pm$ 30.5 x LD_{50}) than for those who died (138.9 $\pm$ 30 x LD_{50}). None of the differences were statistically significant.

Please see Dr. Hongling Zhou's statistical review for details of these analyses. The study was not powered to detect if differences in challenge doses would lead to differences in survival rates in any of the groups or subgroups.

Therapeutic Trigger Symptoms and Timing of Treatment Initiation

Table 8 below summarizes the signs and symptoms in the ITT population including the time to treatment and the documentation of toxemia as defined by quantitative ECL and bacteremia as determined by culture and/or PCR.

	Placebo N = 12	20 mg/kg N = 14	40 mg/kg N = 14	All Groups N = 40	P Value[1]
Time to treatment[2] (hour)					0.1896
n	12	14	14	40	
Mean ± SD	39.5 ± 8.7	45.2 ± 9.1	42.6 ± 4.4	42.6 ± 7.8	
Median	37.5	43.4	42.0	40.3	
Range	28.0-56.2	34.9-58.4	36.2-50.7	28.0-58.4	
Trigger event					0.5040
n	12	14	14	40	
Detectable PA	11 (91.7%)	12 (85.7%)	14 (100.0%)	37 (92.5%)	
PA not detected	1 (8.3%)	2 (14.3%)	0 (0%)	3 (7.5%)	
Toxemia[3] at or before treatment initiation	11 (91.7%)	13 (92.9%)	14 (100.0%)	38 (95.0%)	0.7487
Toxemia at the time of treatment initiation	11 (91.7%)	13 (92.9%)	14 (100.0%)	38 (95.0%)	0.7487
Toxemia at any time	12 (100.0%)	13 (92.9%)	14 (100.0%)	39 (97.5%)	1.0000
Bacteremia[3] at or before treatment initiation	10 (83.3%)	12 (85.7%)	13 (92.9%)	35 (87.5%)	0.8490
No Bacteremia at or before treatment initiation	2 (16.7%)	2 (14.3%)	1 (7.1%)	5 (12.5%)	0.8490
Bacteremia at the time of treatment initiation	10 (83.3%)	12 (85.7%)	13 (92.9%)	35 (87.5%)	0.8490
No Bacteremia at the time of treatment initiation	2 (16.7%)	2 (14.3%)	1 (7.1%)	5 (12.5%)	0.8490
Bacteremia at any time	12 (100.0%)	14 (100.0%)	14 (100.0%)	40 (100.0%)	-
Toxemia and bacteremia at or before treatment initiation	10 (83.3%)	12 (85.7%)	13 (92.9%)	35 (87.5%)	0.8490
Toxemia and bacteremia at the time of treatment initiation	10 (83.3%)	12 (85.7%)	13 (92.9%)	35 (87.5%)	0.8490
Toxemia and bacteremia at any time	12 (100.0%)	13 (92.9%)	14 (100.0%)	39 (97.5%)	1.0000
Toxemia and/or bacteremia at or before treatment initiation	11 (91.7%)	13 (92.9%)	14 (100.0%)	38 (95.0%)	0.7487
Toxemia and/or bacteremia at the time of treatment initiation	11 (91.7%)	13 (92.9%)	14 (100.0%)	38 (95.0%)	0.7487
Toxemia and/or bacteremia at any time	12 (100.0%)	14 (100.0%)	14 (100.0%)	40 (100.0%)	-
Bacteremia without toxemia at or before treatment initiation	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-

1 P-value for comparison of the 3 groups, based on Fisher's exact test for categorical variables, and ANOVA for continuous variables.

2 Time to treatment was calculated relative to the start of challenge.

3 Throughout this table toxemia was determined by quantitative ECL, and bacteremia was determined by culture and/or PCR. Only post-challenge bacteremia and toxemia were counted.

(Adapted from HGS' BLA submission)

Table 8. Summary of signs and symptoms in the ITT population

***MO comment**: For the FDA efficacy analysis, bacteremia was defined as blood culture positivity. Bacteremia as detected by PCR, toxemia as detected by screening PA ECL, and toxemia as detected by quantitative PA ECL are definitions that utilize tests that require qualification. Data have been submitted for qualification of these tests and they are being reviewed. As noted above, there were 5 animals (2 in the placebo group, 2 in the 20 mg/kg raxibacumab*

group, and 1 in the 40 mg/kg raxibacumab group) that were not bacteremic prior to treatment. The FDA analysis for efficacy was based on the outcome of the 35 animals that were bacteremic as defined by culture positivity at the time of treatment. For discussion of the individual animal triggers, see the comment below the efficacy analysis.

Primary Efficacy Analysis

The sponsor analyzed survival at day 28 in the ITT population (see Table 9). Statistically significant survival was demonstrated compared to placebo for both the 20 mg/kg and the 40 mg/kg raxibacumab groups for the ITT population.

Treatment	N	Number(%) of Survivors	P-Value[1]
Control	12	0 (0.0%)	-
20 mg/kg raxibacumab	14	7 (50.0%)	0.0064
40 mg/kg raxibacumab	14	9 (64.3%)	0.0007

[1] P-value based on 2-sided Fisher's exact test for the comparison vs the placebo group.

(Adapted from HGS' BLA submission)

Table 9. Sponsor ITT analysis of survival at day 28

All randomized monkeys were challenged with *B. anthracis* spores and received study agent according to their planned treatment group.

***MO comment**: For the FDA efficacy analysis, bacteremia was defined as blood culture positivity. The results of the FDA statistical analysis performed by Dr. Hongling Zhou are presented in Table 10.*

Table 10. Comparison of Sponsor and FDA Efficacy Analysis (Provided by Dr. Hongling Zhou)

Population	Treatment	N	No. of Survivors (%)	P-value*	95% CI of Raxi-placebo**	97.5% CI of Raxi-placebo**
ITT	Placebo	12	0 (0.0%)			
	20 mg/kg raxibacumab	14	7 (50.0)	0.0064	(19.3, 73.7)	(13.2, 77.0)
	40 mg/kg raxibacumab	14	9 (64.3)	0.0007	(31.6, 84.7)	(27.0, 87.4)
FDA Primary	Placebo	10	0 (0.0%)			
	20 mg/kg raxibacumab	12	5 (41.7%)	0.0396	(7.2, 68.7)	(1.5, 72.4)
	40 mg/kg raxibacumab	13	9 (69.2%)	0.0016	(31.1, 88.9)	(25.7, 90.9)

**P-values are based on two-sided Fisher's exact test for comparisons between the raxibacumab group and placebo.*

*** CIs are exact confidence intervals.*

The following graphic shows the disposition of the animals based on the FDA primary analysis:

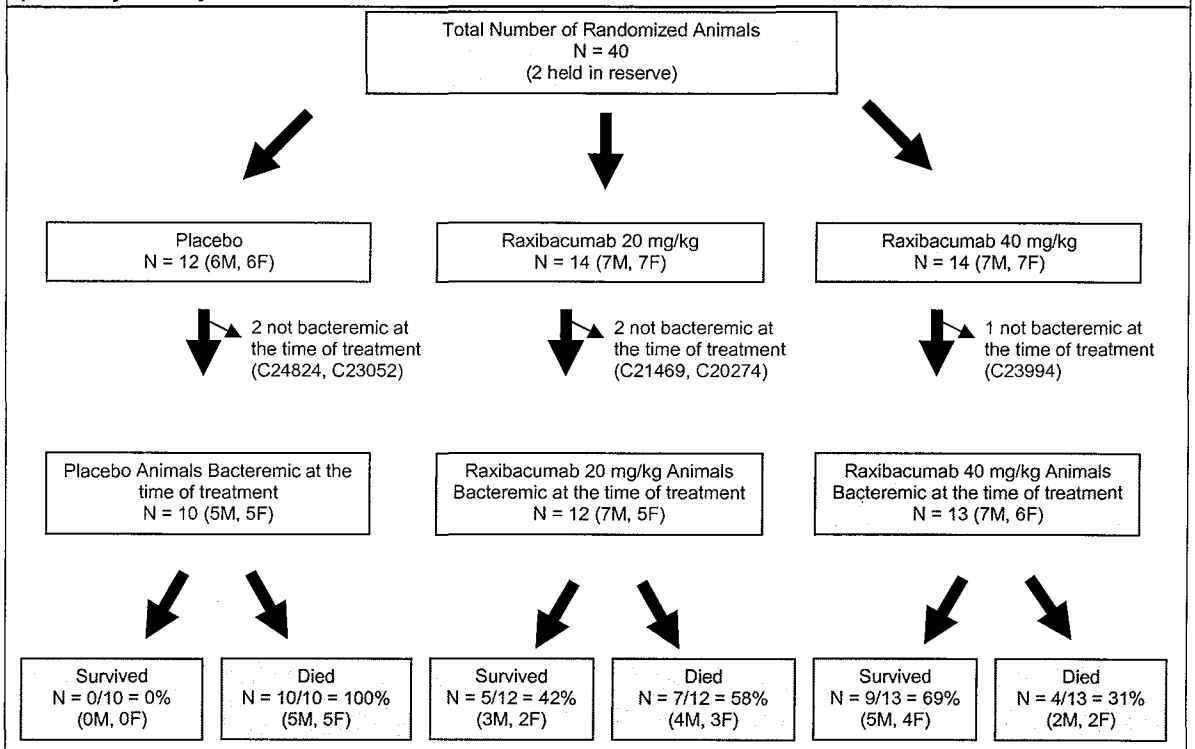


Figure 2. Definition of the animal disposition based on FDA analysis

Each of the animals was treated on the basis of a positive PA screening test or after 54 hours following challenge. The disposition of each individual animal with respect to bacteremia by culture, bacteremia by PCR, and qualitative PA is shown in Figure 3.

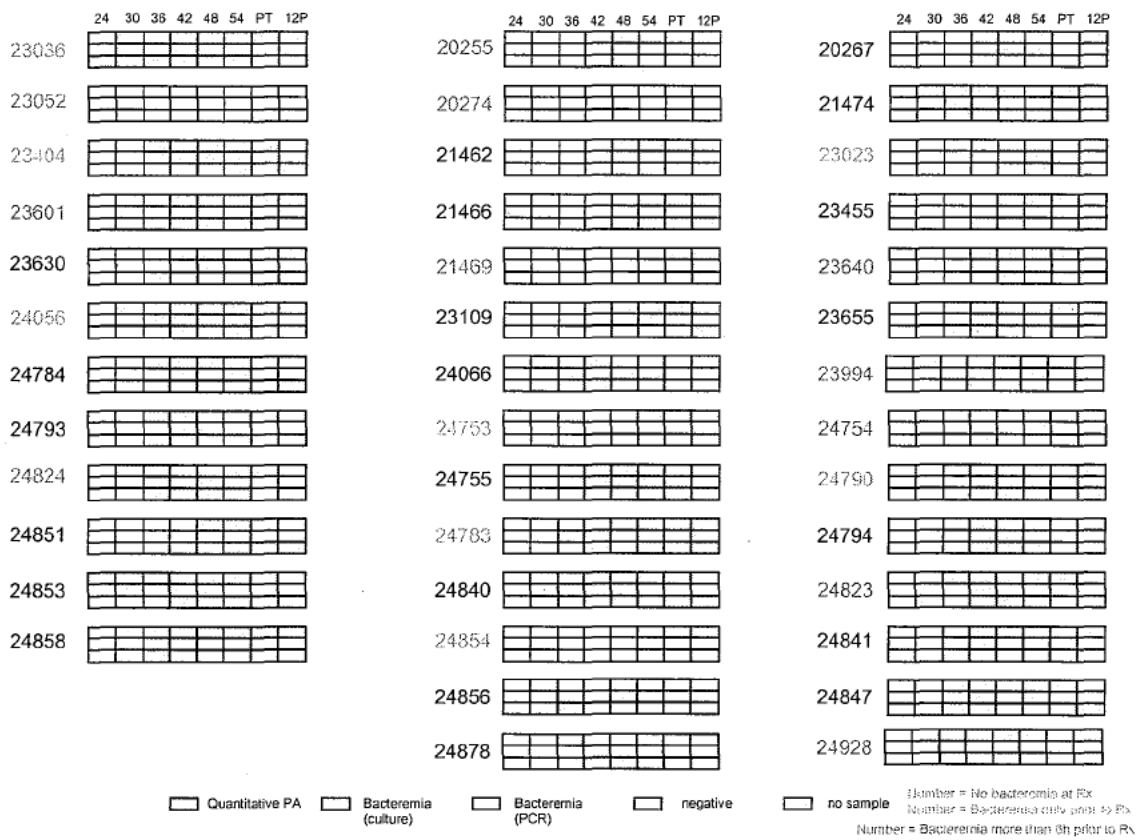


Figure 3. Individual animal disposition with respect to PA and culture.

In figure 3, animals with red numbers were not bacteremic at the time of treatment (N=5). Animals with green numbers were bacteremic only at the blood draw just prior to treatment (N=8). Animals with blue numbers were bacteremic for more than 6 hours prior to treatment (N=6). The disposition for each of these groups is presented in graphic form with outcome data in figure 4.

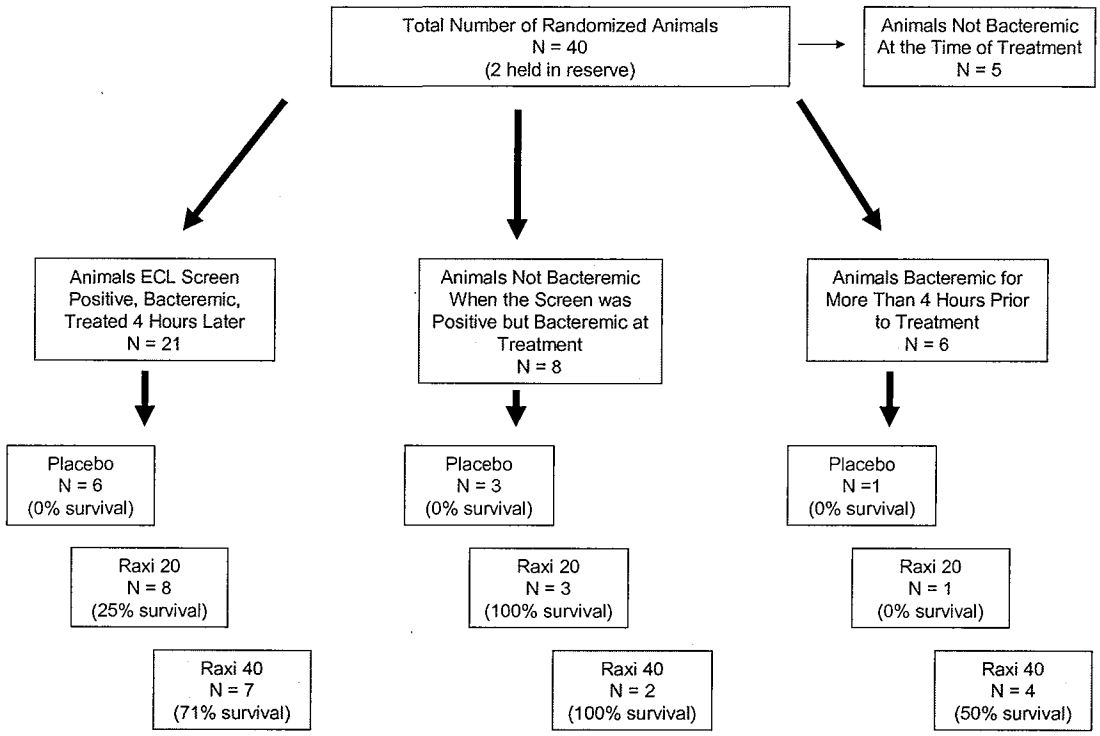
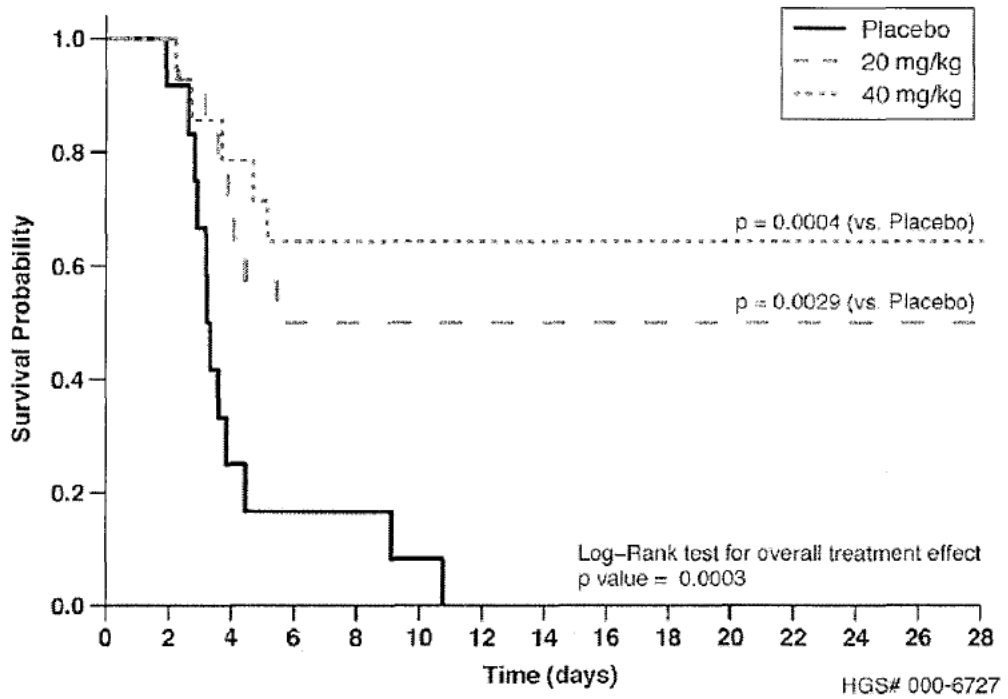


Figure 4. Animal disposition based on timing of bacteremia

These subgroups were not previously proposed so this is an ad hoc *analysis. In addition, the numbers in the subgroups are small, limiting the ability to perform meaningful statistical analysis. The time to treatment was shortest in those who were bacteremic just at the time of treatment (mean 37.6 hours), longer in those who were bacteremic for more than 6 hours prior to treatment (mean 45.8 hours) and longest in those who were never bacteremic (mean 55.8 hours). It is interesting to note that those animals treated just as they became bacteremic had 100% survival in both the 20 mg/kg and the 40 mg/kg raxibacumab groups, while the animals that were bacteremic for more than 6 hours prior to treatment had 0% survival in the 20 mg/kg raxibacumab group and 50% survival in the 40 mg/kg raxibacumab group. This might become more important as we try to define a window of opportunity for human treatment.*

Secondary Endpoint

The secondary efficacy endpoint was survival time, defined as the time from spore challenge to death during the 28 day study period. The following figure (Figure 5) shows the Sponsor's analysis of the ITT population.



(Adapted from HGS' BLA submission)
Figure 5. HGS Kaplan-Meier Curve for Survival

MO Comment: *The figure below, from Dr. Hongling Zhou, is a graph of the Kaplan-Meier survival curves for the three treatment groups of the monkeys in the FDA primary analysis group. The median survival times were 3.3 days in the placebo group, 5.0 days in the raxibacumab 20 mg/kg group and beyond 28 days in the raxibacumab 40 mg/kg group because 69.2% of the animals survived at the end of the study. There was a statistically significant difference in terms of survival time among the three groups (log rank test, p=0.0006). Pairwise comparisons also showed statistically significant differences between each raxibacumab group and the placebo (p = 0.0098 <0.025 for 20 mg/kg group vs. placebo, and p = 0.0005 < 0.025 for 40 mg/kg group vs. placebo) after the Bonferroni adjustment for multiple comparisons (0.025=0.05/2). Please see the statistical review by Dr. Hongling Zhou.*

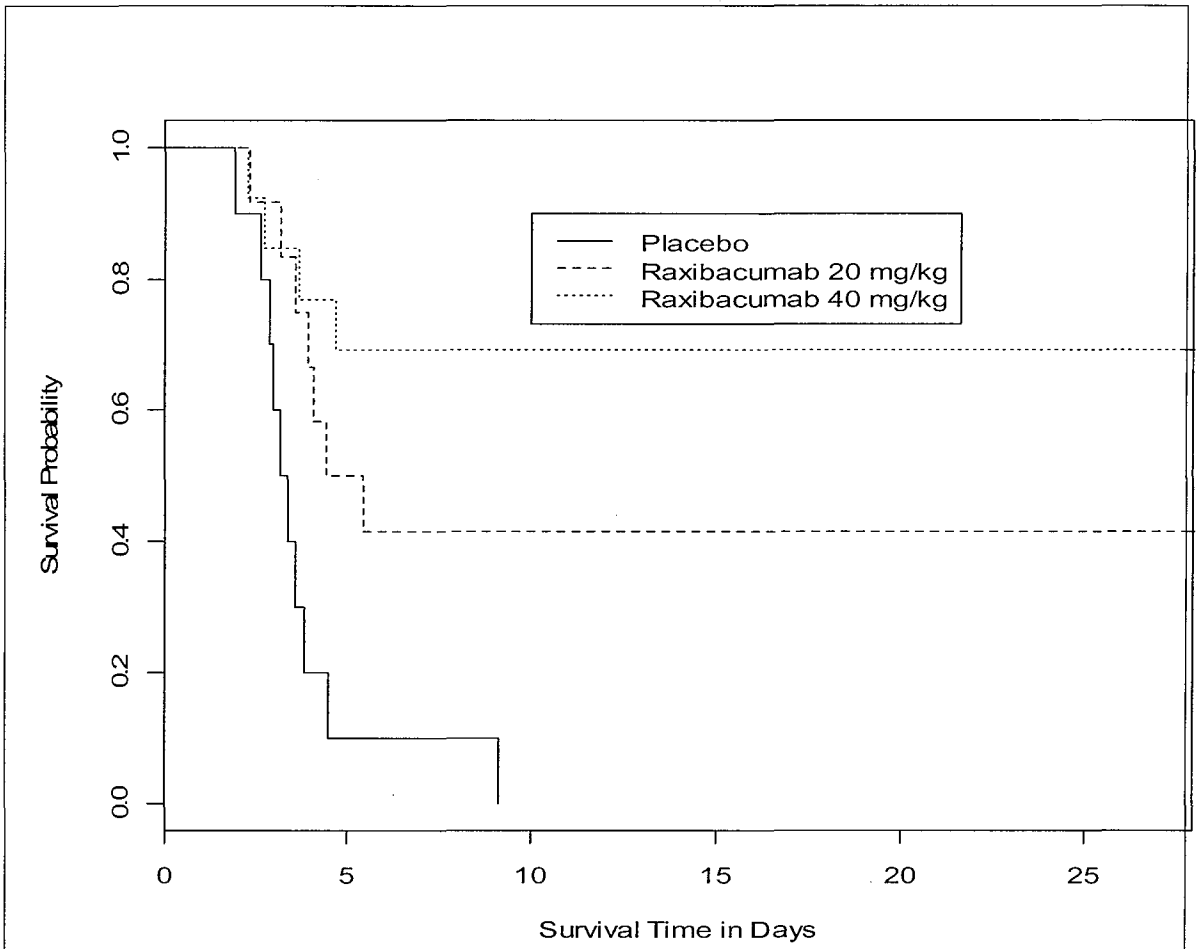


Figure 6. Kaplan-Meier Survival Curves for FDA Primary Analysis

Time to Events:
The summary of time to event information for signs and symptoms in survivors and non-survivors is presented in Table 10.

	Raxibacumab		Placebo
	Survivors N = 14	Non-survivors N = 12	All non-survivors N = 11
Anthrax exposure			
n	14	12	11
Mean ± SD	177.5 ± 38.7	176.4 ± 60.7	201.1 ± 43.8
Median (min, max)	175.2 (112.0, 256.0)	156.1 (106.9, 301.0)	212.0 (121.0, 277.0)
Treatment Trigger			
n	14	12	11
Detectable PA	14 (100.0%)	12 (100.0%)	11 (100.0%)
PA not detected	0 (0.0%)	0 (0.0%)	0 (0.0%)
Time to treatment			
n	14	12	11
Mean ± SD	44.4 ± 7.1	41.1 ± 5.1	38.0 ± 7.3
Median (min, max)	43.2 (34.9, 58.3)	40.1 (35.5, 50.6)	35.8 (28.0, 54.0)
Anti-PA antibody positive at baseline[1]			
n	14	0	0
Positive	0 (0.0%)	-	-
TNA positive at baseline			
n	14	0	0
Positive	0 (0.0%)	-	-
Time to[2] PA detection (quantitative ECL)			
n	14	12	11
Mean ± SD[3]	41.4 ± 6.9	35.5 ± 5.0	33.2 ± 7.2
Median (min, max)[3]	39.6 (31.9, 54.9)	34.9 (30.7, 47.2)	31.4 (25.8, 50.1)
Time to bacteremia (culture)			
n	14	12	11
Mean ± SD	41.0 ± 8.0	36.3 ± 5.9	35.9 ± 7.4
Median (min, max)	40.1 (31.9, 58.3)	35.5 (30.7, 48.6)	35.0 (26.0, 50.1)
Time to bacteremia (PCR)			
n	14	11	10
Mean ± SD	40.3 ± 6.8	_[3]	_[3]
Median (min, max)	39.0 (31.9, 54.9)	35.7 (30.7, _[3])	35.0 (24.0, _[3])
Time to bacteremia (culture and/or PCR)			
n	14	12	11
Mean ± SD	39.9 ± 7.2	36.3 ± 5.9	35.0 ± 7.0
Median (min, max)	37.8 (31.9, 54.9)	35.5 (30.7, 48.6)	35.0 (24.0, 46.6)

(Adapted from HGS' BLA submission)

Table 10. Summary of Time to Event (hours) of Signs and Symptoms Between Survivors and Non-survivors

***MO comment**: The time to event data for this study were similar to data presented in the monkey natural history study. There are no apparent differences in time to event between survivors and non-survivors. Time to death for those animals that died during the study was calculated. The mean time to death for placebo animals was 4.33 ± 2.7 days, for animals in the 20 mg/kg*

raxibacumab group was 3.85 ± 1.0 days and for animals in the 40 mg/kg raxibacumab group was 3.71 ± 1.2 days. This compares with a time to death of 4.89 ± 1.3 days in the natural history study.

Other Parameters Measured:
The majority of animals exhibited clinical signs consistent with anthrax following the spore challenge. The most common clinical features were lethargy, not eating, and respiratory abnormalities.

The MICs for levofloxacin ranged from 0.5 to 4.0 μg/mL and for ciprofloxacin ranged from 0.25 to 2.0 μg/mL. The MICs confirmed that the bacteria cultured from the blood of aerosol challenged animals was inhibited by similar concentrations of levofloxacin and ciprofloxacin as the original challenge material.

Body temperature was monitored in all animals. An elevation in body temperature or loss of diurnal rhythm was noted at 2 to 4 days post-challenge and appeared to coincide with decreased activity in the majority of animals. Body weights were not collected after day 0.

The incidence of positive blood cultures was 100% across all dose groups at some point post-challenge. The positive blood cultures confirm that all monkeys were infected with *B. anthracis*. Five of 40 monkeys did not show bacteremia by PCR prior to treatment. The *pagA* PCR assay was more sensitive than the chromosomal assay for detecting bacterial DNA, as positive samples in the *pagA* assay were detected at earlier time points in 28% of the monkeys. The *pagA* assay data showed correlation with bacteremia by culture at all time points in 75% of the monkeys. In six monkeys, bacteremia was detected by PCR prior to culture while in 4 animals, culture was positive prior to PCR.

Anti-PA antibodies were collected pre-dose and at 28 days following treatment for surviving animals. The data are presented in Table 11. Values for animals in the 40 mg/kg raxibacumab group are more than 2-fold higher than those in the 20 mg/kg raxibacumab group.

		Serum Anti-PA Antibody Concentration (ng/mL)	
		Predose	28 Day Postdose
20 mg/kg	N	7	7
	Mean	0	302
	95% CI	(0,0)	(-39,643)
40 mg/kg	N	9	9
	Mean	0	775
	95% CI	(0,0)	(292,1257)

(Adapted from HGS' BLA submission)

Table 11. Serum anti-PA antibody concentrations in surviving monkeys

Serum TNA titers were drawn for all surviving animals and the data are presented in Table 12. The mean titer for animals in the 40 mg/kg raxibacumab group was nearly 3-fold higher than the mean titer for animals in the 20 mg/kg raxibacumab group.

		Serum TNA Titer	
		Predose	28 Day Postdose
20 mg/kg	N	7	7
	Mean	0	5328
	95% CI	(0,0)	(523,10133)
40 mg/kg	N	9	9
	Mean	0	14827
	95% CI	(0,0)	(5163,24490)

(Adapted from HGS' BLA submission)

Table 12. Serum TNA titers in surviving monkeys

Serum PA levels were monitored over time in all monkeys. There were no measurable PA levels in specimens collected 3 days prior to challenge. In general, PA values increased sharply just prior to 40 hours post-challenge and reached a plateau between 40 to 80 hours. For non-survivors, the plateau was generally followed by a second spike in PA that increased until death. For survivors, the second spike did not occur and levels declined to undetectable levels for most animals by the end of the study. Higher serum PA levels at the first phase plateau were noted in non-survivors in the 40 mg/kg raxibacumab group compared with placebo animals. The sponsor speculates that this likely reflects the ability of raxibacumab to block the lethal effects of PA so that monkeys with no exposure to raxibacumab died at lower serum PA levels than those who received raxibacumab.

***MO comment**: Review of the PA screening test, the quantitative PA assay, and the PCR assay is concurrent with this review. Please see the Microbiology review for details.*

While there were some increases in temperature noted in the monkeys, this was not used as a trigger for treatment in the monkeys in this study.

There was a significant amount of inter-animal variability in the quantitative PA response. While there was an increase in the first phase plateau noted in the 40 mg/kg raxibacumab group compared with placebo in non-survivors, the range was very broad and the number of animals in the 40 mg/kg raxibacumab group was small.

It is not clear why there was a 2-fold higher level of anti-PA and an almost 3-fold higher level of TNA in the survivors in the 40 mg/kg raxibacumab group compared to the 20 mg/kg raxibacumab group. This will need to be followed in subsequent studies.

For information on raxibacumab PK, please see the Clinical Pharmacology review.

Safety Results

Limited safety information was provided during this study. Gross pathology was available on all animals that died with initial histopathology information on 2 animals that did not have gross pathologic evidence of anthrax disease. In addition, serial CBCs and C-reactive protein levels were drawn. Under protocol 901-G005829, Battelle reviewed histopathology of all animals that died and EPL provided independent pathology review of these tissues. None of the surviving animals were reported to have persistent neurologic findings, and neurologic findings were not more common in treated animals that died compared to placebo animals that died.

WBC levels were stable in all monkeys through 24 hours post spore challenge, spiked and then declined to baseline levels between 24 to 48 hours post-challenge for most monkeys. For non-survivors, this decline was followed by WBC increases until the time of death. For the majority of survivors, WBC levels approached baseline levels for the remainder of the study.

Platelet counts showed a decline after approximately 24 hours post challenge. In non-survivors platelet counts continued to decline until the time of death. In survivors, the platelet count decline ended approximately at 72 hours and was followed by an upward trend through the end of the study.

RBC levels began to decline in all animals through 24 hours post-challenge. In non-survivors, the decline continued until death. In survivors, the RBC levels started to trend in a positive direction approximately 168 hours post-challenge.

Serial WBC differentials were recorded for all animals. The neutrophil percent increased following challenge but then subsided in surviving animals. Animals demonstrated decreasing levels of lymphocytes following a rapid spike post-challenge. Monocytes and eosinophils were slightly decreased with a trough at 24 hours post treatment. There was a "marked" increase in the basophil percentages in raxibacumab treated animals on day 5 post treatment.

The sponsor stated that the hematology data collected on this study suggested that the animals were demonstrating non-specific indicators of illness post-challenge.

MO comment: *One animal in the placebo group had an initial WBC that was outside the normal range (low). That animal showed increasing WBCs until death. A number of animals had elevated WBCs during infection, independent of survival. The lowest platelet count while on study was 214 x 10^3/µL in an animal in the 40 mg/kg raxibacumab group. The sponsor commented that there was a "marked" increase in basophil percentages in treated animals on day 5. The highest basophil percentage was 2.1% in an animal in the 40 mg/kg raxibacumab group. There were only 2 animals in the placebo group that had basophil levels drawn on day 5 and their percentages of basophils were 0.3% and 1.5%.*

The reviewer agrees with the sponsor's assessment that the hematology data are most suggestive of a non-specific process associated with the acute anthrax infection in these animals.

Necropsy and Histopathology:
All animals found dead or euthanized had necropsies performed. In addition, all animals who did not survive had terminal blood cultures drawn and all animals that died had positive cultures for *B. anthracis.* Two monkeys did not have evidence of anthrax disease on gross examination. Histopathology was done on these two animals, and bacteria were noted (Table 13). Group 1 is raxibacumab 40 mg/kg and group 3 is placebo. Nephrosis and splenic lymphoid depletion were present in both monkeys. Large rod-shaped bacteria consistent with *B. anthracis* were present in multiple organs in both monkeys, particularly within the vasculature and in the splenic parenchyma. Lesions were more apparent in the control animal compared to the raxibacumab treated animal.

ORGAN/Lesion Group	1	3
BRAIN		
Bacteria	1	2
LYMPH NODE, MEDIASTINAL		
Bacteria	0	1
KIDNEY		
Bacteria	1	1
Nephrosis	1	2
LIVER		
Bacteria	1	2
SPLEEN		
Bacteria	3	3
Lymphoid depletion	1	3
LUNG		
Bacteria	1	3
[a] Scale: 0 = not present, 1 = minimal, 2 = mild, 3 = moderate		

(Adapted from HGS' BLA submission)
Table 13. Microscopic findings in 2 monkeys

Tissue cultures of liver, spleen, and mediastinal lymph node were done for all animals that died. These tissue cultures were positive for all three tissues in 23 of the 24 animals. One animal had a negative lymph node culture but the other tissues were positive for bacteria.

***MO comment**: The animal that had a negative lymph node tissue culture was one of the animals that underwent histopathologic analysis. While the mediastinal lymph node was not positive, the brain, kidney, liver, spleen and lung were all positive for bacteria. Based on the information in the submission, it is suggested that the disease process might be slightly more intense in the control animal compared to the animal in the 40 mg/kg raxibacumab group. However, based on this preliminary information, it is not possible to evaluate differences between the two animals.*

Necropsies were performed on all animals that died. Table 14 outlines the gross findings.

Group	1		2		3	
Number in group	14		14		12	
Mortality (%)	36%		50%		100%	
Sex	M	F	M	F	M	F
ORGAN/Lesion # dead	2	3	4	3	6	6
No Gross Lesions		1				1
ADRENAL GLAND Discoloration	1					
BRAIN Discoloration/foci/accumulation (red/dark)	1	2	3	3	1	2
CAVITY, ABDOMINAL Fluid					1	1
CAVITY, PERICARDIAL Fluid		1	1			1
CAVITY, THORACIC Fluid					1	1
LYMPH NODE, BRONCHIAL Enlarged	1	1	2	1	5	2
LYMPH NODE, MEDIASTINAL Enlarged		1	2		4	1
LYMPH NODE, MESENTERIC Enlarged	1				3	
SKELETAL MUSCLE Cyst			1			

Cells left blank have an incidence of 0

(Adapted from HGS' BLA submission)

Table 14. Gross necropsy findings by treatment group

***MO comment**: Table 15 gives the single animal breakdown of the necropsy findings. The orange highlight identifies the two animals that had histopathological analysis.*

Table 15. Gross necropsy individual animal findings

Group	ID	Brain/ Meninges	Bronch LN	Mes LN	Med LN	Adrenal	Pericard	Abd Cavity	Skeletal muscle	Thoracic Cavity
Placebo	23036									X
	23404	X								
	23601		X							
	23630									
	24056	X	X							
	24784		X	X	X			X		
	24793	X	X							
	24851		X							
	24853		X	X	X					
	24858			X	X					
	23052				X		X	X		
	24824		X		X					
20m/kg	20255	X	X							
	21466	X								

	24066	X								
	24755	X	X							
	24840	X			X		X			
	24856		X							
	24878	X			X				X	
40mg/kg	23640									
	23655	X	X		X					
	24847					X				
	24928	X	X	X						
	23994	X					X			

Of note, a number of animals had discoloration/foci/accumulation (red/dark) in the brain. It is interesting to note brain involvement in 3 of the 12 placebo animals, 6 of 7 animals in the 20 mg/kg raxibacumab group, and 3 of 5 animals in the 40 mg/kg raxibacumab group. There is a statistically significant difference between placebo and treated animals with respect to brain gross lesions, 3/12 in the placebo group compared to 9/12 in the treated groups (p=0.0391). Individual comparisons show significance for the placebo (3/12) compared to the 20 mg/kg raxibacumab group (6/7) (p=0.0198). No significance was seen for placebo (3/12) compared the 40 mg/kg raxibacumab group (3/5) (p=0.2801). These numbers are small but there is concern for the trend in placebo vs. treated animals. Based on this information, the sponsor was asked to perform histopathologic analysis on all tissues (with particular emphasis on the brains) for all animals that died during the study.

Protocol 901-G005829 and EPL Independent Pathology Assessment

The results of the histopathologic analysis of the tissues of all animals that died are presented in Table 16. All protocol-required tissues were evaluated with the exception of the mediastinal lymph node from animal C23036 which was not successfully processed to a slide. Microscopic findings were graded semi-quantitively according to the scale:

- Grade 1 = minimal
- Grade 2 = mild
- Grade 3 = moderate
- Grade 4 = marked

Microscopic findings were considered consistent with anthrax disease in all animals that died. Animals treated with raxibacumab had a greater incidence and/or severity of CNS lesions than did placebo treated animals. "More

numerous and widespread bacteria were observed in the brain of Raxibacumab-treated animals, while bacteria in the brain of untreated control animals were often confined to blood vessels or the immediate perivascular space. However, untreated control animals had greater incidence and/or severity of parenchymal organ lesions did Raxibacumab-treated animals." The conclusion by Battelle was that raxibacumab treated animals that died had a greater incidence of inflammation, hemorrhage, necrosis and bacteria in the brain. Further they argue that the median time to death for monkeys without inflammation was 3.2 days compared to 4.5 days in those that had inflammation. They also state that there was a significant improvement in survival time in treated animals based on the inclusion of time of survival data for animals that survive the challenge.

EPL also analyzed the histopathology slides and the summary of their data compared with the Battelle data is included in Table 17. For the most part, the findings were similar in both incidence and severity with a correlation coefficient of > 0.85. EPL concluded, "By the EPL reading, the incidence of findings in the brain is not higher in the raxibacumab treatment groups compared with the placebo treatment groups, but the severity of the findings, particularly in the meninges, tends to be greater."

Tissue/Observation	Incidence (Severity) Among Non-Survivors Placebo (n = 12)	20 mg/kg Raxibacumab (n = 7)	40 mg/kg Raxibacumab (n = 5)
Kidney			
Bacteria	11 (1.75)	3 (0.43)	3 (0.60)
Inflammation	0	1 (0.14)	0
Liver			
Bacteria	11 (1.67)	3 (0.43)	3 (0.60)
Lung			
Bacteria	11 (2.17)	3 (0.43)	4 (1.20)
Hemorrhage	3 (0.42)	1 (0.14)	0
Inflammation	6 (0.58)	2 (0.29)	1 (0.20)
Lymph node, bronchial			
Bacteria	8 (1.25)	2 (0.67)	1 (0.50)
Hemorrhage	5 (1.50)	1 (0.33)	1 (0.50)
Inflammation	1 (0.25)	1 (0.33)	0
Lymph node, mediastinal			
Bacteria	11 (1.36)	5 (0.86)	2 (0.40)
Hemorrhage	7 (1.09)	1 (0.14)	2 (0.40)
Lymph node, mesenteric			
Bacteria	3 (1.0)	0	1 (1.0)
Hemorrhage	2 (2.0)	0	0
Inflammation	1 (1.0)	0	1 (2.0)
Spleen			
Bacteria	10 (3.0)	4 (0.86)	3 (1.60)
Brain			
Bacteria	11 (1.17)	5 (2.29)	5 (2.0)
Hemorrhage	2 (0.33)	6 (2.57)	3 (2.0)
Inflammation	1 (0.25)	6 (2.57)	2 (1.4)
Necrosis	1 (0.17)	3 (0.71)	1 (0.40)

(Adapted from HGS' BLA submission)

Table 16. Summary of Histopathologic Findings by Battelle

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Incidence (Severity[1]) Among Non-Survivors

Tissue/Observation	Battelle Placebo (n = 12)	Battelle 20 mg/kg Raxibacumab (n = 7)	Battelle 40 mg/kg Raxibacumab (n = 5)		EPL Placebo (n = 12)	EPL 20 mg/kg Raxibacumab (n = 7)	EPL 40 mg/kg Raxibacumab (n = 5)
Lymph node, bronchial	(n=8)	(n=3)	(n=5)	Lymph node, bronchial	(n=3)	(n=3)	(n=3)
Bacteria	8 (1.3)	2 (0.7)	1 (0.5)	Bacteria	3 (1.6)	3 (1.3)	1 (0.5)
Hemorrhage	5 (1.5)	1 (0.3)	1 (0.5)	Hemorrhage	5 (1.4)	1 (0.7)	1 (0.5)
Inflammation	1 (0.3)	1 (0.3)	0	Inflammation	1 (0.3)	0	0
Necrosis	8 (2.1)	3 (1.0)	0	Necrosis	3 (2.4)	1 (0.3)	1 (0.5)
Lymph node, mediastinal	(n=11)			Lymph node, mediastinal	(n=11)		
Bacteria	11 (1.4)	5 (0.9)	2 (0.4)	Bacteria	10 (1.4)	4 (0.6)	2 (0.6)
Hemorrhage	6 (1.1)	1 (0.1)	2 (0.4)	Hemorrhage	4 (0.3)	0	1 (0.2)
Inflammation	0	0	0	Inflammation	1 (0.1)	0	0
Necrosis	10 (1.8)	4 (0.9)	4 (1.0)	Necrosis	10 (2.2)	5 (1.4)	0
Lung				Lung			
Bacteria	11 (2.2)	3 (0.4)	4 (1.2)	Bacteria	11 (2.5)	0	2 (0.8)
Hemorrhage	3 (0.4)	1 (0.1)	0	Hemorrhage	1 (0.3)	0	1 (0.2)
Inflammation	6 (0.6)	2 (0.3)	1 (0.2)	Inflammation (chronic active)	4 (0.4)	4 (0.6)	1 (0.2)

Tissue/Observation	Battelle Placebo (n = 12)	Battelle 20 mg/kg Raxibacumab (n = 7)	Battelle 40 mg/kg Raxibacumab (n = 5)		EPL Placebo (n = 12)	EPL 20 mg/kg Raxibacumab (n = 7)	EPL 40 mg/kg Raxibacumab (n = 5)
Kidney				Kidney			
Bacteria	11 (1.8)	3 (0.4)	4 (0.6)	Bacteria	10 (0.3)	1 (0.1)	3 (0.8)
Hemorrhage	0	0	0	Hemorrhage (tubular)	1 (0.1)	0	1 (0.1)
Inflammation	0	1 (0.1)	0	Inflammation	0	1 (0.1)	0
Necrosis	0	0	0	Necrosis	-	-	-
Liver				Liver			
Bacteria	11 (1.76)	3 (0.4)	5 (0.6)	Bacteria	3 (1.3)	1 (0.1)	2 (0.4)
Hemorrhage	-	-	-	Hemorrhage	1 (0.3)	0	0
Inflammation	-	-	-	Inflammation	1 (0.2)	1 (0.1)	0
Necrosis	2 (0.3)	1 (0.1)	0	Necrosis	5 (0.6)	3 (0.7)	0
Spleen				Spleen			
Bacteria	10 (3.9)	4 (0.9)	5 (1.6)	Bacteria	9 (2.8)	2 (0.3)	3 (1.2)
Hemorrhage	0	0	0	Hemorrhage	7 (1.2)	1 (0.3)	3 (0.8)
Inflammation	0	0	0	Inflammation (acute)	4 (0.4)	2 (0.5)	4 (1.6)
Necrosis	11 (2.9)	5 (0.9)	5 (1.4)	Necrosis	12 (3.0)	7 (2.0)	4 (2.2)
Fibrin exudation	7 (0.3)	3 (0.6)	2 (0.6)	Fibrin accumulation	4 (0.4)	0	6 (1.4)

Tissue/Observation	Battelle Placebo (n = 12)	Battelle 20 mg/kg Raxibacumab (n = 7)	Battelle 40 mg/kg Raxibacumab (n = 5)		EPL Placebo (n = 12)	EPL 20 mg/kg Raxibacumab (n = 7)	EPL 40 mg/kg Raxibacumab (n = 5)
Brain				Brain			
Bacteria	11 (1.2)	5 (2.3)	5 (2.0)	Bacteria	9 (1.1)	6 (2.9)	5 (2.2)
Hemorrhage	2 (0.5)	6 (2.6)	4 (2.0)	Hemorrhage	4 (0.5)	7 (2.6)	4 (2.2)
Inflammation	1 (0.3)	6 (2.6)	3 (1.4)	Inflammation	0	1 (0.4)	0
				Inflammation (chronic active)	1 (0.3)	4 (1.7)	2 (1.4)
Necrosis	1 (0.2)	3 (0.7)	2 (0.4)	Necrosis	0	5 (0.7)	0

[1] Severity is measured on a scale of 1 to 4, where 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.

(Adapted from HGS' BLA submission)

Table 17. Histopathologic Analysis Battelle and EPL

***MO comment**: There are no significant differences in histopathologic findings in any tissues other than the brain. Analysis was undertaken to explain the differences in inflammation, and hemorrhage in the brains of raxibacumab treated animals compared to controls. HGS has suggested that the increased*

inflammation seen in the treated animals was secondary to longer time of survival in the treated animals, since the increased inflammation is seen in animals with longer time of survival.

A number of factors were examined, including survival time from the end of challenge, time to development of bacteremia, time between the onset of bacteremia and treatment, and time between the onset of bacteremia and death. Table 17 is an overview of the Battelle data. Tables 18 and 19 present the severity of CNS findings by treatment group.

Table 17. Overview of Battelle Data

	Placebo	**Raxi 20/kg**	**Raxi 40/kg**
Survival Time from the End of Challenge in Hours	N=12 Median=78.8 Mean=103.6±65.2	N=7 Median=93.5 Mean=92.1±23.9	N=5 Median=88.6 Mean=88.8±29.8
Time to Bacteremia from the End of Challenge in Hours	N=12 Median=35 Mean=43.3±27.6	N=7 Median=34.2 Mean=35.2±5.7	N=5 Median=35.6 Mean=37.2±6.5
Time Bacteremic Before Treatment in Hours	N=12 Median=3.8 Mean=-4±22	N=7 Median=4.5 Mean=4.9±2.1	N=5 Median=9.9 Mean=4.5±9.5
Time Bacteremic Prior to Death in Hours	N=12 Median=41.1 Mean=60.3±49.3	N=7 Median=57.4 Mean=56.9±19.8	N=5 Median=53.6 Mean=51.7±25.5

There is a trend toward increased survival time in the treated groups when the medians are compared but these differences are not statistically significant. This increased survival trend is reflected in the time that the animals were bacteremic prior to treatment as well as the time that the animals were bacteremic before death but not in the time to bacteremia following the end of the challenge.

Table 18. Histopathology Results by Treatment Group – Battelle Data

		Placebo	**Raxi 20/kg**	**Raxi 40/kg**
CNS Bacteria	**Category 0/1**	10/12	2/7*	2/5
	Category 2/3/4	2/12	5/7*	3/5
Inflammation	**Category 0**	11/12	1/7*	3/5
	Category 3/4	1/12	6/7*	2/5
Hemorrhage	**Category 0/1**	11/12	1/7*	2/5
	Category 3/4	1/12	6/7*	3/5

Necrosis	***Category 0/1***	*11/12*	*5/7*	*4/5*
	Category 2	*1/12*	*2/7*	*1/5*

**** p < 0.05 compared to placebo***

Table 19. Histopathology Results – Placebo vs. Treatment – Battelle Data

		Placebo	***Treated***
CNS Bacteria	***Category 2/3/4***	*2/12*	*8/12**
Inflammation	***Category 3/4***	*1/12*	*8/12**
Hemorrhage	***Category 3/4***	*1/12*	*9/12**
Necrosis	***Category 2***	*1/12*	*3/12*

**** p < 0.05 compared to placebo***

There are significant differences in the most severe levels of bacteria, inflammation and hemorrhage between the raxibacumab 20 mg/kg animals and the placebo animals. These differences are also seen when all the treated animals are compared with the placebo animals.

Tables 20 and 21 examine the effect of length of survival and time of bacteremia on CNS bacteria and CNS inflammation.

Table 20. CNS Bacteria – Battelle Data

		Placebo	Raxi 20/kg	Raxi 40/kg
Survival Time from the End of Challenge in Hours	No bacteria	N=1 Median=257.7 Mean=257.7	N=2 Median=92.8 Mean=92.8±53.6	N=0
	Bacteria	N=11 Median=77.6 Mean=89.6±45.7	N=5 Median=93.5 Mean=91.8±11.9	N=5 Median=88.6 Mean=88.8±29.8

		Placebo	Raxi 20/kg	Raxi 40/kg
Time to Bacteremia from the End of Challenge in Hours	No bacteria	N=1 Median=128 Mean=128	N=2 Median=38.8 Mean=38.8±11.3	N=0
	Bacteria	N=11 Median=34.8 Mean=35.6±7.4	N=5 Median=34.2 Mean=33.8±2.7	N=5 Median=35.6 Mean=37.2±6.5

		Placebo	Raxi 20/kg	Raxi 40/kg
Time Bacteremic Before Treatment in Hours	No bacteria	N=1 Median=-72 Mean=-72	N=2 Median=4 Mean=4±0.8	N=0
	Bacteria	N=11 Median=4 Mean=2.1±5.5	N=5 Median=4.5 Mean=5.3±2.4	N=5 Median=9.9 Mean=4.5±9.5

		Placebo	Raxi 20/kg	Raxi 40/kg
Time Bacteremic Prior to Death in Hours	No bacteria	N=1 Median=129.7 Mean=129.7	N=2 Median=54 Mean=54±42.2	N=0
	Bacteria	N=11 Median=40.6 Mean=54±46.4	N=5 Median=57.4 Mean=58.1±11.5	N=5 Median=53.6 Mean=51.7±25.5

There do not appear to be differences in the treatment groups with respect to the presence or absence of CNS bacteria. However, this analysis is severely limited by the small number of animals that did not have CNS bacteria.

Table 21. CNS Inflammation – Battelle Data

		Placebo	Raxi 20/kg	Raxi 40/kg
Survival Time from the End of Challenge in Hours	**No inflammation**	N=11 Median=77.6 Mean=103.3±68.4	N=1 Median=55 Mean=55	N=3 Median=65.3 Mean=69.3±17.6
	Inflammation	N=1 Median=107 Mean=107	N=6 Median=95.8 Mean=98.3±19	N=2 Median=118.2 Mean=118.2±7.6

		Placebo	Raxi 20/kg	Raxi 40/kg
Time to Bacteremia from the End of Challenge in Hours	**No inflammation**	N=11 Median=34.8 Mean=44±28.8	N=1 Median=30.8 Mean=30.8	N=3 Median=35 Mean=33.9±2.5
	Inflammation	N=1 Median=35.1 Mean=35.1	N=6 Median=35.2 Mean=36±5.8	N=2 Median=42.1 Mean=42.1±8.8

		Placebo	Raxi 20/kg	Raxi 40/kg
Time Bacteremic Before Treatment in Hours	**No inflammation**	N=11 Median=4 Mean=-4.4±23.1	N=1 Median=4.6 Mean=4.6	N=3 Median=9.9 Mean=8.2±3.2
	Inflammation	N=1 Median=0.05 Mean=0.05	N=6 Median=4.2 Mean=5±2.3	N=2 Median=-1.1 Mean=-1.1±15.5

		Placebo	Raxi 20/kg	Raxi 40/kg
Time Bacteremic Prior to Death in Hours	**No inflammation**	N=11 Median=40.6 Mean=59.3±51.6	N=1 Median=24.2 Mean=24.2	N=3 Median=34.3 Mean=35.4±17.6
	Inflammation	N=1 Median=71.9 Mean=71.9	N=6 Median=62.6 Mean=62.4±14.8	N=2 Median=76.1 Mean=76.1±1.2

There is a trend toward longer time of survival in all treatment groups for animals that had evidence of inflammation. This appears to be due to a longer time of bacteremia prior to death and not to a difference in time to bacteremia or time of

bacteremia prior to treatment. However, within each of the treatment groups, the numbers are small.

Tables 22 through 25 examine the time to survival and time of bacteremia with respect to animals with and without CNS bacteria, with and without CNS inflammation, with and without hemorrhage, and with and without necrosis.

Table 22. Presence of Bacteria – Battelle Data

	Time to Survival	***Time to Bacteremia***	***Time from Bacteremia to Treatment***	***Time from Bacteremia to Death***
No Bacteria	*N=3* *Median=130.7* *Mean=147.8±102.5*	*N=3* *Median=46.8* *Mean=68.5±52.1*	*N=3* *Median=3.4* *Mean=-21.3±43.9*	*N=3* *Median=83.9* *Mean=79.2±52.9*
Bacteria	*N=21* *Median=85.4* *Mean=90±35.3*	*N=21* *Median=35* *Mean=35.5±6.2*	*N=21* *Median=4.3* *Mean=3.4±6*	*N=21* *Median=53.6* *Mean=54.4±35.2*

There are no differences in the times to survival or time of bacteremia in the animals that had CNS bacteria compared to those that did not have CNS bacteria. However, the analysis is limited by a small number of animals that did not have CNS bacteria.

Table 23. Presence of Inflammation – Battelle Data

	Time to Survival	***Time to Bacteremia***	***Time from Bacteremia to Treatment***	***Time from Bacteremia to Death***
No Inflammation	*N=15* *Median=76.5* *Mean=93.3±60.7*	*N=15* *Median=34.8* *Mean=41.1±24.9*	*N=15* *Median=4.3* *Mean=-1.3±20.3*	*N=15* *Median=37.3* *Mean=52.2±45.8*
Inflammation	*N=9* *Median=106.5** *Mean=103.7±17.6*	*N=9* *Median=35.9* *Mean=37.2±6.2*	*N=9* *Median=3.9* *Mean=3.1±6.4*	*N=9* *Median=70** *Mean=66.5±13.3*

**** p < 0.05 compared to no inflammation***

When the means are compared, there is a statistically significant difference in survival time and in time of bacteremia prior to death in animals with inflammation of any level of severity compared to those without inflammation. . However, all the treatment groups are lumped together and the finding of increased inflammation is mainly driven by the fact that this was seen within the raxibacumab treatment groups. It does not explain however, why very little inflammation was seen in placebo or why more was seen in the raxibacumab groups.

Table 24. Presence of Hemorrhage = Battelle Data

	Time to Survival	***Time to Bacteremia***	***Time from Bacteremia to Treatment***	***Time from Bacteremia to Death***
No Hemorrhage	*N=13* *Median=70.5* *Mean=94.2±65.6*	*N=13* *Median=34.8* *Mean=42.4±26.6*	*N=13* *Median=4.2* *Mean=-2.2±21.7*	*N=13* *Median=34.3* *Mean=51.8±49.5*
Hemorrhage	*N=11* *Median=98.1** *Mean=100.7±17.1*	*N=11* *Median=35.1* *Mean=36.5±5.9*	*N=11* *Median=4.4* *Mean=3.3±5.8*	*N=11* *Median=67.7** *Mean=64.2±12.9*

**** p < 0.05 compared to no hemorrhage***

When the means are compared, there is a statistically significant difference in survival time and in time of bacteremia prior to death in animals with hemorrhage of any level of severity compared to those without hemorrhage. . However, all the treatment groups are lumped together and the finding of increased hemorrhage is mainly driven by the fact that this was seen within the raxibacumab treatment groups. It does not explain however, why very little hemorrhage was seen in placebo or why more was seen in the raxibacumab groups.

Table 25. Presence of Necrosis – Battelle Data

	Time to Survival	***Time to Bacteremia***	***Time from Bacteremia to Treatment***	***Time from Bacteremia to Death***
No Necrosis	*N=19* *Median=77.6* *Mean=95.9±54.8*	*N=19* *Median=35* *Mean=41.2±22.1*	*N=19* *Median=4.2* *Mean=-1±18.2*	*N=19* *Median=41.4* *Mean=54.7±41.5*
Necrosis	*N=5* *Median=106.5* *Mean=102±10.6*	*N=5* *Median=35.1* *Mean=33.9±2.7*	*N=5* *Median=4.8* *Mean=5.6±4.1*	*N=5* *Median=70* *Mean=68.1±8.7*

When the means are compared, there are no statistically significant differences in animals with necrosis of any level of severity compared to those without necrosis.

Tables 27 to 30 examine the time of survival and time of bacteremia based on the severity of CNS bacteria, inflammation, hemorrhage, and necrosis.

Table 27. Severity of CNS Bacteria – Battelle Data

Severity Level	Time to Survival	Time to Bacteremia	Time from Bacteremia to Treatment	Time from Bacteremia to Death
0	N=3 Median=130.7 Mean=147.8±102.5	N=3 Median=46.8 Mean=68.5±52.1	N=3 Median=3.4 Mean=-21.3±43.9	N=3 Median=83.9 Mean=79.2±52.9
1	N=11 Median=70.5 Mean=83.7±46.8	N=11 Median=33.2 Mean=34.3±6.6	N=11 Median=4.3 Mean=5±3.6	N=11 Median=37.3 Mean=49.4±47
2	N=2 Median=83.1 Mean=83.1±7.7	N=2 Median=40.7 Mean=40.7±8	N=2 Median=-3.7 Mean=-3.7±11.7	N=2 Median=42.4 Mean=42.4±15.7
3	N=7 Median=98.1 Mean=99.4±16.4	N=7 Median=35.1 Mean=36±5.8	N=7 Median=3.9 Mean=2.1±6.9	N=7 Median=67.7 Mean=63.5±13.1
4	N=1 Median=106.5 Mean=106.5	N=1 Median=36.5 Mean=36.5	N=1 Median=9.5 Mean=9.5	N=1 Median=70 Mean=70

Table 28. Severity of Inflammation – Battelle Data

Severity Level	Time to Survival	Time to Bacteremia	Time from Bacteremia to Treatment	Time from Bacteremia to Death
0	N=15 Median=76.5 Mean=93.3±60.7	N=15 Median=34.8 Mean=41.1±24.9	N=15 Median=4.3 Mean=-1.3±20.3	N=15 Median=37.3 Mean=52.2±45.8
3	N=8 Median=102.3 Mean=102.5±18.5	N=8 Median=35.6 Mean=37.4±6.6	N=8 Median=3.8 Mean=2.2±6.3	N=8 Median=68.9 Mean=65.2±13.5
4	N=1 Median=112.8 Mean=112.8	N=1 Median=35.9 Mean=35.9	N=1 Median=9.9 Mean=9.9	N=1 Median=76.9 Mean=76.9

Table 29. Severity of Hemorrhage – Battelle Data

Severity Level	Time to Survival	Time to Bacteremia	Time from Bacteremia to Treatment	Time from Bacteremia to Death
0	N=13 Median=70.5 Mean=94.2±65.6	N=13 Median=34.8 Mean=42.4±26.6	N=13 Median=4.2 Mean=-2.2±21.7	N=13 Median=34.3 Mean=51.8±49.5
1	N=1 Median=86 Mean=86	N=1 Median=31.2 Mean=31.2	N=1 Median=4.4 Mean=4.4	N=1 Median=54.8 Mean=54.8
3	N=9 Median=98.1 Mean=101±17.9	N=9 Median=35.1 Mean=37.1±6.2	N=9 Median=3.9 Mean=2.5±5.9	N=9 Median=67.7 Mean=63.9±13.2
4	N=1 Median=112.8 Mean=112.8	N=1 Median=35.9 Mean=35.9	N=1 Median=9.9 Mean=9.9	N=1 Median=76.9 Mean=76.9

Table 30. Severity of Necrosis – Battelle Data

Severity Level	Time to Survival	Time to Bacteremia	Time from Bacteremia to Treatment	Time from Bacteremia to Death
0	N=19 Median=77.6 Mean=95.9±54.8	N=19 Median=35 Mean=41.2±22.1	N=19 Median=4.2 Mean=-1±18.2	N=19 Median=41.4 Mean=54.7±41.5
1	N=1 Median=98.1 Mean=98.1	N=1 Median=30.4 Mean=30.4	N=1 Median=4.8 Mean=4.8	N=1 Median=67.7 Mean=67.7
2	N=4 Median=106.8 Mean=102.9±12	N=4 Median=35.5 Mean=34.8±2.1	N=4 Median=6.6 Mean=5.8±4.7	N=4 Median=71 Mean=68.1±10

Comparison of Battelle and EPL Analyses								
Treatment Group	CNS Bacteria		Inflammation		Hemorrhage		Necrosis	
	Battelle	EPL	Battelle	EPL	Battelle	EPL	Battelle	EPL
Placebo	11/12	9/12	1/12	2/12	2/12	4/12	1/12	1/12
Raxi 20/kg	5/7	6/7	6/7	6/7	6/7	7/7	3/7	5/7
Raxi 40/kg	5/5	5/5	3/5	2/5	3/5	4/5	1/5	1/5

EPL Analysis

The EPL findings were essentially similar to the Battelle findings. Of the 24 animals that were examined, there were differences between EPL and Battelle in 17 animals. However, the differences were primarily changes in one level of severity for single observations, ie. from minimal to mild. In looking at the overall severity of involvement for each of the animals (including observations of CNS bacteria, inflammation, hemorrhage, and necrosis), there was no difference between Battelle and EPL with respect to minimal involvement compared to significant CNS involvement. Neither analysis showed differences in the treated groups for presence of CNS bacteria. Both analyses showed significant differences in the treatment groups with respect to inflammation and hemorrhage. When any location of necrosis was considered, the EPL analysis showed a significant difference between the treatment groups with respect to necrosis, whereas the Battelle analysis did not show a difference.

In an effort to present consolidated histopathologic data across multiple studies, the following tables (30 and 31) for the Battelle and EPL data were developed. Low pathology level was determined to be grades 0-2, and high pathology level was determined to be grades 3 and 4.

Table 30. CNS Pathology Battelle

Rx group	Animal ID	Bacteria grade	Inflammation grade	Hemorrhage grade	Necrosis grade	Highest pathology grade	Pathology Level
Placebo	23036	1	0	0	0	1	Low
	23052	0	0	0	0	0	Low
	23404	1	0	0	0	1	Low
	23601	1	0	0	0	1	Low
	23630	1	0	0	0	1	Low
	24056	3	3	3	2	3	High

	24784	1	0	0	0	1	Low
	24793	1	0	0	0	1	Low
	24824	2	0	0	0	2	Low
	24851	1	0	0	0	1	Low
	24853	1	0	0	0	1	Low
	24858	1	0	1	0	1	Low
Raxi 20mg/kg	20255	4	3	3	2	4	High
	21466	0	3	3	0	3	High
	24066	3	3	3	2	3	High
	24755	3	3	3	0	3	High
	24840	3	3	3	1	3	High
	24856	0	0	0	0	0	Low
	24878	3	3	3	0	3	High
	20274					lived	
	21462					lived	
	21469					lived	
	23109					lived	
	24753					lived	
	24783					lived	
	24854					lived	
Raxi **40mg/kg**	23640	1	0	0	0	1	Low
	23655	2	0	3	0	3	High
	23994	3	3	3	0	3	High
	24847	1	0	0	0	1	Low
	24928	3	4	4	2	4	High
	20267					lived	
	21474					lived	
	23023					lived	
	23455					lived	
	24754					lived	
	24790					lived	
	24794					lived	
	24823					lived	

	24841					*lived*	

Table 31. CNS Pathology (EPL)

Rx group	Animal ID	Bacteria grade	Inflammation grade	Hemorrhage grade	Necrosis grade	Highest pathology grade	Pathology Level
Placebo	23036	1	0	0	0	1	Low
	23052	0	0	1	0	1	Low
	23404	2	0	0	0	2	Low
	23601	2	1	0	0	2	Low
	23630	0	0	0	0	0	Low
	24056	3	3	3	1	3	High
	24784	1	0	1	0	1	Low
	24793	1	0	0	0	1	Low
	24824	1	0	0	0	1	Low
	24851	1	0	1	0	1	Low
	24853	0	0	0	0	0	Low
	24858	1	0	0	0	1	Low
Raxi 20mg/kg	20255	4	3	3	1	4	High
	21466	3	2	3	1	3	High
	24066	4	3	3	0	4	High
	24755	3	3	3	0	3	High
	24840	3	3	3	2	3	High
	24856	0	0	1	0	1	Low
	24878	3	3	3	0	3	High
	20274					lived	
	21462					lived	
	21469					lived	
	23109					lived	
	24753					lived	
	24783					lived	
	24854					lived	
Raxi 40mg/kg	23640	1	0	1	0	1	Low
	23655	2	0	3	0	3	High

	23994	3	3	3	0	3	High
	24847	1	0	0	0	1	Low
	24928	4	4	4	1	4	High
	20267					lived	
	21474					lived	
	23023					lived	
	23455					lived	
	24754					lived	
	24790					lived	
	24794					lived	
	24823					lived	
	24841					lived	

No association between severity of CNS bacteria and length of survival or time of bacteremia was seen on either analysis. Severity of inflammation was associated with a longer time of survival in the Battelle analysis and a longer time of bacteremia before death in both Battelle and EPL analyses. There was an association between severity of necrosis and time of survival and time of bacteremia in the EPL analysis. The differences between Battelle and EPL hinge on one or two animals changing groups.

In looking at the presence compared to the absence of CNS bacteria, inflammation, hemorrhage and necrosis, both analyses demonstrated differences in time to survival and time of bacteremia for the presence of hemorrhage. There was no association between length of survival for CNS bacteria in either analysis. The Battelle analysis showed a correlation between length of survival and time of bacteremia for inflammation while the EPL analysis did not. The EPL analysis showed a correlation between length of survival and time of bacteremia with the presence of necrosis, while the Battelle analysis did not. The differences between Battelle and EPL hinge on one or two animals changing groups. Of note, there were only 2 levels of necrosis in the EPL analysis while there were 3 levels in the Battelle analysis.

For all the analyses, the findings, in general, were more pronounced in the raxibacumab 20mg/kg group than in the 40 mg/kg group. The sponsor concluded that the severity of the pathology could be correlated to time of survival. Table 32 lists the medians of survival for all the non-surviving animals by treatment group and based on the Battelle definition of low or high pathology (Table 30). Within the treatment groups, there appears to be a trend to longer survival, however, the numbers of low pathology animals in the raxibacumab treated groups are small, as are the number of high pathology animals in the placebo group.

Table 32. Median Survival Times for All Non-Surviving Animals (Hours)

	CNS Pathology Level*	Placebo (N)	Raxibacumab 20 mg/kg (N)	Raxibacumab 40 mg/kg (N)
Survival Time		103.9 ± 65.1 (12)	92.5 ± 24 (7)	89.1 ± 29.8 (5)
Total Time of Survival	**High**	107.3 (1)	98.6 ± 19.2 (6)	108.6 ± 17.9 (3)
	Low	103.6 ± 68.3 (11)	55.3 (1)	59.9 ± 8.1 (2)
Time of Bacteremia Prior to Treatment	**High**	0.05 (1)	5.0 ± 2.3 (6)	0.8 ± 11.4 (3)
	Low	-4.4 ± 23.1 (11)	4.6 (1)	10 ± 0.1 (2)
Total Time of Bacteremia	**High**	71.9 (1)	62.3 ± 14.8 (6)	68.6 ± 13 (3)
	Low	59.3 ± 51.6 (11)	24.2 (1)	26.4 ± 11.2 (2)
Time from Treatment to Death	**High**	71.9 (1)	57.4 ± 14.6 (6)	67.8 ± 19.1 (3)
	Low	63.7 ± 64.7 (11)	19.6 (1)	16.4 ± 11.2 (2)

** Low = Levels 0-2, High = Levels 3-4*

STUDY 724-G005829 CONCLUSIONS

This was a parallel-group, double-blind, randomized, placebo-controlled GLP study in 40 healthy male and female cynomolgus monkeys to evaluate the therapeutic efficacy of a single IV dose of raxibacumab in anthrax spore inhalation-challenged monkeys experiencing symptoms of inhalation anthrax.

Efficacy conclusions:

- Raxibacumab at both 20 mg/kg and 40mg/kg given by IV bolus dose was superior to placebo in preventing inhalational anthrax induced mortality in bacteremic nonhuman primates (cynomolgus monkeys) (FDA primary efficacy analysis population) with a survival of 41.7% (p=0.0396) in the 20 mg/kg raxibacumab group and 69.2% (p=0.0016) in the 40 mg/kg raxibacumab group.
- In the intention to treat (ITT) efficacy analysis population (as treated challenged nonhuman primates regardless of bacteremia status) used by the sponsor, raxibacumab at 20 mg/kg demonstrated a 50% survival (p=0.0064) and raxibacumab at 40 mg/kg demonstrated a 64.3% survival (p=0.0007).

Safety conclusions:

- Safety data were limited in the study to findings on gross necropsy, histopathology, and serial CBCs.
- Gross brain lesions appeared to be more common in treated (9/12) compared to placebo (3/12) animals (p=0.0391). Two animals did not have

gross evidence of anthrax disease and histopathologic analysis was performed on those animals. Both animals had evidence of bacteria in the brain by histopathology. In these two animals, it was suggested that the disease was more prominent in the placebo treated animal compared to the raxibacumab treated animal.

- Based on the concern for an increase in the number of brain lesions in the treated animals, a complete histopathologic analysis was performed on all tissues from all animals that died. There were no significant differences in treated compared to control animals in the severity of pathologic lesions in any tissues other than the brain. In the brain, there were significant differences between treated and control animals in the incidence and severity of inflammation and hemorrhage in both the Battelle and the EPL analyses (of note, these were more pronounced in the raxibacumab 20 mg/kg group, and there did not seem to be a dose related effect although the numbers are small). While there was some correlation between severity of inflammation and hemorrhage with survival time and time of bacteremia to death in all animals when the groups were condolidated, the overall survival time and time from bacteremia to death based on treatment group would not seem to explain these differences. Based on the available analyses, the pathophysiologic basis for these differences is unclear. In addition, the number of animals analyzed is small and statistical differences hinge on single animals changing assigned groups. While it is reassuring that no gross neurologic findings were noted in the surviving animals (no formal testing was performed), and no brain abnormalities were seen in toxicologic studies, a potential interaction between anthrax and raxibacumab contributing to CNS inflammation and hemorrhage cannot be ruled out. It will be important to examine the histopathology in animals that die in any subsequent studies and it would be reassuring to examine the brains of those animals that survive in order to establish normal brain histology in survivors. If it is not possible to sacrifice survivors, then more formal neurologic assessments including imaging studies should be performed in subsequent studies.

9.8 Individual Study Report

CLINICAL REVIEW OF THE ANIMAL STUDY REPORT 682-G005758

BB-IND: 11069, SN057
Document Type: GI
Supplement Modification: CR

Submitted: 04/30/2008
Reviewed: 01/05/2009

Product: Raxibacumab (Human monoclonal antibody to *B. anthracis* protective antigen)
Sponsor: Human Genome Sciences, Inc.
PI/Study Location: Battelle Biomedical Research Center (BBRC)
State Route 142
West Jefferson, OH 43162
Histopathology Lab: Battelle Biomedical Research Center (BBRC)
505 King Ave
Columbus, OH 43201
Independent Pathology Lab (IPRP): Experimental Pathology Laboratories, Inc.
Herndon, VA
Reference Lab: (PCR for *B. anthracis,* PA ELISA) Human Genome Sciences, Inc.
14200 Shady Grove Rd
Rockville, MD 20850

Submission content: Complete Study Report of Protocol 682-G005758 Evaluation of Raxibacumab Efficacy as Therapeutic Treatment Against Inhalation Anthrax in the Rabbit Model

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Nonclinical Study Report 682-G005758: Evaluation of Raxibacumab Efficacy as Therapeutic Treatment Against Inhalation Anthrax in the Rabbit Model

INVESTIGATOR: Jason Mott, DVM, Ph.D.

STUDY CENTER: Battelle Biomedical Research Center (BBRC)

STUDY PERIOD: 26-February-2007 to 26-March-2007

OBJECTIVES:
Primary objective:

- To evaluate of Raxibacumab Efficacy as Therapeutic Treatment Against Inhalation Anthrax induced lethality in the Rabbit Model.

Secondary objectives:

- To evaluate raxibacumab PK following a single IV raxibacumab dose
- To evaluate the kinetics of *B. anthracis* in rabbits with inhalational anthrax

METHODOLOGY: Single-blinded, single center, randomized, placebo controlled dose-ranging study

NUMBER OF ANIMALS: Three cohorts of 18 animals each. Total enrollment of 54 New Zealand White rabbits

STUDY DRUG: IV raxibacumab given as a single bolus infusion at either 20 or 40 mg/kg dose.
Raxibacumab, also known as HGS1021, PA mAb, or ABthrax, is a recombinant, fully human $IgG_1\lambda$ mAb that binds *B. anthracis* PA with high affinity and inhibits its biological activity. Raxibacumab is expressed in the NS0 mouse myeloma cell line and secreted into culture media, from which it is purified by a series of chromatography and filtration steps.

The Appearance, Formulation, and Dosage Strength of the Study Drug used in the Study 682-G005758

Study Drugs	Appearance	Formulation	Dose Unit	Batch numbers
Raxibacumab	Clear liquid	Raxibacumab sterile solution for injection	50mg/ml	71044
Placebo (raxibacumab formulation buffer)	Clear liquid	n/a	n/a	71043

MO comment: *The test article was raxibacumab (Lot #71044). Vials were labeled as X, Y, and Z. There is no reference to M11 in the study report. A confirmation of M11 manufacturing process used for raxibacumab product in this study was received form the Sponsor in submission SN072 to the IND.*

DURATION OF TREATMENT: Raxibacumab given as a single IV bolus at either 20 or 40mg/kg dose to NZW rabbits at the time when inhalational anthrax disease was diagnosed

TREATMENT COMPLIANCE: Study drug infusion information, including dosage and volume prescribed, and volume administered, was recorded.

STUDY DESIGN: This study was designed as a single-blinded, single center, randomized, placebo controlled dose-ranging study of raxibacumab given as a

single IV bolus at either 20 or 40mg/kg dose to NZW rabbits at the time when inhalational anthrax disease was diagnosed.

Background: To date the Sponsor has conducted evaluation of raxibacumab in several animal species and under various conditions. Efficacy of raxibacumab was established in a proof-of concept rat model of Lethal Toxin infusion in pre-exposure and post-exposure setting. In addition, when used for pre-exposure prophylaxis raxibacumab showed dose-dependent survival benefit in rabbits and monkeys. Time-related effect on survival was also observed in post-exposure prophylaxis setting in a rabbit model.

PROOF OF CONCEPT (RODENTS)

PRE EXPOSURE PROPHYLAXIS
Rat Toxin Model[a]

- 100% survival when administered IV w/ LT or 3wks to 24h pre LT infusion
- 80% survival when administered 4wks before LT infusion
- Prolonged time to death when administered IV 8 wks pre LT infusion

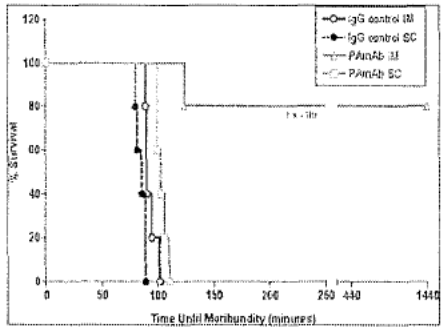


Figure 1 Effect of PA mAb on survival of Fisher 344 rats challenged with lethal toxin, SC or IM administration of antibody at t = -60 minutes

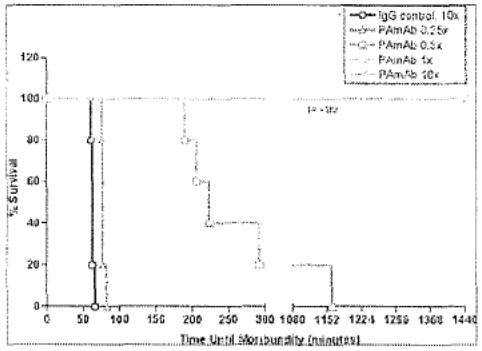


Figure 2 Effect of PA mAb on survival of Fisher 344 rats challenged with lethal toxin, administration of antibody at t = -60 minutes

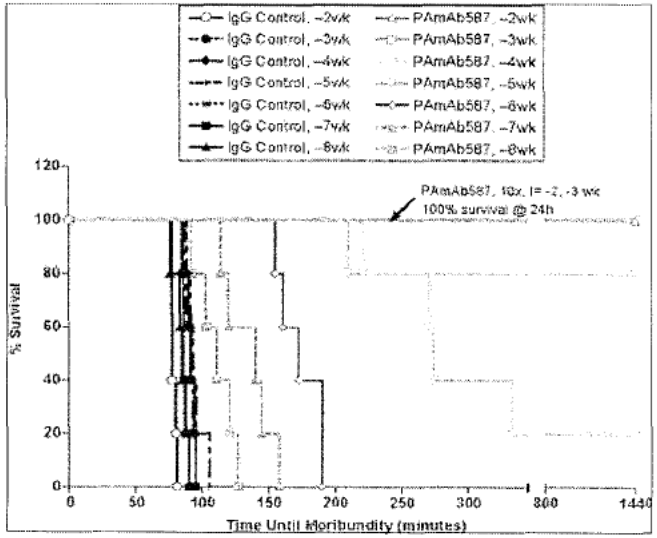
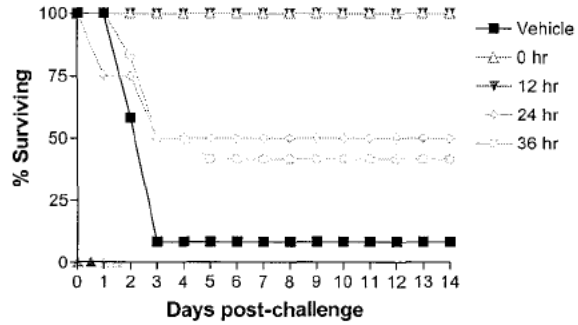


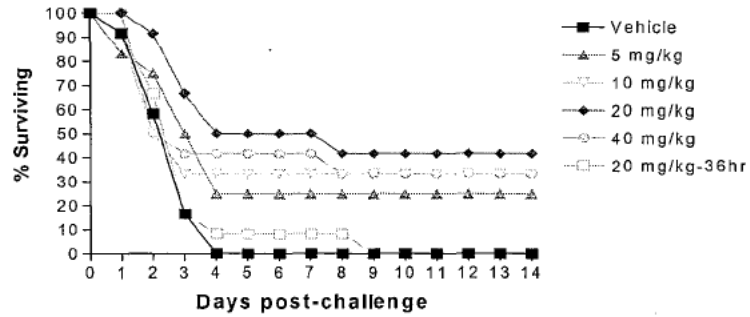
Figure 4 Effect of PA mAb on survival of Fisher 344 rats challenged with lethal toxin administration of antibody at t = -2 to -8 weeks

POST EXPOSURE PROPHYLAXIS
Rat Toxin Model[b] (placebo survival 60%,

Dose –response survival (100% survival 6-12h post toxin)

median time to death 16h)	

SMALL ANIMALMODELS	
PRE EXPOSURE PROPHYLAXIS (48h PRE-exposure) **Rabbit Anthrax Inhalational Model**[c]	• Dose response in survival, time to death, extent of disease (histopath • Superior to placebo (12.5% to 100%) in survival and clearance of bacteremia • Survival correlated with PK parameters (Cmax and AUC)
POST EXPOSURE PROPHYLAXIS (1h POST-exposure) **Rabbit Anthrax Inhalational Model**[c]	• Superior to placebo (100%) in survival. • Superior to placebo in clearance of bacteremia
POST EXPOSURE PROPHYLAXIS **Rabbit Anthrax Inhalational Model**[d] n=60 (358N005999) 40 mg/kg at 12, 24, 36 h post exposure (8% placebo survival rate, median survival time 3 days)	• Time dependent response (42-100%) in time to death & survival to 28 days • Superiority to placebo in survival. • Survivors cleared bacteremia; similar trend for non-survivors  Rabbits received a single dose at 0, 12, 24 or 36 hours post -spore challenge

TREATMENT (at expected bacteremic phase) **Rabbit Anthrax Inhalational Model**[e] n=72, (374N006101) Treatment started 24h post exposure for 5, 10, 20, 40 mg/kg w/ add'l 36 h post exposure for 20 mg/kg (0% survival in placebo, median survival time 3 days)	• Dose response in endpoints of survival and extent of disease (histopath) • Superior to placebo in survival, clearance of bacteremia. • Time to death better than placebo at 24 but not 36 h intervention • Survival correlated with PK parameters (Cmax and AUC)  PA mAb was administered 24 or 36 hrs post challenge to 12 rabbits/group

NON HUMAN PRIMATE MODEL	
PRE EXPOSURE PROPHYLAXIS (48h pre exposure) **Inhalational anthrax in macaques**[f] (290N005433) 0, 10, 20, and 40mg/kg of Raxibacumab SC 48h pre-exposure n=40 Phase 1 -placebo survival was 0%, median survival was 4 days, 9/10 bacteremic at death phase 2 - placebo survival 0%, all bacteremic	• Dose response in survival rates (60-90%) and time to death • All doses superior to placebo in survival. at28 days & clearance of bacteremia • NO dose response in extent of histopath lesions • Survival correlated with PK parameters (Cmax and AUC) Phase 2 - 1 year after initial treatment, all 21 mAb survivors survive reinfection-

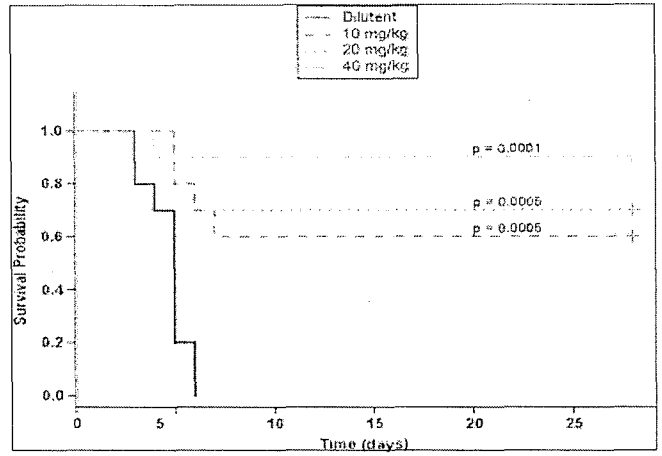


Figure 6 Survival curves for pre-exposure proplylaxis in monkeys

At Regulatory Briefing on the applicability of the Animal Rule to the licensing of anthrax anti-toxin monoclonal antibodies held at CDER on October 10, 2006, a consensus was reached that the efficacy of the monoclonal antibody against anthrax toxin should be demonstrated in 2 animal species (rabbit and NHP). Both models must show a significant benefit of the monoclonal over placebo. In addition, both models should explore the magnitude of benefit of an antitoxin therapeutic when combined with an antimicrobial over antimicrobial alone.

This submission addresses the evaluation of raxibacumab efficacy over placebo in one of two proposed recommended animal models – rabbit.

The Sponsor provided the results inhalational anthrax natural history study in rabbit. The Sponsor claims the natural history of inhalational anthrax disease in the characterization studies in NZW rabbits conducted thus far demonstrated reproducible clear and predictable clinico-laboratory profile (body temperature, clinical hematology, PA toxemia, and bacteremia) that correlates with mortality (see following Table). In addition, it is clamed that correlation of PA toxemia and temperature elevation of ≥2˚F to onset of bacteremia has been established in these studies.

MO comment: *The Division has not yet reviewed the data from the natural history characterization study, but agrees that the completed efficacy study (the subject of this review), proved the above claimed correlations are true, could support the conclusions derived regarding raxibacumab efficacy in established inhalational anthrax.*

Table 7 Correlation between survival time and other time to event parameters (N = 8)

	Spearman Correlation Coefficients (P Values)			
	Time to 1st Serum PA Detected	Time to 1st Bacteremia (Culture)	Time to 1st Bacteremia (PCR)	Time to 2°F Temperature Increase
Survival time	0.86446 (0.0056)	0.80995 (0.0148)	0.92729 (0.0009)	0.77846 (0.0229)
Time to 1st PA detected	-	0.94341 (0.0004)	0.96290 (0.0001)	0.96978 (< .0001)
Time to 1st bacteremia (culture)	-	-	0.96304 (0.0001)	0.85391 (0.0070)
Time to 1st bacteremia (PCR)	-	-	-	0.87958 (0.0040)

Design:
This is a randomized, open-label, parallel-group, placebo controlled study.
Inclusion criteria:

- Disease free New Zealand White (NZW) rabbits 50% male and 50% female weighing 3-4kg supplied by [redacted] (b) (4)
- Implanted femoral catheters and vascular access ports
- Quarantine period of 7 days
- 2 temperature transponder chips implanted prior to study

Exclusion criteria:

- Malformations
- Clinical signs and symptoms of disease

Treatments and Study Groups:

Table 1 Treatment Group Designation

		Raxibacumab Treatment		
Group	**# of Animals**	**Dose (mg/kg)**	**Dose Schedule and Route**	**Treatment Point Post-Challenge**
1 (Control Group)	18	Raxibacumab buffer [a]	Single IV	Treated as cohort based on onset of 1st symptoms in Groups 2 or 3 or upon 1st death in Group 1
2	18	20	Single IV	Individual times will be based on serum PA levels or body temperature [b]
3	18	40	Single IV	Individual times will be based on serum PA levels or body temperature [b]

a Dose equivalent in ml/kg volume to Group 3.
b Each individual rabbit in groups 2 and 3 will receive raxibacumab treatment immediately upon either its first positive PA result, or its first two consecutive time points of a body temperature 2 or more degrees F higher than the baseline average (whichever occurs first).

Study procedures: Study was conducted under GLP.

- Animals were housed in the individual cages.

- Study animals were randomized by dose group and gender prior to challenge in 2 sets of 27.
- Aerosol challenge at targeted 200 x LD50 of *B. anthracis* Ames strain spores will occur on Day0).
- Temperature will be measured Q1h from 12h to 72h post-challenge.
- Single dose IV study treatment will be administered as per Table 1.

MO comment: *The treatment was administered as a bolus injection. In human studies raxibacumab is to be administered by 2.5 hour infusion. As we are trying to make a leap from animal to human efficacy, it is imperative to ensure that it will be possible to match animal and human PK parameters, i.e. Cmax and the duration of infusion may significantly impact the pharmacokinetics of the product.*

- Clinical observations were collected bid after day 7

MO comment: *Available information (sponsor's summary of the natural history of inhalational anthrax characterization study in rabbits, scientific literature on the subject, and presentations from veterinary laboratories conducting these studies) on the inhalational anthrax model in rabbits points to the very rapid progress of anthrax disease in rabbits (3-4 day survival post-challenge). Twice daily observations of the animals appear to be insufficient to determine the time of the animal's death if euthanasia was not performed. This will have a significant impact on time to death analyses in view of the fact that even 2-3hours of additional survival time recorded might be crucial.*

- Animals were euthanized if found moribund on D14
- Necropsy and histopathology were conducted on all euthanized and all dead animals

MO comment: *It is important to confirm end-organ bacillary damage. Quantitative post mortem cultures of relevant organs were recommended to be provided. However, the Sponsor resorted only to identify bacteria in dead animals only on microscopic examination.*

The FDA also asked for independent pathology review of the slides. The independent evaluation of the pathology slides was completed by Experimental Pathology Laboratories, Inc. (EPL). The draft report of their results was submitted as serial 0093 on December 23, 2008. At EPL, all the slides were read in a blinded manner and then peer reviewed.

Blood collection and assay schedule

Time Point post-challenge	Bacteremia (Culture)	Bacteremia (PCR – ship DNA to HGS)	Serum PA level	CBC/CRP	PK (ship serum to HGS)
Study Day -3				X	
Study Day 0 (Pre-challenge)	X	X	X	X	X
12 hours followed by Q4 hours up to Rx	X	X	X	X	
IV treatment time (pre)	X	X		X	
5 minutes after IV Rx			X		X
4 hours after IV treatment	X		X	X	X

10h after IV treatment	X		X	X	X
24h after IV treatment	X		X	X	X
36h after IV treatment	X		X	X	X
48h after IV treatment	X		X		
72h after IV treatment	X		X		X
144h after IV treatment	X		X		X
216h after IV treatment	X		X	X	X
Terminal	X		X	CRP only	X

*Post-exposure bleed time points are calculated from the time each animal's aerosol challenge exposure ended. All blood samples will occur within 1 hour of the stated time point, except for the first two post-treatment blood samples which will occur within 5 and 10 minutes of the stated time, respectively. Exact blood draw times will be documented.

Raxibacumab PK

PK analyses of raxibacumab concentration-time profiles for all raxibacumab-dosed rabbits were conducted using population analysis techniques with NONMEM software. Serum raxibacumab concentration-time data, serum PA concentration-time data, individual raxibacumab PK parameters, and individual PA kinetic parameters were summarized using descriptive statistics.

MO comment: *Please refer to the Clinical Pharmacology Review for PK analysis.*

Endpoints:

Primary efficacy endpoint was survival in the ITT study population on Day 14

Analysis Populations:

- ITT population is defined as all randomized rabbits that received the study agent
- mITT population is defined as a subset of the ITT population that received raxibacumab or placebo (based on treatment group assigned, not actual treatment received
- As randomized population defined as all rabbits that were randomized regardless of whether or not they were spore-challenged
- As treated population defined as the set of rabbits that received the study agent with the assignment to treatment group that was based on the actual treatment administered to the rabbits

MO comment: *Because this pivotal study report will also be submitted for licensing application (BLA) for the treatment of inhalational anthrax in humans under the Animal Rule, it is important to ensure that the study population is sufficiently reflective of the human population with anthrax disease. Therefore, the Per Protocol population (all animals that were confirmed to have anthrax disease as evident by the presence of bacteremia at the time of treatment initiation) will be the study population the reviewer will be focusing efficacy assessments on. The outcomes in the ITT and mITT should trend in the same direction as the Per Protocol analysis.*

Statistical Plan:

The sample size of 18 animals per study group provides 80% power at overall significance level of 0.05 to detect an absolute difference of 45% or more in 14 day survival in one of the raxibacumab treatment groups (20 or 40 mg/kg). Placebo survival is assumed to be 0 with 5% deviation. Raxibacumab survival is assumed to be 50%

The primary efficacy analysis will be performed using 2-sided likelihood ratio test or Fisher's exact test. Adjustment is made for multiple comparisons

MO comment: *Please refer to the Statistical review by Zhou, Hongling, Ph. D.*

PROTOCOL CHANGES:

Original protocol was submitted and reviewed by the Division on February 23, 2007. Subsequently, protocol was amended 8 times.

Amendment 1 (28 February 2007)

• Corrections were made to typographical errors and misidentified SOP numbers.

Amendment 2 (02 March 2007)

• At the request of FDA rabbits in Placebo group are to be treated based on the same individual criteria as for both active treatment groups (either its 1st detectable serum PA result or its first 2 consecutive timepoints of a body temperature ≥2 °F than the baseline average temperature (whichever comes first) after inhalation challenge.

MO comment: *Sponsor's original design called for the placebo animals to be treated as a cohort when the first placebo animal was found to have anthrax disease.*

• At the request of FDA clinical observation frequency was increased during the period of expected death

• At the request of FDA MIC determination for levofloxacin and ciprofloxacin was added to micro evaluation of *B. anthracis* challenge material

• Storage samples for PCR bacteremia was modified

• An additional PK sample was to be collected at 48h post IV treatment

• Increase in blood sample volumes from 1.0ml to 1.4ml except for the screening sample

• Wording clarifying the difference between screening and quantitative PA ECL assay was added.

Amendment 3 (08 March 2007)

• One animal in the 20mg/kg raxibacumab group died prior to challenge and was substituted with an unchallenged animal from the placebo group. Statistical analysis plan was adjusted to reflect a change in the number of placebo animals: 17 instead of 18. The smaller number rabbits resulted in a slight decrease in statistical power (76% vs 80%) to detect improvement in treated rabbits.

Amendment 4 (26 March 2007)

• For consistency with analytical plan, the primary efficacy analysis was to be performed on an intention-to-treat population, defined as all randomized rabbits that receive spore challenge.

• Modification of the description of terminal blood sample collection to maximize the use of the terminal blood samples.

Amendment 5 (17 October 2007)

• Reinstated inadvertent change in the clinical observation past day 6 resulted from Amendment 4.

Amendment 6 (14 December 2007)

• Description of rabbit exclusion due to non-specific PA assay interference at pre-screening

Amendment 7 (4 January 2008)

•Clarifications that the primary efficacy analysis was to be performed on an intention-to-treat population not mITT

Amendment 8 (26 February 2008)

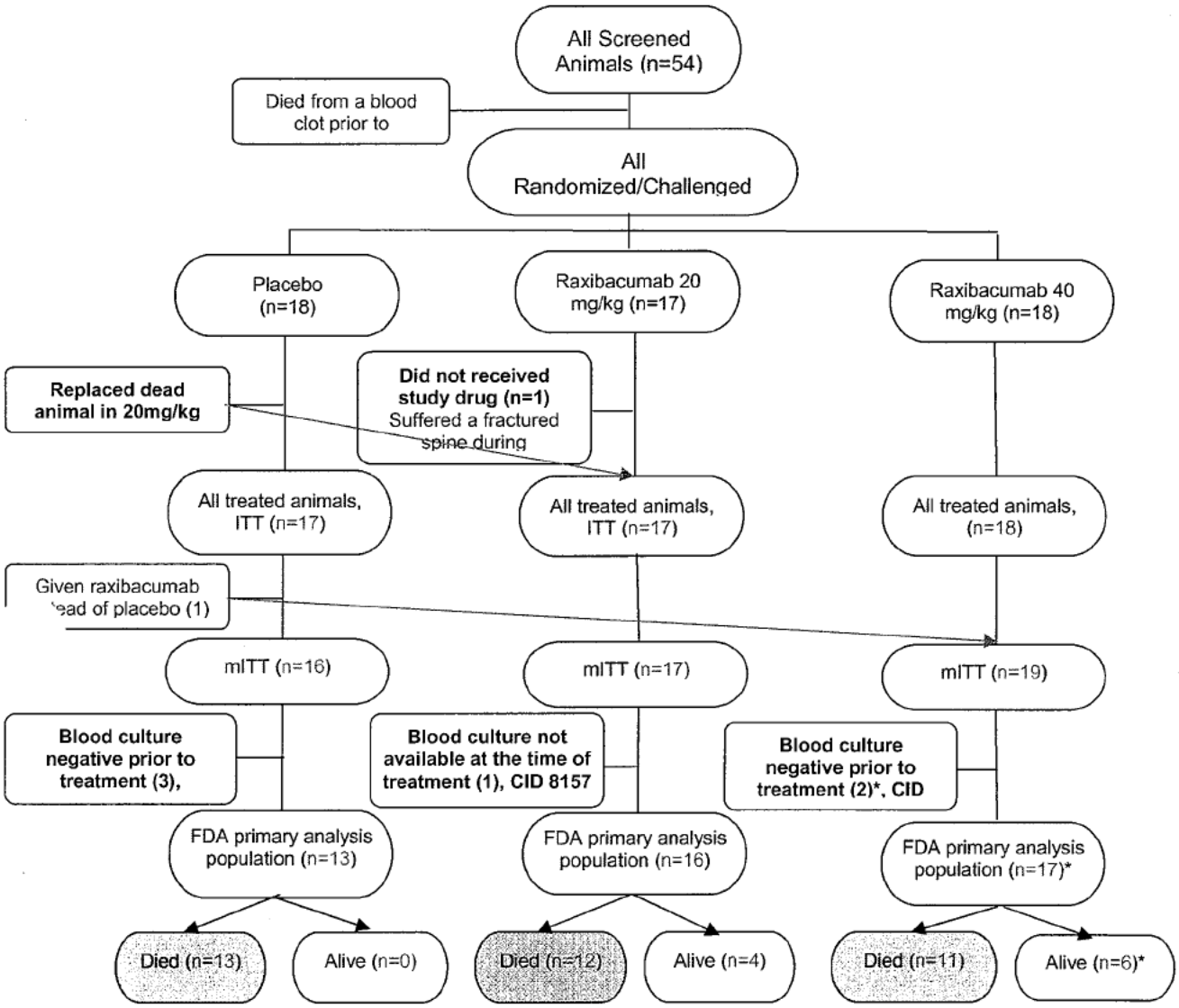
•Reinstated inadvertent omission of the secondary analysis of survival time

RESULTS

Animal Disposition
Study centers: 1 study center, (b) (4)

Total animals screened: 54 (53 received study treatment).
The following flowchart and the tables summarize the composition of the applicant's and reviewer's populations for analysis, the primary reasons for exclusion and the demographics of the study population.

Animal's Disposition



- ***Animal L08147 was bacteremic by culture 1.4hours prior to treatment with raxibacumab 40mg/kg; however, sample drawn immediately prior to raxibacumab administration did not grow bacteria. Blood cultures remained negative for the remainder of the study period. Animal was febrile for 5 hours prior to treatment and remained febrile for additional 17 hours after treatment. Animal's PA levels by ELISA at the time of treatment were at lower limit of detection. Changes on CBC compared to baseline normal values noted 1.4 hours prior to treatment were neutrophilia, lymphopenia, and anemia. These abnormalities resolved within 24 hours posttreatment. This animal was considered to be bacteremic prior to treatment administration by clinical and microbiology reviewers.***

- ***All 3 placebo animals negative for bacteremia by culture were febrile and positive for PA by quantitative ECL. Bacteremia in these animals was detected after placebo treatment administration/necropsy.***

> ***MO comment**: The reviewer elects to focus her examination of efficacy on animals that were bacteremic prior to treatment administration (the FDA primary analysis population). Sensitivity analyses will include ITT, mITT. For the evaluation of the qualitative and quantitative assays for PA toxemia determination employed in this study please refer to Microbiology Review.*

Demographic and Baseline Characteristics

Summary of demographics and baseline characteristics (in the ITT population)

	Placebo N = 17	20 mg/kg N = 18	40 mg/kg N = 18	All Active N = 36	All Groups N = 53	P value[1]
Sex						0.9842
Male	9 (52.9%)	10 (55.6%)	10 (55.6%)	20 (55.6%)	29 (54.7%)	
Female	8 (47.1%)	8 (44.4%)	8 (44.4%)	16 (44.4%)	24 (45.3%)	
Weight(kg)						0.8733
Mean ± SD	3.0 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	
Median	3.0	3.1	3.1	3.1	3.1	
Range	(2.8, 3.3)	(2.8, 3.4)	(2.7, 3.3)	(2.7, 3.4)	(2.7, 3.4)	
Age at randomization (Days)						
Mean ± SD	205.0 ± 0.0	205.0 ± 0.0	205.0 ± 0.0	205.0 ± 0.0	205.0 ± 0.0	
Median	205.0	205.0	205.0	205.0	205.0	
Range	(205.0, 205.0)	(205.0, 205.0)	(205.0, 205.0)	(205.0, 205.0)	(205.0, 205.0)	

1. P value based on likelihood ratio test for categorical variables and 1-way ANOVA, for continuous variables for comparison among all treatment groups.

> ***MO comment**: It appears that all study animals had the same age. As reported in the demographics dataset their birth date was August 5, 2006. The Sponsor confirmed that individual animals' age in days was the same for all animals in this study.*

Extent of Anthrax Exposure

Anthrax Exposure Level by Challenge Date

Inhalational Exposure to	03/05/07 N = 27	03/12/07 N = 26 (25)	P-value

anthrax spores			
Mean ± SD (xLD_{50})	248.6 ± 38.6	206.0 ± 32.7	< 0.0001
Median	250	206	
Range	174.0-352.0	144.0-296.0	

MO comment: *The challenge spore dose was significantly higher on 03/05/07 as compared to 03/12; however, all three study arms were equally represented on both challenge days to minimize intergroup variability.*

Summary of the extent of anthrax exposure (mITT)

Inhalational Exposure to anthrax spores	**Placebo N = 16**	**20 mg/kg N = 17**	**40 mg/kg N = 19**	**All Active N = 36**	**All Groups N = 52**	**P value[1]**
Mean ± SD (xLD_{50})	221 ± 32	230 ± 37	233 ± 53	231.3 ± 45.3	228.1 ± 41.5	0.7134
Median	220.0	223.0	230.	226.5	222.5	
Range	(174.0, 298.0)	(175.0, 299.0)	(144.0, 352.0)	(144.0, 352.0)	(144.0, 352.0)	

MO comment: *Mean actual exposures were consistent with the targeted exposure of approximately 200 x LD50. There was no significant difference in the inhaled median and mean doses among the 3 treatment groups (p = 0.7). However, animals in 40mg/kg dose group had a wider range of anthrax exposures as compared to placebo and 20mg/kg groups. Although the lowest exposure in this study was in the 40mg/kg group, it was well above x100 LD50.*

Animals challenged on 3/12/07 received a lower mean dose of anthrax spores than animals challenged on 3/5/07. Animals in each treatment group challenged on 3/12/07 also had lower mean dose of anthrax exposure than the corresponding treatment group challenged on 3/5/07. But the survival rates for each treatment group challenged on 3/12/07 were higher than the survival rates for the corresponding treatment group challenged on 3/5/07. The increased level of anthrax exposure does not seem to have a negative impact on the survival rates.

Survival Rate (%) and Mean Exposure level (±SD) by Challenge Date

Challenge Date /mean exposure	**Placebo**	**Raxibacumab 20 mg/kg**	**Raxibacumab 40 mg/kg**
3/5/07	0/9 (0)	3/9 (33.3)	5/9 (55.6)
248.6 (±38.6)	223.6 (±25.7)	256.7 (±25.7)	265.6 (±49.2)

3/12/07	0/8 (0)	2/9 (22.2)	3/9 (33.3)
206.0 (±32.7)	219.3 (±37.3)	199.1 (±18.9)	200.4 (±37.7)

Prepared by Hongling Zhou, statistical Reviewer

Therapeutic Trigger Symptoms and Timing of Treatment Initiation

The table below outlines trigger event (detectable serum PA or increased body temperature) along with time to treatment intervention among treatment groups and summarizes the occurrence of post challenge bacteremia, toxemia, and temperature increase.

Summary of signs and symptoms in the ITT population (Sponsor's)

	Placebo N = 17	20 mg/kg N = 18	40 mg/kg N = 18	All Groups N = 53	P value[1]
Time to treatment[2] (hour)	-	-	-	-	0.2641
Mean ± SD	26.4 ± 4.4	28.2 ± 4.7	26.3 ± 3.9	26.9 ± 4.3	-
Median	27.2	28.1	24.8	25.6	-
Range	19.5-34.9	20.9-35.9	21.3-35.7	19.5-35.9	-
Trigger event	-	-	-	-	0.6625
Detectable PA	12 (70.6%)	10 (58.8%)	13 (72.2%)	35 (67.3%)	-
Significant temperature increase[3]	5 (29.4%)	7 (41.2%)	5 (27.8%)	17 (32.7%)	-
Toxemic[4,5] at or before treatment initiation	16 (94.1%)	17 (94.4%)	16 (88.9%)	49 (92.5%)	1.0000
Toxemic at treatment initiation	15 (88.2%)	16 (88.9%)	16 (88.9%)	47 (88.7%)	1.0000
Toxemic at anytime	17 (100.0%)	17 (94.4%)	17 (94.4%)	51 (96.2%)	1.0000
Bacteremic[4,6] at or before treatment initiation	14 (82.4%)	16 (88.9%)	16 (88.9%)	46 (86.8%)	0.7797
Bacteremic at treatment initiation	14 (82.4%)	16 (88.9%)	15 (83.3%)	45 (84.9%)	0.8978
Bacteremic at any time	17 (100.0%)	16 (88.9%)	17 (94.4%)	50 (94.3%)	0.7649
Toxemic and bacteremic at or before treatment initiation	14 (82.4%)	16 (88.9%)	15 (83.3%)	45 (84.9%)	0.8978
Toxemic and bacteremic at treatment initiation	13 (76.5%)	15 (83.3%)	15 (83.3%)	43 (81.1%)	0.8258
Toxemic and bacteremic at any time	17 (100.0%)	16 (88.9%)	16 (88.9%)	49 (92.5%)	0.5298
Toxemic and/or bacteremic at or before treatment initiation	16 (94.1%)	17 (94.4%)	17 (94.4%)	50 (94.3%)	1.0000
Toxemic and/or bacteremic at treatment initiation	16 (94.1%)	17 (94.4%)	16 (88.9%)	49 (92.5%)	1.0000
Toxemic and/or bacteremic at any time	17 (100.0%)	17 (94.4%)	18 (100.0%)	52 (98.1%)	1.0000
Temperature increased at or before treatment initiation	11 (64.7%)	13 (72.2%)	10 (55.6%)	34 (64.2%)	0.5786
Temperature increased but with no PA toxemia or bacteremia at or before treatment initiation	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-

1. P values are based on the Kruskal-Wallis test, likelihood ratio test or Fisher's exact test for comparison among all treatment groups.
2. Time to treatment was calculated relative to the start of challenge.
3. First 2 consecutive time points of a body temperature ≥ 2°F higher than the baseline average temperature.
4. Throughout this table, only post-challenge bacteremia and toxemia were counted.
5. Throughout this table, toxemia was determined by quantitative ECL assay.
6. Throughout this table, bacteremia was determined by culture.

***MO comment**: The reviewer based her analyses below on the as treated population of animals: 16 in the placebo arm, 17 in the raxibacumab 20mg/kg, and 19 in the raxibacumab 40mg/kg arms. 55 to 76.4% of rabbits were febrile at or before the time of treatment initiation. The highest number of febrile rabbits (13/17, 76.4%) was observed in the 20mg/kg study group and the lowest (10/18, 55%) was in the 40mg/kg study group. 29/34(85.3%) of febrile animals (3 groups) were simultaneously toxemic and bacteremic before treatment initiation.*

Toxemia was observed in approximately >87% of all challenged rabbits at or before treatment initiation in each of the study groups. 43/46(93.5%) toxemic animals were bacteremic prior to treatment.

Bacteremia prior to treatment was documented by culture in greater than 89% of rabbits in both active treatment groups 20 and 40mg/kg and in 87.5% of placebo animals. 43/45 (95.6%) of bacteremic animals were also toxemic prior to treatment. Only positivity for

PA by ECL was not a good predictor of bacteremia. Only 32/45 (71.1%) of bacteremic animals were positive by ECL screen assay. And 30/45(66.7%) of bacteremic animals were febrile prior to treatment. However, PA screen in combination with temperature elevation was able to capture all animals bacteremic prior to treatment 45/45.
Median time to treatment administration as determined by the trigger (PA toxemia (ECL and or temperature rise) was longest for the 20mg/kg treatment group (28.1 hours) and shortest for the 40mg/kg (24.3hours). As the course of inhalational anthrax in rabbits is quite short; every hour from the time of diagnosis to treatment administration is critical.

Time from diagnosis (PA ECL) to treatment

Time from diagnosis (PA ECL) to treatment (h)	**Placebo N=16**	**Raxibacumab 20mg/kg N=17**	**Raxibacumab 40mg/kg N=19**
All treated animals			
Mean ±SD	5.8±4.2	5.1±1.6	4.7±1.8
Median	4.1	4.7	4.4
Range	2.6-18.9	3.3-9.6	1.5-9.6
All animals toxemic at treatment administration	**N=14/16 (87.5%)**	**N=16/17 (94.1%)**	**N=17/19 (89.5%)**
Mean ±SD	5.9±4.5	5.2±1.5	4.8±1.7
Median	4.1	4.8	4.4
Range	2.6-18.9	3.8-9.6	1.6-9.6

***MO comment**: Greater than 87% of all treated animals were positive for PA toxemia. On average, animals in 20mg/kg treatment group had greater delay in treatment administration as compared to 40mg/kg and placebo groups. In order to evaluate treatment delay in study cohorts relative to other evidence of anthrax disease, the Reviewer will examine time from blood culture positivity to treatment administration and the differential between the outcomes for the animals who received intervention within 4 hours of diagnosis versus those who were treated later within the treatment groups*

Time from diagnosis (Blood Culture) to treatment

Time from diagnosis (Bl Cx) to treatment (h)	**Placebo**	**Raxibacumab 20mg/kg**	**Raxibacumab 40mg/kg**
All treated animals	**N=16**	**N=17**	**N=19**
Mean ±SD	-8.1±22.8	-9.1±53.5	-9.7±50.0
Median	0.25	3.4	1.2
Range	-71.9 - 7	-216 - 15.9	-216-5.5
All bacteremic animals at treatment administration	**N=13/16 (87.5%)**	**N=16/17 (94.1%)**	**N=17/19 (89.5%)**
Mean ±SD	2±2.2	3.8±4.2	2.1±2.0
Median	0.4	3.65	1.4

Range	0.1 - 7	0-15.9	0.1-5.5

***MO comment**: A greater number of animals on active treatment arms was bacteremic at treatment administration. Animals in the 20mg/kg raxibacumab cohort appear to be bacteremic for a longer period of time (mean and median) prior to treatment administration (mean 3.8 versus 2.1 hours and median 3.65 versus 1.2 hours) as compared to 40mg/kg raxibacumab cohort.*
Among bacteremic animals that survived (n=9) median time to treatment administration from the time of positive blood culture was 1.4 versus 3.4 hours among who died (n=23); mean of 2.6 versus 3.1 hours. There were no survivors among animals that were bacteremic for >7.4 hours prior to treatment.
Survival outcomes in the animals where treatment was administered before or after arbitrary cut-off of 4 hours after the first positive blood culture were as follows:
Raxibacumab 20mg/kg
All treated animals <4h 3/10 (30%) and >4h 2/7(28.6%)
Animals bacteremic prior to treatment <4h 2/9 (30%) and >4h 2/7(28.6%)
Raxibacumab 40mg/kg
All treated animals <4h 7/16 (43.8%) and >4h 1/3(33.3%)
Animals bacteremic prior to treatment <4h 5/14(35.7%) and >4h 1/3(33.3%)
All raxibacumab treated animals
All treated animals <4h 10/26 (38.5%) and >4h 3/10(30%)
Animals bacteremic prior to treatment <4h 7/23(30.4%) and >4h 3/10(30%)

The numbers of survivors in the subgroups evaluated are small; however, it does not appear that treatment administration of >4h post first positive blood culture negatively affected survival in this animal model.

Time to treatment from the time of challenge

Time to treatment (h)	**Placebo**	**Raxibacumab 20mg/kg**	**Raxibacumab 40mg/kg**
All treated animals	**N=16**	**N=17**	**N=19**
Mean ± SD	26.0 (± 4.4)	28.2 (± 4.7)	26.6 (± 3.9)
Median	25.8	28.1	24.9
Range	19.5 – 34.9	20.9 – 35.9	21.3 – 35.7

***MO comment**: The above findings are consistent with the earlier studies of raxibacumab of delayed post-exposure prophylaxis rabbit model that rabbits develop anthrax disease between 20-36 hours post inhalational challenge.*

Other Parameters Measured:
The majority of animals exhibited clinical signs consistent with anthrax following the spore challenge. The most common clinical features were lethargy, not eating, and respiratory abnormalities.
Body temperature was monitored in all animals. The readings were stable until approximately 30 hours post challenge, and then exhibited sharp increases above baseline, and

remained high until death among placebo-treated and raxibacumab-treated non-survivors. Among raxibacumab-treated survivors, body temperatures also increased around 30 hours post challenge, then gradually declined to and remained at baseline levels, with the exception of a second increase above baseline at approximately 80 hours post challenge from which they recovered by approximately 160 hours post challenge.
The *B. anthracis* MICs for levofloxacin, prior to treatment and the terminal MICs ranged from 0.5 to 4μg/mL. For ciprofloxacin, the MICs prior to treatment ranged from 0.25 to 2μg/mL, and the terminal MICs ranged from 0.125 to 2μg/mL, demonstrating that the original MICs to these antibiotics did not change during the course of the study.
Body temperature was monitored in all animals.
No data on anti-PA antibody levels and TNA post-challenge were collected during the study.

All animals that survived were monitored for 14 days post-challenge. Clinical signs and symptoms of anthrax, including body temperature elevations, resolved following resolution of bacteremia. Animals exhibited normal baseline behavior at the time of study termination.
Animals found moribund were euthanized. All animals that died underwent pathological examination.

***MO comment**: Although the Division has recommended performing necropsy on all animals at the end of the study in communications with the Sponsor in the planning phase of the study, pathology data was collected only for the non-survivors, limiting reviewer's ability to perform analyses of the pathology findings that involved all animals that were treated with raxibacumab.*

Causes of Death, Gross and Histopathology Results

SID	Sex	LD_{50}	Bacteremia PTT	PA PTT	Outcome Day 14	Necropsy
acebo						
)8109	F	250	0	0	Natural Death	+bacteria/histo kidney, lung, lns, spleen
)8110	F	222	1	1	Natural Death	+histo lns spleen, +bacteria in lns, kidneys, spleen lung
)8111	F	176	1	1	Natural Death	+bacteria/histo kidney, lung, lns, spleen
)8114	F	298	1	1	Natural Death	meningitis, pneumonia/effusion, thymus, renal abscess, +histo/bact kidney, liver, lns, lung, append
)8119	F	194	1	1	Natural Death	mediastenitis, pneumonia/effusion, appendicitis, +histo/bact kidney, lns, lung, spleen, appendix
)8122	F	226	1	1	Moribund Sacrifice	meningitis, appendicitis, +histo/bact brain, lns, lung spleen, appendix
)8123	F	174	1	1	Natural Death	+histo/bact lns, spleen, +bact kidney, lung, +histo liv
)8127	F	218	1		Natural Death	pleural effusion, +histo/bact kidney, lns, lung, splee
)8134	M	230	1	1	Moribund Sacrifice	+histo/bact lns, spleen, +bact kidney, lung,
)81	M	191	1	1	Natural Death	meningitis, appendicitis, +histo/bact brain, appendi +histo lns, liver, spleen,
)81	M	218	0	1	Natural Death	pericardial/pleural effusion, thymic lns edema,

						+histo/bact kidneys, lns, lung, spleen
)8149	M	229	1	1	Natural Death	appendicitis, +histo/bact kidney, lns, spleen, +bact lung, +histo liver
)8151	M	264	1	1	Moribund Sacrifice	cerebellitis, +histo/bact brain, kidney, lns, spleen, +histo liver, +bact lung
)8155	M	227	1	1	Natural Death	pleural effusion, mediastenitis, thymic edema, +histo/bact lns, lung, spleen
)8156	M	206	1	1	Natural Death	pleural effusion, mediastenitis, thymic edema, +histo/bact lns, spleen, +bact kidney, lung
)8161	M	213	0	1	Natural Death	meningitis, thymic edema, + histo/bact lung, lns, + histo brain, spleen, +bact kidney
axibacumab 20mg/kg						
)8108	F	262	1	1	Natural Death	+ histo/bact lns, lung, spleen, + histo brain
)8112	F	250	1	1	lived	
)8120	F	272	1	1	Moribund Sacrifice	meningitis?,+ histo/bact lns, lung, spleen, kidney
)8124	F	206	1	0	lived	PA by ECL and ELISA was positive PTT, but ECL primary and secondary plates failed
)8130	F	263	1	1	lived	
)8131	F	223	1	1	Natural Death	meningitis, + histo/bact brain, + histo lns, lung
)8132	F	299	1	1	Moribund Sacrifice	meningitis, + histo/bact brain, kidney, + histo lns, lur
)8	M	179	1	1	Natural Death	meningitis, thymic hemorrhage, , + histo/bact brain lns, + histo kidney, spleen
)8136	M	263	1	1	Natural Death	meningitis, lad, + histo/bact brain, + histo heart, lun lns
)8137	M	186	1	1	Moribund Sacrifice	meningitis, pericarditis, +histo brain, heart, liver, lns spleen
)8142	M	230	1	1	Moribund Sacrifice	meningitis, lad, + histo/bact brain, kidney, + histo live lung, lns, spleen
)8143	M	190	1	1	Natural Death	meningitis, appendicitis, thymic edema, +histo/bac brain, appendix, + histo spleen, lung lns, kidney
)8153	M	175	1	1	Moribund Sacrifice	meningitis, appendicitis, +histo/bact brain, appendi + histo spleen, lns, kidney
)8157	M	215		1	lived	blood cx prior to treatment was not found
)8158	M	263	1	1	Natural Death	meningitis, + histo/bact brain, kidney, + histo lns, live lung, spleen
)8159	M	208	1	1	lived	
)8160	1	219	1	1	Moribund Sacrifice	meningitis, appendicitis, +histo/bact brain, appendi + histo spleen, lns, kidney, liver
axibacumab 40mg/kg						
)8113	F	217	1	1	Natural Death	meningitis, appendicitis, thymic edema, kidney discoloration +histo/bact brain, + histo appendix, lns + bact spleen
)8	F	199	1	1	Natural Death	lad, + histo/bact lns, lung, + histo kidney, spleen, liv
)8	F	204	1	1	Natural Death	meningitis, + histo/bact brain, kidney, lns, + histo co

						liver, spleen
)8117	F	144	1	1	lived	
)8118	F	281	0	1	lived	
)8121	F	316	1	1	Natural Death	+histo cor, lung, spleen, lns, blood cx +6h prior to death, and terminal
)8125	F	242	1	1	Natural Death	meningitis, appendicitis, +histo/bact brain, appendi kidney, lns + histo spleen, cor, lung
)8126	F	154	1	1	Moribund Sacrifice	+histo/bact lns, +histo lung, spleen
)8135	M	242	1	1	Moribund Sacrifice	meningitis, + histo/bact brain, + histo lns, lung, splee
)8138	M	249	1	1	lived	
)8139	M	259	1	1	Moribund Sacrifice	meningitis, appendicitis, +histo/bact brain, appendi + histo spleen, cor, lung, lns
)8140	M	203	1	1	Moribund Sacrifice	meningitis, appendicitis, +histo/bact brain, + histo cc lung, lns, appendix,
)8141	M	352	1	1	lived	
)8145	M	230	1	1	Moribund Sacrifice	meningitis, appendicitis, +histo/bact brain, appendi +histo lns, liver, lung, spleen
)8146	M	204	1	1	lived	
)8147	M	177	0	0	lived	animal had 1-5 colonies on the bl Cx 1.4 hours prio to rx, sample from immediately before rx was negati
)8	M	208	1	1	Natural Death	meningitis, + histo/bact brain, lns, + histo liver, lung spleen
)8152	M	256	1	1	lived	
)8154	M	287	0	0	lived	

Meningitis finding refers to gross pathologic changes in the meninges.

***MO comment**: All animals that died and were necropsized had either gross or histopathology findings or both consistent with disseminated anthrax infection. Only in one animal (L08121) the pathologist did not document presence of the bacteria in the organs examined. However, this animal had 2 blood cultures that were positive prior to raxibacumab treatment and one 4 hours after. Also, the animal's terminal blood culture was positive, while 10 hour post-treatment one was reported to be negative.*
All animals that died had positive blood culture either prior to or post treatment.

Efficacy results

Primary Efficacy Analyses

Study population	Placebo	Raxibacumab 20mg/kg	Raxibacumab 40mg/kg
FDA Primary analysis population (as treated bacteremic animals)			
	N=13	**N=16**	**N=17**
Survival, n (%)	**0**	**4 (25)**	**6 (35)**
P value*		**0.1067**	**0.0237**
Modified ITT(as treated animals regardless of bacteremia)			

	N=16	N=17	N=19
Survival, n (%)	0	5 (29.4)	8 (42.1)
P value*		0.0445	0.0038
ITT (as randomized animals regardless of bacteremia)			
	N=18	**N=18**	**N=18**
Survival, n (%)	0	5 (27.8)	8 (44.4)
P value*		0.0455	0.0029

*P-values are based on two-sided Fisher's exact test for comparisons between the treatment and placebo

***MO comment**: HGS proposed the Hochberg method to adjust for multiple comparisons in the protocol. The adjustment for multiple comparisons is needed when interpreting the study results. Using this method for the bacteremic population, FDA analysis shown in the shaded area of the table above, only the results for 40mg/kg raxibacumab group are statistically significant.*
MITT population above represents animals that were either febrile or toxemic prior to treatment administration and grouped by the actual treatment they received.

Additional exploratory efficacy analyses
To determine utility of PA screening and quantitative assays relative to bacteremia the following exploratory analyses were performed.

Study population	Placebo	Raxibacumab 20mg/kg	Raxibacumab 40mg/kg
As Treated Toxemic Animals (Screening Assay*)			
	N=10	**N=9**	**N=11**
Survival, n (%)	**0**	**2 (22)**	**4 (36)**
P value[a]		0.2105	0.0902
As Treated Toxemic Animals (Quantitative Assay)**			
	N=15	**N=17**	**N=18**
Survival, n (%)	0	5 (29.4)	7 (39)
P value[a]		0.0456	0.0090
As Treated Toxemic (Quantitative Assay) and Bacteremic Animals			
	N=13	**N=16**	**N=17**
Survival, n (%)	0	4 (25)	6 (35)
P value[a]		**0.1067**	**0.0237**

a P-values are based on two-sided Fisher's exact test for comparisons between the raxibacumab group
and placebo.
* both plate and sample had to pass quality controls for the results to be considered positive (7 additional animals found to be positive for PA by screening assay, but failed

plate quality control, therefore were excluded from the analyses. Also, 2 additional animals found to be PS positive by screening assay only at baseline, but not after challenge were excluded from the above analyses.
**3 animals (2 placebo and 1 raxibacumab 20mg/kg) had positive PA at the time of pre-study screening, but went on to be challenged and continued to be positive at all remaining timepoints, and eventually succumbed to death from anthrax. These animals were included in the above analyses. If they were to be excluded, the results would look as follows:

- As treated toxemic animals (quantitative assay) placebo 0/11, raxibacumab 20mg/kg 5/16 (31.3%)
- As treated toxemic (quantitative assay) and bacteremic animals placebo 0/11, raxibacumab 20mg/kg 4/15 (26.7%)

***MO comment**: Based on these analyses an improved survival is seen in the raxibacumab 40mg/kg treated animals as compared to placebo, and is similar to the results of the FDA primary efficacy analyses.*

Efficacy conclusions:

- **Raxibacumab at 40mg/kg IV bolus dose was superior to placebo in preventing inhalational anthrax induced mortality in bacteremic NZW rabbits (FDA primary efficacy analysis population) with achieved survival of 35% versus 0%(p=0.0237).**
- **Animal survival in raxibacumab 20mg/kg IV was not statistically different (p=0.1) from survival in the placebo group for the FDA primary efficacy analysis population of bacteremic NZW rabbits with achieved survival of 25% versus 0%**
- **In the modified intention to treat efficacy analysis population (as treated challenged NZW rabbits regardless of bacteremia status) raxibacumab at both dosing regimens of 20mg/kg IV single bolus and 40mg/kg IV single bolus was superior to placebo in preventing inhalational anthrax induced mortality with achieved survival of 29.4% versus 0% (p=0.0445) and 42.1% (p=0.0038), respectively.**
- **In the intention to treat analysis(as randomized challenged NZW rabbits regardless of bacteremia status) raxibacumab at both dosing regimens of 20mg/kg IV single bolus and 40mg/kg IV single bolus was superior to placebo in preventing inhalational anthrax induced mortality with achieved survival of 27.8% versus 0% (p=0.0455) and 44.4% (p=0.0029), respectively.**
- **Additional exploratory analyses (as treated toxemic animals, all treated toxemic and bacteremic animals) revealed similar trend of improved survival in the raxibacumab treated animals without reaching statistical significance.**

Safety Results

A survival benefit of raxibacumab administration at 40mg/kg IV bolus dose was observed in this animal study. The reviewer examined limited laboratory data and pathology finding in order to gain some insight of raxibacumab safety in animals.

Hematology abnormalities PTT (Prior to Treatment)

Hematology Findings PTT	Placebo	Raxibacumab 20mg/kg	Raxibacumab 40mg/kg	All challenged animals
All challenged rabbits	**N=16**	**N=17**	**N=19**	**N=52**
Leukocytosis	6 (37.5)	3 (17.6)	10 (52.6)	19 (36.5)
Neurophilia	11 (68.8)	9 (52.9)	10 (52.6)	30 (57.7)
Lymphopenia	11 (68.8)	10 (58.8)	10 (52.6)	31 (59.6)

***MO comment**: The most frequently observed hematologic abnormalities that are reflected in the above table are consistent with the ongoing infectious disease process.*

Hematology abnormalities EOS (End of Study or Prior to Death)

Hematology Findings at EOS/PTD	Placebo	Raxibacumab 20mg/kg	Raxibacumab 40mg/kg
Survivors	**0**	**5**	**8**
Lymphopenia		3 (60)	5 (62.5)
Thrombocytosis		3 (60)	4 (50)
Deceased	**16**	**12**	**11**
Leukocytosis	9 (56.3)	2 (16.7)	5 (45.5)
Anemia	4 (25)	4 (50)	6 (55.5)
Thrombocytopenia	1 (6.3)	6 (50)	4 (36.4)
Lymphopenia	7 (43.8)	6 (50)	5 (45.5)
Low basophils	8 (50)	4 (33.3)	3 (27.3)

***MO comment:** Greater than half of all survivors exhibited persistent lymphopenia at EOS (14 day post-treatment). Also, a prominent thrombocytosis (>500,000/ml) was observed in approximately 55% of all animals that survived.*
Among animals that died there was no significant differences in hemogram parameters between the animals in the placebo group versus those treated with raxibacumab except for more frequent finding of thrombocytopenia in animals in raxibacumab groups.

The animals that succumbed to disease or were sacrificed due to moribundity were necropsized. The internal organs were examined for gross pathology and then sections were examined microscopically. The necropsy and histopathology readings were initially performed by the pathologist at Battelle in a blinded fashion. Upon Division's request, the organs and histopathology slides were re-examined and read by an Independent Peer Review Pathology Panel (IPRP). Below analyses were performed for both Battelle and IPRP findings, as there were discrepancies in the histopathology readouts of the slides.

Incidence of pathology findings on necropsy, Battelle

Pathology Findings/necropsized animals	Placebo N=16 (%)	Raxibacumab 20mg/kg	Raxibacumab 40mg/kg

		N=17 (%)	N=19 (%)
Brain (gross brain pathology and/or histopathology+ bacteria)	4/16 (25)	12/12 (100)	8/11 (72.7)
Heart	2/16 (12.6)	2/12 (16.7)	5/11 (45.5)
Kidney	14/16 (87.5)	4/12 (33.3)	3/11 (27.3)
Liver	1/16 (6.3)	0/12 (0)	0/11 (0)
Bronchial/Mediastinal Lymph Nodes	15/16 (93.8)	5/12 (41.7)	5/11 (45.5)
Lung	15/16 (93.8)	2/12 (16.7)	1/11 (9.1)
Spleen	14/16 (87.5)	2/12 (16.7)	1/11 (9.1)
Appendix	6/16 (37.5)	3/12 (25)	3/11 (27.3)

***MO comment**: Among rabbits that received raxibacumab, fewer organs contained visible bacteria than seen in placebo rabbits. It is important to note, however, that greater number of animals that succumbed to anthrax in either of the active treatment groups had brain pathology findings, including those consistent with meningitis (100% for raxibacumab 20mg/kg and 72.7% for raxibacumab 40mg/kg) as compared to placebo (25%). Also, greater number of animals in 40mg/kg raxibacumab group had pathologic changes in the heart as compared to placebo and 20mg/kg raxibacumab groups. Per calculations performed by statistical reviewer Hongling Zhou, Ph.D., the rates of rabbits with brain pathology were significantly different between the placebo group and the raxibacumab 20 mg/kg group (p = 0.0003, Fisher's exact test) even after the Bonferroni correction (0.05/2=0.025). The difference between the placebo group and the raxibacumab 40 mg/kg group was not significantly different (p = 0.0542, Fisher's exact test).*

The study report states that the likely explanation for these findings is longer survival of the diseased animals in the active treatment groups as compared to placebo (in the 20 mg/kg raxibacumab group median = 3.5 days, and 40 mg/kg group median = 3.8 days, and in the placebo group median = 2.7 days). Also, the average time to death in rabbits with moderate inflammation in the brain was 4.5 days compared with 3.1 days in all rabbits that died, suggesting that the increased survival time allowed for additional pathology to develop in the brain. This finding is reported to be consistent with the reported observation that cynomolgus monkeys that survive longer post challenge have increased rates of meningeal hemorrhage and cerebral vasculitis (Vasconcelos et al, 2003).

***MO comment:** The Reviewer examined data and manipulations performed by the Sponsor that led to the above conclusion (submitted upon Reviewer's request) and determined that case ascertainment of moderate inflammation in the brain of the diseased animals was not consistent among individual animals, and, as disease progression in rabbits is very fast, rounding to a full day for the times to deaths is not acceptable.*

Below presented is reviewer's finding on the incidence of minimal-mild (grade 0-1) and moderate-severe pathology findings in the brains of deceased animals.

Histopathology Results by Treatment Group, Battelle

		Placebo N=16 (%)	Raxi 20/kg N=12 (%)	Raxi 40/kg N=11 (%)	Active Rx N=23 (%)
CNS Bacteria	**Grade 0**	13 (81)	3 (25)	4 (36)	7 (30)
	Grade 1	1 (6)	0 (0)	1 (9)	1 (4)
	Grade 2	2 (13)	2 (17)	3 (27)	5 (22)
	Grade 3-4	0 (0)	7 (58)	3 (27)	10 (43)
Inflammation	**Grade 0**	14 (87)	4 (33)	6 (54)	10 (43)
	Grade 1	0 (0)	3 (25)	0 (0)	3 (13)
	Grade 2	2 (13)	2 (17)	2 (18)	4 (17)
	Grade 3-4	0 (0)	3 (25)	3 (27)	6 (26)
Hemorrhage	**Grade 0**	12 (75)	2 (17)	4 (36)	6 (26)
	Grade 1	2 (13)	2 (17)	0 (0)	2 (8)
	Grade 2	0 (0)	3 (25)	3 (27)	6 (26)
	Grade 3-4	2 (13)	5 (42)	4 (36)	9 (39)
Necrosis	**Grade 0**	16 (100)	7 (58)	5 (45)	12 (51)
	Grade 1	0 (0)	2 (17)	2 (18)	4 (17)
	Grade 2	0 (0)	3 (25)	3 (27)	6 (26)
	Grade 3-4	0 (0)	0 (0)	1 (9)	1 (4)

MO comment: *Moderate-severe brain pathology was more common in deceased animals treated with raxibacumab, although without any particular dose-response pattern.*

Incidence of pathology findings on necropsy, Independent Peer Review Panel (IPRP)

Pathology Findings/necropsized animals	Placebo N=16 (%)	Raxibacumab 20mg/kg N=17 (%)	Raxibacumab 40mg/kg N=19 (%)
Brain	16 (100)	12 (100)	10 (91)
Heart	Pending	Pending	Pending
Kidney	16 (100)	12 (100)	10 (91)
Liver	16 (100)	12 (100)	11 (100)
Bronchial/Mediastinal Lymph Nodes	16 (100)	12 (100)	11 (100)
Lung	16 (100)	12 (100)	11 (100)
Spleen	16 (100)	12 (100)	11 (100)

MO comment: *Although, IPRP report documents a greater frequency of pathologic findings consistent with anthrax in different organs, it is in agreement with Battelle that severity of pathologic findings was greater for all organs except brain in placebo animals, as compared to those treated with raxibacumab.*

Histopathology Results by Treatment Group, Independent Peer Review Panel (IPRP)

		Placebo N=16 (%)	Raxi 20/kg N=12 (%)	Raxi 40/kg N=11 (%)	Active Rx N=23 (%)
CNS Bacteria	**Grade 0**	1 (6)	3 (25)	4 (36)	7 (30)
	Grade 1	13 (82)	3 (25)	1 (9)	4 (17)
	Grade 2	2 (12)	2 (17)	2 (18)	4 (17)
	Grade 3-4	0 (0)	4 (33)	4 (36)	8 (35)
Inflammation	**Grade 0**	15 (94)	4 (33)	5 (45)	9 (39)
	Grade 1	0 (0)	4 (33)	1 (9)	5 (22)
	Grade 2	1 (6)	2 (17)	1 (9)	3 (13)
	Grade 3-4	0 (0)	2 (17)	3 (27)	5 (22)
Hemorrhage	**Grade 0**	11 (69)	1 (8)	3 (27)	4 (17)
	Grade 1	3 (19)	6 (50)	1 (9)	7 (30)
	Grade 2	2 (13)	3 (25)	2 (18)	5 (22)
	Grade 3-4	0 (0)	2 (17)	4 (36)	6 (26)
Necrosis	**Grade 0**	14 (87)	4 (33)	4 (36)	8 (35)
	Grade 1	2 (13)	2 (17)	3 (27)	5 (22)
	Grade 2	0 (0)	5 (42)	3 (27)	8 (35)
	Grade 3-4	0 (0)	1 (8)	1 (9)	2 (9)

MO comment: *Although the Reviewer views the presence of bacteria in the brain as an all-or-none finding, the above table is still in general agreement with the findings from Battelle where frequency of moderate-severe inflammation is higher in raxibacumab as compared to placebo treated animals that underwent necropsy.*

To better understand the relationship between the timing of death and development of brain pathology, including meningitis, the reviewer performed the following analyses.

Brain Pathology and Time to Death (days)

Time to Death (days)	**Placebo**	**Raxibacumab 20mg/kg**	**Raxibacumab 40mg/kg**	**All active treatment**	**All treated animals**
All treated animals* (Sponsor's) (Kaplan Meyer) survival curve)	**N=16**	**N=17**	**N=19**	**N=36**	**N=52**
Median	**2.7**	**3.8**	**3.5**	**3.65**	**3.05**
All necropsized animals**	**N=16**	**N=12**	**N=11**	**N=23**	**N=39**
Mean	**3.0**	**3.4**	**2.7**	**3.1**	**3.1**
M	**2.7**	**3.0**	**2.9**	**2.9**	**2.8**

A[illegible]ls with Brain Pathology on necropsy (Battelle)	N=5	N=12	N=8	N=20	N=25
Mean	3.2 ± 0.8	3.4 ± 1.5	3.1 ± 0.7	3.3 ± 1.3	3.3 ± 1.2
Median	2.7	2.95	2.95	2.95	2.9
Min-Max	2.6 - 4.2	1.4 - 6.9	1.8 - 4.1	1.4 - 6.9	1.4 - 6.9
Animals with bacterial meningitis	N=3	N=9	N=7	N=16	N=19
Mean	2.7 ± 0.1	3.9 ± 1.5	3.2 ± 0.5	3.6 ± 1.2	3.4 ± 1.1
Median	2.7	3.1	3.0	3.1	3.0
Animals without Brain Pathology on necropsy (Battelle)	**N=11**	**N=0**	**N=3**	**N=3**	**N=14**
Mean	3.0 ± 0.9	-	1.8 ± 0.4	1.8 ± 0.4	2.7 ± 1.0
Median	2.7	n/a	1.8	1.8	2.6
Min-Max	1.4 – 4.8	n/a	1.5 - 2.2	1.5 - 2.2	1.4 - 4.8

Animals with Brain Pathology on necropsy (IPRP)	**N=16**	**N=12**	**N=10**	**N=22**	**N=38**
Mean	3.0 ± 0.9	3.4 ± 1.5	2.8 ± 0.8	3.1 ± 1.3	3.1 ± 1.1
M[illegible]	2.7	3.0	2.9	2.9	2.9
Mi[illegible]ax	1.4 - 4.9	1.4 – 6.9	1.5 – 4.1	1.4 - 6.9	1.4 - 6.9
Animals with bacterial meningitis	N=15	N=9	N=7	N=16	N=31
Mean	2.9 ± 0.8	3.7 ± 1.7	3.2 ± 0.5	3.5 ± 1.3	3.2 ± 1.1
Median	2.7	3.1	3.0	3.1	2.9
Animals without Brain Pathology on necropsy (IPRP)	**N=0**	**N=0**	**N=1**	**N=1**	**N=1**
Mean	-	-	1.8	1.8	1.8
Median	n/a	n/a	1.8	1.8	1.8
Min-Max	n/a	n/a	1.8	1.8	1.8

*Rabbit L08128 died during the challenge and was excluded from the as-treated population.
**Only animals died of anthrax were necropsized.

MO comment: *Although there is a numerical difference in mean values of time to death between the animals with and without brain pathology on necropsy, this difference becomes less pronounced when median values are compared, particularly, in placebo animals. Also, there is a significant overlap of times to death ranges between meningitis and no meningitis animal subgroups.*
Although plausible, it does not appear that just the duration of illness alone could account for greater pathologic findings in the brain of animals treated with raxibacumab versus placebo.

In the attempt to further delineate the relationship between the pathological findings in the brain and the duration of anthrax illness (duration of documented bacteremia) as opposed to survival from the time of anthrax challenge, the Reviewer performed the following analyses.

uration of bacteremia prior to treatment and prior to death in animals with and without CNS findings n necropsy/histopathology, Mean ± SD (Median)

	N	Placebo	N	Raxi 20	N	Raxi 40	N	Active Rx
Duration of bacteremia prior to treatment (hr)								
Lived	0	n/a	4**	2.9±3.6 (2.2)	6**	2.3±2.0 (1.4)	10**	2.5±2.6 (1.4)
Died	13*	2.0±2.2 (0.4)	12	4.2±4.5 (3.7)	11	2±2.0 (0.5)	23	3.1±3.6 (3.4)
CNS pathology	4*	2.7±3.3 (1.8)	12	4.2±4.5 (3.7)	8	2.6±2.1 (3.6)	20	3.6±3.7 (3.6)
No CNS pathology	9*	1.7±1.8 (0.4)	0	n/a	3	0.4±0.1 (0.3)	3	0.4±0.1 (0.3)
Bac[illegible]al me[illegible]tis	3	3.5±3.5 (3.5)	9	5.1±4.7 (3.9)	7	2.5±2.2 (3.8)	16	3.9±4.0 (3.9)
No bacterial meningitis	10*	1.6±1.8 (0.4)	3	1.6±2.5 (0.2)	4	1.1±1.5 (0.4)	7	1.3±1.8 (0.3)
Duration of bacteremia prior to death (hr)								
All necropsized animals	16	37.7±15.6 (37.3)	12	58.7±38.1 (45.4)	11	40.5±20.1 (46.6)	23	50.0±31.5 (46.4)
Battelle								
CNS pathology	5	44.0±20.3 (40.8)	12	58.7±38.1 (45.4)	8	48.3±17.1 (48.2)	20	54.6±31.2 (47.2)
No CNS pathology	11	34.8±13.1 (35.1)	0	n/a	3	19.5±9.5 (18.2)	3	19.5±9.5 (18.2)
Bacterial meningitis	3	40.9±2.5 (40.8)	9	69.6±37.7 (58.2)	7	53.0±11.7 (48.7)	16	62.4±29.7 (49.1)
No bacterial meningitis	13	37.0±17.3 (35.1)	3	25.9±12.2 (30.8)	4	18.6±8.0 (17.0)	7	21.7±9.8 (18.2)
CNS inflammation (mod/severe)	0	n/a	3	109.1±42.3 (119.5)	3	57.4±17.8 (47.7)	6	71.6±38.0 (56.0)
CN[illegible]lammation (min[illegible]ild)	16	37.7±15.6 (37.3)	9	41.9±16.7 (39.8)	8	34.1±17.8 (36.6)	17	38.2±17.1 (39.8)

IPRP								
Bacterial meningitis	15	35.1±12.1 (36.1)	9	64.5±42.3 (46.4)	7	53.0±11.7 (48.7)	16	59.4±32.3 (48.2)
No bacterial meningitis	1	76.7	3	41.3±14.8 (34.8)	4	18.5±8.0 (17.0)	7	28.3±15.9 (29.6)
CNS inflammation (mod/severe)	0	n/a	2	103.9±58.5 (103.9)	4	55.5±15.1 (48.6)	6	83.3±40.5 (70.3)
CNS inflammation (min/mild)	16	37.7±15.6 (37.3)	10	49.7±29.1 (42.1)	7	31.9±18.0 (29.6)	17	42.4±26.1 (39.8)
Time to Bacteremia from challenge start (hr)								
CNS pathology	5	33.3±21.7(24.0)	12	23.4±5.5 (22.6)	8	24.8±5.1(24.1)	20	24.0±5.2 (24.1)
No CNS pathology	11	34.6±25.6(23.9)	0	n/a	3	24.4±0.1(24.4)	3	24.4±0.1(24.4)

MO comment: *The Reviewer attempted to explore whether the treatment with raxibacumab might have caused accelerated bacterial translocation into meninges (shorter duration of bacteremia prior to treatment) and, therefore, brain pathology (the first 3 analyses). In fact, raxibacumab treated animals that developed meningitis had slightly longer duration of bacteremia prior to treatment as compared to those without meningitis. On the other hand, additional analyses of bacteremia duration prior to death seem to support the Sponsor's claim for the longer duration of illness (in this case duration of bacteremia) almost 2.5-3 fold prior to death for animals with CNS findings, including bacteria, as compared to those animals that died without CNS involvement. Additional analyses were performed to explore the correlation between the duration of bacteremia and severity of CNS inflammation. A trend towards numerically longer duration of bacteremia prior to death was observed in animals with moderate/severe inflammation as compared to those with minimal/mild CNS inflammatory changes. Also, raxibacumab treated animals that succumbed to anthrax had numerically longer mean and median duration of bacteremia prior to death as compared to placebo treated animals. Unexplained, however, remains the finding of longer time to bacteremia after the spore challenge in the placebo animals that died as compared to raxibacumab treated animals. Sampling bias should not have occurred as blood samples for culture were collected per protocol every 4 hours starting 12 hours post-challenge in all animals regardless of the group assignment.*

Safety conclusions:

- Raxibacumab at 40mg/kg IV offered a statistically significant survival benefit to the NZW rabbits with established systemic inhalational anthrax disease evident by bacteremia prior to therapeutic intervention.
- Frequent and more extensive findings of CNS pathology in raxibacumab treated animals on necropsy as compared to placebo are possibly associated with a longer

duration of illness, i.e. documented bacteremia by culture, prior to death in the raxibacumab treated animals. However, monoclonal-disease interaction could not be excluded. This pathology finding should be described in the resultant label of raxibacumab if approved, especially if corroborated by the similar findings in NHP anthrax treatment study.

CLINICAL REVIEW OF THE ANIMAL STUDY REPORT 724-G005829 and the HISTOPATHOLOGY STUDY PROTOCOLS 901-G005829 (Battelle) and 866-001 (EPL)
and
CLINICAL REVIEW OF THE ANIMAL STUDY REPORT 789-G923702

Susan K. McCune, M.D.
Medical Team Leader
Office of Counterterrorism and Emergency Coordination
(Reviewer for DSPTP)

10/19/2009
Date

Yuliya Yasinskaya, M.D.
Medical Team Leader
Division of Special Pathogens and Transplant Products

10/19/2009
Date

BLA 125,349 (Raxibacumab)

Signatory page

Reviewer: Sue Lim, M.D.
Discipline: Clinical
Date: October 19, 2009
Team Leader: Yuliya Yasinskaya, M.D.

Sue Lim

Sue Lim

19 October 2009

Date

Yuliya Yasinskaya

10.19.09.

Date

REVIEW OF REQUEST FOR PRIORITY REVIEW

To:	Edward Cox, MD, MPH Director, Office of Antimicrobial Products
Through:	Renata Albrecht, M.D Director, DSPTP, OAP
From:	Yuliya Yasinskaya, M.D. Acting Medical Team Leader, DSPTP, OAP
BLA:	125349
Submission Date:	5/14/09
Date Review Completed:	5/29/09
Product:	Raxibacumab (monoclonal antibody against Protective Antigen of *B. anthracis*)
Sponsor:	Human Genome Sciences, Inc. Rockville, MD
Proposed Indication:	Treatment of anthrax
Proposed Dosing Regimen:	A single intravenous infusion of 40 mg/kg dose

A Biologics Licensing Application (BLA) under 21 CFR 601, Subpart H. for raxibacumab, a fully human monoclonal antibody against Protective antigen of *B. anthracis*, for the treatment of anthrax was submitted on 13 May 2009. This submission contained a request for a Priority Review. However, the Sponsor did not provide written justification for such a request. In response to the Division's letter dated May 21, 2009, HGS provided additional information justifying their request that the raxibacumab BLA be considered for Priority Review, as discussed below.

Under BB-IND 11,069 initiated in June 2003 raxibacumab was assigned Fast Track status and was granted Orphan Drug designation (03-17550) for the treatment of anthrax on 15 August 2003 and 12 November 2003, respectively. Both Fast Track status and Orphan Drug designation carry the requirement that the drug addresses an unmet medical need (Guidance for Industry Fast Track Drug Development Programs - Designation, Development, and Application Review [January 2006] and 21 CFR 316.20, respectively).

MO comment: *Anthrax disease is associated with 3 know virulence factors of B. anthracis: antiphagocytic capsule and two protein exotoxins: lethal and edema toxins. As in most infections*

antibacterial treatment will result in killing and eradication of the susceptible bacteria. However, in case of anthrax antimicrobials alone are not sufficient to abort the part of the disease pathogenesis that is triggered by the toxins. A crucial component of B. anthracis toxins is PA (protective antigen) that results in a heptamer formation on the target cell membrane and allows entry of the lethal and edema factors intracellularly to exert their cytopathic effect. Raxibacumab has a novel mechanism of action distinct from the action of antimicrobials; by binding with high affinity to PA of B. anthracis it prevents formation of active toxins and diminishes their downstream pathologic effects.

HGS requested consideration for Priority Review for the raxibacumab BLA application for therapeutic treatment of inhalation anthrax as the application satisfies the criteria for Priority Review outlined in CDER MAPP 6020.3 on Priority and Standard Review, that is, raxibacumab provides safe and effective therapy where no satisfactory alternative therapy exists and provides significant improvement compared to marketed products in treating anthrax disease.

Demonstration of effectiveness: Raxibacumab provided a statistically significant improvement in survival rate as compared to placebo in pivotal efficacy studies in 2 different species when raxibacumab was administered to the animals with confirmed inhalational anthrax disease. The demonstration of efficacy in 2 animal species is consistent with the requirements outlined in the Animal Rule (21CFR 601 Subpart H).

MO comment: *The evidence of efficacy of raxibacumab as a single 40mg/kg IV dose as compared to placebo in a setting of established inhalational anthrax disease in two animal models (NZW rabbits and cynomolgus monkeys) was reviewed previously for the raxibacumab acquisition by Strategic National Stockpile. The reviewers concurred with the Sponsor's conclusions that administration of raxibacumab in this setting in both animal species resulted in statistically significant survival benefit as compared to placebo. The Sponsor collected and provided sufficient information to conclude that the requirements for demonstration of efficacy of raxibacumab under the Animal Rule (21CFR 601, Subpart H) are met.*

Demonstration that no satisfactory alternative therapy exists and that raxibacumab provides a significant improvement compared to marketed products in treating anthrax disease: Raxibacumab is a fully human monoclonal antibody against the PA of *Bacillus anthracis*. It has a novel mechanism of action of binding and inhibiting the action of bacterial toxin as compared with the currently marketed products that act by virtue of their bactericidal or bacteriostatic activity (see prescribing information for CIPRO, Levaquin and Vibramycin). While vaccine containing PA has been licensed to promote the generation of antibodies to PA, vaccines do not provide immediate anti-toxin activity and require weeks and multiple injections to provide significant protection (see prescribing information for AVA [Biothrax]). There is no satisfactory alternative therapy which provides immediate anti-toxin (anti-PA) activity leading to a survival benefit. Moreover, the effectiveness of raxibacumab in improving survival has been demonstrated in the setting of therapeutic treatment when animals have documented anthrax disease, in contrast to the currently marketed products in where their effectiveness in treating anthrax was derived from either *in vitro* activity data or from the studies in animals in the setting of post-exposure prophylaxis (see prescribing information for CIPRO, Levaquin and Vibramycin, and Friedlander et al, 1993) and anthrax vaccine (Friedlander et al, 1993) or in

humans in the setting of post-exposure prophylaxis with ciprofloxacin following the anthrax attacks of 2001 (see CIPRO prescribing information) or in the setting of pre-exposure prophylaxis with vaccine (see Biothrax prescribing information).
In addition, raxibacumab provides an alternative therapy when antibiotics or vaccines are contraindicated (see contraindications and warnings in the prescribing information for CIPRO, Levaquin, Vibramycin and Biothrax), and for subjects who are non-compliant.
Furthermore, in the event of an anthrax disease due antibiotic-resistant bacteria, which can be bioengineered (Anthamna et al, 2004), raxibacumab might be the only therapy to provide immediate effective treatment.

MO comment: *As discussed previously the novel mechanism of action allows raxibacumab to be used for treatment of inhalational anthrax not only in combination with antibiotics as it is anticipated, but also in the settings where antimicrobials are poorly tolerated, contraindicated, or are ineffective due to resistance.*

Demonstration of safety: Raxibacumab was evaluated in over 400 subjects, of whom over 300 received raxibacumab at the to-be-marketed dose and route of administration. Raxibacumab was safe, well tolerated, and non-immunogenic when administered as single or repeat dose. The incidence of all adverse events (AEs), related AEs, severe AEs and serious AEs (SAE) was in raxibacumab-treated subjects was comparable to subjects who received placebo. SAEs were rare with only a single SAE (cholecystitis) reported as possibly related to raxibacumab, although the investigator judged the event to be most likely related to the subject's pre-existing, underlying medical condition. Concomitant administration of raxibacumab with antibiotics in healthy humans did not alter the safety or pharmacokinetics (PK) of either antibiotic or raxibacumab and in animals did not alter antibiotic efficacy or PK.

MO comment: *Safety data limited to 82 healthy subjects that received single 40mg/kg IV dose of raxibacumab alone and in combination with ciprofloxacin were reviewed prior to the product acquisition by the SNS. The reviewers concurred with the Sponsor that the use of product was associated with mild histamine mediated infusion reactions and now requires diphenhydramine premedication. Otherwise, its safety profile was comparable to that of placebo. The BLA application contains additional safety data >200 additional healthy subjects, that brings the total safety database for this product to over 300.*

Conclusion: Based on the evidence provided above, the Reviewer agrees with the Sponsor's proposal that raxibacumab satisfies the criteria for Priority Review because raxibacumab provides safe and effective therapy where no satisfactory alternative therapy exists and provides a significant improvement compared to marketed products in treating anthrax disease.

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