Application Type	Original BLA
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Division / Office	DHCR/OBRR
Committee Chair	Robert Fisher, PhD
Clinical Reviewer(s)	Leland Ross Pierce, MD
Project Manager	Thomas Maruna
Priority Review	Yes
Reviewer Name(s)	Jiang Hu, PhD
Review Completion Date / Stamped Date	
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Applicant	Cangene Corporation (Emergent Biosolutions)
Established Name	Anthrax Immune Globulin Intravenous (Human)
(Proposed) Trade Name	Anthrasil
Pharmacologic Class	Hyperimmune immunoglobulin
Formulation(s), including Adjuvants, etc	The formulation contains purified human antibodies to Bacillus anthracis stabilized with 10 g% maltose and 0.03% (w/w) polysorbate 80.
Dosage Form(s) and Route(s) of Administration	Liquid for Intravenous Administration in 50 mL glass vials
Dosing Regimen	Single dose
Indication(s) and Intended Population(s)	Anthrax Immune Globulin Intravenous (Human) is indicated for the treatment of adult and pediatric patients with toxemia associated with inhalational anthrax. AIGIV is beneficial in combination with appropriate antibacterial drugs.

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GLOSSARY

AE	Adverse event
AIGIV	Anthrax immune globulin intravenous (Human)
ANOVA	Analysis of variance
ARDS	Acute respiratory distress syndrome
AUC	Area under the concentration curve
AVA	Anthrax vaccine adsorbed
(b) (4)	
BMI	Body mass index
bw	Body weight
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	Confidence interval
CL	Clearance
Cmax	Maximum drug concentration observed
CV	Coefficient of variation
(b) (4)	
GCP	Good Clinical Practices
GLP	Current Good Laboratory Practice
ITT	Intent to treat
IR	Information request
IV	Intravenous
LD_{50}	Lethal dose 50%
LF	Lethal factor
LOD	Limit of detection
LLOQ	Lower limit of quantitation
LT	Lethal toxin
MITT	Modified intent to treat
MMAD	Mass median aerodynamic diameter
NAT	Nucleic acid testing
ND	Not determined
NHP	Non-human primate
PA	Protective antigen
SAE	Serious adverse event
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean
TNA	Toxin neutralization assay
TRALI	Transfusion-related acute lung injury
U	Units
VAP	Vascular access port
Vd	Volume of distribution
WBC	White blood Cells

1. Executive Summary

This original Biologics License Application was submitted by Cangene for Anthrasil®, Anthrax Immune Globulin Intravenous (Human) (AIGIV), as a treatment of adult and pediatric patients with toxemia associated with inhalational anthrax. The proposed product, AIGIV, is a sterile gamma globulin (IgG) fraction of human plasma containing antibodies to *Bacillus anthracis*.

Considering the rare occurrence of natural anthrax and inability to design ethical studies exposing human beings to anthrax toxins, it is infeasible to conduct human clinical efficacy trials. Three animal studies (b) (4)-828, (b) (4)-1182 and (b) (4)-1207) were conducted to establish the efficacy of AIGIV and one healthy volunteer study (AX-001) was conducted for safety. This statistical memo focuses on efficacy analyses and safety analyses of these four controlled studies.

Study (b) (4)-828 was a dose selection study for AIGIV in cynomolgus monkeys. It demonstrates that a single dose of AIGIV was efficacious in improving the survival rate of inhalational anthrax. Both studies (b) (4)-1182 and 1207 tested a single dose (15U/kg) administration in rabbits, but Study (b) (4)-1207 was designed to evaluate the efficacy of AIGIV when treatments were administered after the first detection of protective antigen in serum while Study (b) (4)-1182 was designed to assess the efficacy of added benefit of AIGIV over the use of levofloxacin when treatment was initiated at 96 h after the aerosol exposure. Study (b) (4)-1207 shows a statistically significant improvement in the survival rate of the AIGIV group compared with the control group. Although in Study (b) (4)-1182 the survival rate in the AIGIV treated arm is numerically higher compared to the control, the difference is not statistically significant. The overall efficacy of AIGIV is established by considering the results of all three studies.

No serious safety concern was detected through the healthy volunteer study AX-001.

2. Clinical and Regulatory Background

The polyclonal immune globulin G in Anthrax Immune Globulin Intravenous (Human) is a passive immunizing agent that neutralizes anthrax toxin. AIGIV binds to protective antigen (PA) and other potential antigens in anthrax vaccine adsorbed (BioThrax®) to neutralize the pathogenic effects of anthrax toxin.

2.1 Disease or Health-Related Condition(s) Studied

Anthrax is a serious and life-threatening disease caused by *Bacillus anthracis*, a Gram positive, rod shaped bacterium that forms highly resistant spores under stressful environmental conditions. Resulting from the inhalation of aerosolized *Bacillus anthracis* spores, inhalational anthrax is the most serious form of the disease.

Inhalational anthrax has a short incubation, rapid progression and high mortality. Early symptoms of infection appear after a 1 to 5 day incubation period and are consistent with a mild respiratory tract infection and include malaise, fever, fatigue, myalgias, non-

productive cough, lethargy, nausea and vomiting. These early symptoms usually persist for 2-3 days and may temporarily improve, only to be followed by an acute, sudden onset of severe respiratory symptoms. The disease is usually fatal within 24-36 hours after the onset of respiratory symptoms.

The course of inhalational anthrax disease begins when inhaled spores are taken up by alveolar macrophages and are subsequently transported via the lymphatic system to the intrathoracic lymph nodes. Infection ensues when surviving spores germinate and multiply in the lymph nodes. As the phagocytic capacity of the lymph node is overwhelmed, bacilli pass through the efferent lymphatics, infect successive nodes, and ultimately enter the blood stream through the thoracic duct. After germination, the replicating *Bacillus anthracis bacilli* release three toxin components, protective antigen (PA), lethal factor (LF) and edema factor (EF). Two toxins, lethal toxin (LT) and edema toxin (ET), are formed when PA binds to the LF and EF, respectively. PA forms a heptomeric cell surface receptor that transports LF and EF across the cellular membrane. Intracellularly, LF and EF interrupt signaling pathways, inhibit immune function and lyse target cells resulting in hemorrhage, edema and necrosis. Both the high concentration of replicating bacteria and the toxemia contributes to the pathogenesis.

2.2 Currently Available, Pharmacologically Unrelated Treatment(s)/Intervention(s) **for the Proposed Indication(s)**

Currently, symptomatic anthrax patients are treated with antimicrobial agents with known activity against *Bacillus anthracis*.

Raxibacumab (GlaxoSmithKline), a human monoclonal antibody targeting the PA component of the lethal toxin of *Bacillus anthracis*, is approved for the treatment of adult and pediatric patients with inhalational anthrax due to *Bacillus anthracis* in combination with appropriate antibacterial drugs and for prophylaxis of inhalational anthrax when alternative therapies are not available or not appropriate. While antibiotics target the anthrax bacteria, Raxibacumab offers an additional mechanism by blocking the activity of the anthrax toxin, which plays a key role in the progression of the disease.

The anti-toxin mechanism of action for AIGIV is similar to Raxibacumab; however, the polyclonal nature of AIGIV provides several differences to the monoclonal product. Raxibacumab specifically blocks PA binding to the cellular receptor, thus with Raxibacumab treatment PA levels remain elevated in serum several days post-dosing. In contrast, polyclonal anthrax immune globulins will contain antibodies to a wider range of anthrax antigens and has been demonstrated to neutralize or clear PA to undetectable levels post-administration.

AIGIV is being developed for the treatment of toxemia associated with inhalational anthrax disease. The product is a human polyclonal antisera produced from the serum of individuals vaccinated with Anthrax Vaccine Adsorbed (AVA, BioThrax™). AIGIV contains antibodies against PA and has toxin neutralizing capabilities. Individuals vaccinated with 4-6 injections of AVA develop significant anti-PA titers, and anti-PA titers of (b) (4) are typically correlated with protective immunity. Intravenous

administration of AIGIV offers the same immunity provided by the vaccine, but is immediately effective whereas it may take weeks to months to confer protection through vaccinations.

AIGIV is a clear or slightly opalescent colorless liquid essentially free of foreign particles that is formulated in 10 g% maltose and 0.03% polysorbate 80. It contains no preservatives and is intended for single use by intravenous (IV) administration. It is anticipated that AIGIV will be used in conjunction with antimicrobial therapy in patients presenting with symptomatic anthrax. The treatment of toxemia with AIGIV is expected to complement the bactericidal actions of antimicrobial therapy in patients with symptomatic anthrax disease.

2.4 Previous Human Experience with the Product (**Including Foreign Experience**)

Due to the rare occurrence of natural anthrax infections and the inability to design ethical studies exposing humans to anthrax toxins in sufficient quantities to cause anthrax, it is not feasible to conduct clinical efficacy studies in humans.

Besides the healthy volunteer study AX-001, AIGIV had been applied to 19 subjects in IND 13026 which is sponsored by the Centers for Disease Control and Prevention (CDC). In this study, 13 patients survived and 6 died. The IND was originally submitted in May 2006 and was allowed to proceed for release of AIGIV under an emergency IND.

2.5 Summary of Pre- and Post-submission Regulatory Activity Related to the Submission

The applicant held a pre-IND meeting with CBER regarding the development plan for Anthrax Immune Globulin on August 26, 2004, and received considerable input from CBER. On October 8, 2004 the applicant submitted IND 11982 for AIGIV.

The applicant has held several meetings and teleconferences with FDA regarding the design and progress of their animal efficacy model studies, given the intention of the applicant to submit a BLA under the Animal Rule.

- CRMTS 8095, August 29, 2011: FDA agrees to use the modified intent to treat (MITT) population instead of the intent to treat (ITT) population for the primary analysis of the primary efficacy endpoint for studies (b) (4)-1182 and (b) (4) 1207. The MITT population excludes subjects in ITT population who were not toxemic and bacteremic at least once prior to the treatment.
- CRMTS 9053, August 15, 2013: FDA suggested that the applicant not proceed to the second stage of study (b) (4)-1182 after reviewing the interim analysis report, because the power analysis indicated that continuation to the second stage would provide only approximately 48% power to demonstrate added benefit resulting from the AIGIV treatment (combined with levofloxacin as designed in study (b) (4)-1182).
- CRMTS 9270, February 20, 2014: Several regulatory issues were clarified in the pre BLA meeting.

3. SUBMISSION QUALITY AND GOOD CLINICAL PRACTICES

3.1 Submission Quality and Completeness

The submission was adequately organized for conducting a complete statistical review without unreasonable difficulty.

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

4.2 Assay Validation

The applicant submitted validation reports for the Toxin Neutralization Assay (TNA) quantification of AIGIV as a standard requirement by FDA guidelines. The applicant analyzed the data following bioanalytical methods. These reports were reviewed by the product reviewer.

FDA/CBER sent an information request (IR) on November 26, 2014 to the applicant requesting they reanalyze the assay precision data using a log-transformation. The applicant replied that non-clinical and clinical versions of TNA are bioanalytical methods analogous to the standard (b) (4) These assays fall under the scope of the Draft FDA Guidance for Industry *Bioanalytical Method Validation* (September 2013, v1). Log-transformation of precision data was not mandatory in support of the method validation. In contrast to the potency determination for AIGIV product, these bioanalytical methods employ a calibration curve that is generated using a reference standard, typically modeled using a non-linear 4 or 5 parameter curve fit. The signal response (b) (4) of one or more dilutions of the test sample is then interpolated from the standard curve fit model, adjusted for dilution, and averaged to report the test sample result. Therefore, only precision data from the AIGIV product potency assay were re-analyzed by the applicant using a log-transformation.

The applicant reanalyzed data from the validation studies B55-B and B55-F in using a log-transformation. B55-B was completed in November 2005 and B55-F was completed in October 2006. For B55-F an additional test article was included at a concentration of 1875 μg/mL in order to further assess precision within the range of filled drug product lots. Both validation addendums evaluated assay accuracy and precision across the validated range of the method.

Table 1: Summary of Re-Analysis of Validation Data from B55-B and B55-F for the AIGIV Product Potency Assay

(b) (4)
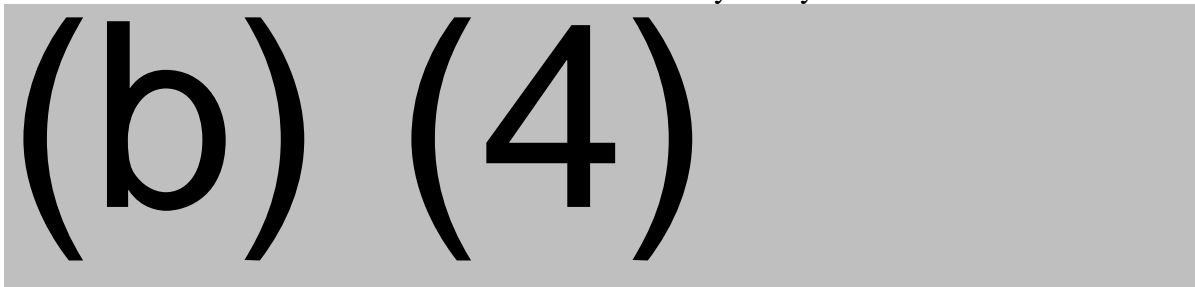
The applicant claimed that the re-analyzed precision data meets the pre-determined validation criteria outlined in the original B55 validation protocol, i.e., the coefficient of variation (CV) is (b) (4) therefore, the method is considered suitable for its intended purpose in supporting the lot release and stability of the AIGIV product.

5. SOURCES OF CLINICAL DATA AND OTHER INFORMATION CONSIDERED IN THE REVIEW

All data sources are included in the applicant's eCTD submission located in the FDA/CBER Electronic Document Room (EDR).

5.1 Review Strategy

Table 2 summarizes all the studies submitted in support of the product efficacy. Table 3 summarizes demographics of studies relevant to product efficacy in animal models and Table 4 reports clinical studies which provide safety data for AIGIV. The objective of these studies is to pursue the product for licensure under the Animal Rule.

This review memo focuses on the efficacy analyses of one animal study conducted in cynomolgus monkeys (b) (4)-828), two animal studies conducted in rabbits (b) (4)-1182 and (b) (4)-1207), as well as the safety analyses of a healthy volunteer study (AX-001).

5.2 BLA/IND Documents That Serve as the Basis for the Statistical Review

This BLA is based on IND 11982 which included studies (b) (4)-828, (b) (4)-1182, (b) (4)-1207, and AX-001.

The following documents in the BLA submission were reviewed:
- 2.2 Introduction
- 2.5 Clinical Overview
- 2.7 Summary of Clinical Efficacy
- 2.7.4 Summary of Clinical Safety
- 2.7.6 Synopses of Individual Studies
- 5.3.3.1 Report of Human Pharmacokinetic Studies (AX-001)
- 5.3.5.1 Study Reports of Controlled Clinical Studies Pertinent to the Claimed Reports of Efficacy and Safety Studies ((b) (4)-828, -1182, (b) (4)-1207)

5.3 Table of Studies/Clinical Trials

Table 2: Summary of All Studies Providing Information Relevant to Product Efficacy

Study No.	Study Title	Study Location	Study Objective	Regulatory Status
Rabbits				
(b) (4)-677-G005681	Determination of Dose and Time Range Efficacy of Anthrax Immune Globulin (AIG), AIGIV In Rabbits Exposed to Inhalation Anthrax	4.2.1.1 Primary Pharmacodynamics	Dose range efficacy	GLP
(b) (4)-1207-100005104	Therapeutic Efficacy of Anthrax Immune Globulin Intravenous (AIGIV), AIGIV in Rabbit Model of Inhalation Anthrax	5.3.5.1 Controlled Studies Pertinent to the Claimed Indication	Pivotal therapeutic efficacy in symptomatic rabbits	GLP
(b) (4)-898-G005681	Determination of Delayed Time course Efficacy of Anthrax Immune Globulin (AIG), AIGIV given in Combination with Levofloxacin In Rabbits Exposed To Inhalation Anthrax	4.2.1.1 Primary Pharmacodynamics	Added benefit study	Non-GLP
(b) (4)-1079-G005681	Determination of Added Benefit of AIGIV over Levofloxacin by Delayed Treatment in New Zealand White Rabbits Exposed to Inhalational Anthrax	4.2.1.1 Primary Pharmacodynamics	Added benefit study	Non-GLP
(b) (4)-1182-100011472	Efficacy Evaluation of AIGIV (AIGIV) in Combination with Levofloxacin when administered at 96 h Post-exposure in the Rabbit Model of Inhalational Anthrax	5.3.5.1 Controlled Studies Pertinent to the Claimed Indication	Pivotal added benefit study	GLP
Cynomolgus Macaques				
(b) (4)-828-G005780	Determination of Dose Range Efficacy of Anthrax Immune Globulin (AIG), AIGIV in Cynomolgus Monkeys Exposed to Inhalation Anthrax: GLP Study	5.3.5.1 Controlled Studies Pertinent to the Claimed Indication	Pivotal therapeutic efficacy	GLP
(b) (4)-987-G005780	Therapeutic Efficacy of AIGIV given in Combination with Ciprofloxacin against Inhalation Anthrax Challenge in Cynomolgus Monkeys: Non-GLP	4.2.1.1 Primary Pharmacodynamics	Added benefit study	Non-GLP
Human				
BB-IND13026	Investigational New Drug Application (IND) Protocol: Use of Anthrax Immune Globulin Intravenous (Human) (AIGIV) for Treatment of Systemic Anthrax	1.4.4 Cross reference to other applications	Treatment plan for AIGIV in patients with severe, systemic anthrax with collection of anthrax patient data	Expanded access program held by CDC
Patient Experience Report	Anthrax Immune Globulin Intravenous (AIGIV) Patient Experience Report	5.3.5.2 Study Reports of Uncontrolled Clinical Studies	Summary of available data and descriptive information of anthrax patients treated with AIGIV	Expanded access program held by CDC

Source: Section 2.7: Summary-clin-efficacy-treatment-of-toxemia-associated.pdf, pages 22-26.

Table 3: Demographics of All Studies Providing Information Relevant to Product Efficacy in Animal Models

Study Identifier	Animal Weight (kg)	Number of animals	No./ Group/ Dose	No./ Sex	Mean LD_{50} (LD/animal)	Average MMAD (μm)	Trigger for Treatment	AIGIV dose (U/kg)
Rabbits								
(b) (4) 677-G005681 Dose and Time Range Efficacy in Rabbits (GLP)	2.5–3 3	122	14	7	265 ±37	1.17	Fixed time post-exposure; 20 hours and 30 hours	7.5, 15, 30
(b) (4) 1207-1000005104 Efficacy of in Rabbits (GLP)	2.3–3 5	110	50	25	194 ±33	1.2	At the onset of toxemia (PA)	15
(b) (4) 898-G005681 Delayed Time-Course Efficacy in Combination with Levofloxacin in Rabbits (Non-GLP)	2.6–3 2	73	8	4	178 ±40	0.95	Time Course /symptomatic animals	15
(b) (4) 1079-G005681 Added Benefit over Levofloxacin with Delayed Treatment in Rabbits (Non-GLP)	2.4-3.2	246	10–36	5-18	282 ±71	1.11	Time course-delayed administration	15
(b) (4) 1182-100011472 Efficacy in Combination with Levofloxacin at 96 h Postexposure in the Rabbit (GLP)	2.6–3.6	336	168	31-33	238 ±49	1.1	96 hours post-exposure	15
Cynomolgus Macaques								
(b) (4) 828-G005780 Dose Range Efficacy of in Cynomolgus Macaques (GLP)	2.1–3.1	64	16	8	154 ±40	1.05	At the onset of toxemia (PA)	7.5, 15, 30
(b) (4) 987-G005780 Therapeutic Efficacy in Combination with Ciprofloxacin in Cynomolgus Macaques (Non-GLP)	2.0-4.3	72	20	10	366 ±115	1.30	At the onset of toxemia (PA)	15, 30

Source: Section 2.7: Summary-clin-efficacy-treatment-of-toxemia-associated.pdf, pages 24-25.

Table 4: Clinical Studies Providing Safety Data for AIGIV

Study Identifier	Study Type	No. of Subjects	Study Location	Study Objective
AX-001	Phase 1, safety and PK	74 AIGIV 18 Placebo	5.3.3.1 Healthy Subject PK and Initial Tolerability Study Reports	Primary: To assess the pharmacokinetics of three doses of AIGIV (210 U, 420 U and 840 U) Secondary: To evaluate the safety of AIGIV based on adverse events and laboratory values; to determine the pharmacokinetic dose proportionality relation of three different doses.
BB-IND13026	Expanded Access Program	19	Patient Experience Report in 5.3.5.2 Study Reports of Uncontrolled Clinical Studies	To provide a treatment plan for emergency use of AIGIV in a person with severe, systemic anthrax.

Source: Section 2.7: Summary-clin-safety.pdf, pages 6.

5.4 Consultations

5.4.1 Advisory Committee Meeting (if applicable)

The advisory committee meeting has been waived.

6. DISCUSSION OF INDIVIDUAL STUDIES/CLINICAL TRIALS

6.1 Trial #1: (b) (4)-828

6.1.1 Objectives

The objective of this study was to determine the dose related efficacy of treatment with AIGIV in cynomolgus monkeys exposed to *Bacillus anthracis* (Ames strain) spores by aerosol route.

6.1.2 Design Overview

A total of 68 (34 male, 34 female) juvenile specific pathogen-free cynomolgus macaques were purchased. Sixty-four (64) monkeys randomized to 4 groups of 16 animals: the control group, the low dose group (7.5U/kg), the medium dose group (15U/kg), and the high dose group (30U/kg). An equal number of male and female monkeys were included in each group (Table 5). All 64 animals were exposed to 154 ($\pm$ 40) LD_{50} of aerosolized spores. Following challenge, each monkey was monitored for clinical signs of disease including abnormal body temperature, altered activity levels, outward clinical signs of disease, hematological abnormalities, abnormal C-reactive protein (CRP) levels, positive bacteremia cultures, presence of *Bacillus anthracis* DNA via (b) (4), and circulating levels of *Bacillus anthracis* PA as assessed by an (b) (4) .

Table 5: Treatment groups in (b) (4)-828

Group	Treatment	Dose (U/kg)	# of animals
1	IGIV	N/A	16
2	AIGIV	7.5	16
3	AIGIV	15.0	16
4	AIGIV	30.0	16

6.1.3 Population

Juvenile specific pathogen-free cynomolgus monkeys (*Macaca fascicularis*) of Vietnamese origin were purchased from (b) (4)   Age was not a criterion for placement on study. Monkeys weighed a minimum of 2.1 kg prior to aerosol challenge. Females were nulliparous and non-pregnant.

6.1.4 Study Treatments or Agents Mandated by the Protocol

The investigated product was a pre-formulated AIGIV solution in frozen form provided by the applicant at a concentration of approximately 2.73 U/mL of AIG (potency determined by TNA). It was provided in 50 mL glass containers with an extractable drug volume of (b) (4)

The control was normal human IGIV.

All aerosol challenges occurred with a well-characterized, single lot of *B. anthracis* (Ames strain) spores. The target aerosol exposure for this study was 200 LD$_{50}$ *Bacillus anthracis* (Ames strain) spore equivalents. The average aerosol exposure dose for all 64 animals was 154 ± 40 *Bacillus anthracis* (Ames strain) LD$_{50}$ equivalents.

6.1.6 Sites and Centers
This study was performed at the (b) (4)
                                        transmitter surgical implantation, venous access port (VAP) surgical implantation, and tissue preparation and histological assessments were performed at (b) (4)

6.1.8 Endpoints and Criteria for Study Success

Mortality attributed to anthrax through Day 28 post-challenge was the primary endpoint used to determine the protective benefit of AIGIV.

Secondary efficacy endpoints include median time to death for each group and the percentage of animals positive for bacteremia prior to treatment.

6.1.9 Statistical Considerations & Statistical Analysis Plan

For the primary efficacy analysis, Fisher's exact test was applied to compare each of the three treatment group survival rates to the control group using a one-sided alpha level of 0.05. To address the multiple comparisons between the treated groups and the control group, Bonferroni-Holm adjusted p-values were also calculated. No adjustments for multiple comparisons were made for any of the other analyses.

Secondary efficacy analyses include analysis of the median time to death for each group. Point estimation with 2-sided 95% confidence intervals was provided. The difference in time to death between study groups were also assessed through a two-sided log-rank test. In addition, the primary efficacy analysis was repeated for subjects positive for bacteremia prior to treatment.

6.1.10 Study Population and Disposition

6.1.10.1   Populations Enrolled/Analyzed

Sixty-one (61) of the 64 (95%) animals challenged exhibited positive blood cultures following challenge. Three animals (28560 [Group 2], 28943 [Group 4], and 28654 [Group 4]) that did not exhibit a positive culture were excluded from the efficacy analysis.

Animals 28943 and 28584 were not toxemic prior to treatment. Animal 28560 had excised its telemetry implant and was found unresponsive 24 days following challenge. Based on lack of findings consistent with *Bacillus anthracis* infection, the pathologist concluded that this animal's death was not caused by anthrax.

6.1.11 Efficacy Analyses

6.1.11.1 Analyses of Primary Endpoint(s)

Ninety-four (94) percent (15/16) of the untreated control animals (Group 1) died following challenge with median time-to-death of 127.6 h.

Group 2 had 11 deaths out of 15 animals (73% mortality) attributed to anthrax through Day 28 post-challenge with a median time-to-death of 132.8 h. Animal 28560 was excluded from the statistical analysis of survival.

Group 3 exhibited a mortality rate of 56% (9/16 animals died following challenge) with a median time to death of 156.7 h.

Group 4 showed a mortality rate of 29% (4/14 animals died following challenge) and not enough animals died in order to calculate a median time-to-death, as the median time-to-death calculation includes survivors. Animals 28943 and 28584 were excluded from the statistical analysis of survival.

The survival rates for all four groups are summarized in Table 6.

Table 6: Survival rates and p-values from One-sided Fisher's Exact Test

Group	Number animals Survived/Total	Survival rate (95% CI)	Unadjusted p-value	Bonferroni-Holm adjusted p-value
1	1/16	0.06 (0.00, 0.30)	-	-
2	4/15	0.27 (0.08, 0.55)	0.1462	0.1462
3	7/16	0.44 (0.20, 0.70)	0.0186	0.0372
4	10/14	0.71 (0.42, 0.92)	0.0003	0.0009

A secondary mortality analysis was performed that included only animals that were positive for bacteremia prior to treatment. Though PA was detected in all animals prior to treatment, some animals were not bacteremic prior to treatment.

Of the 11 Group 1 animals bacteremic prior to treatment all succumbed to disease (0% survival). Eleven (11) Group 2 animals were positive for bacteremia prior to treatment and 4 animals (36%) survived. Fourteen (14) Group 3 animals were confirmed bacteremic prior to treatment and 6 animals (43%) survived through Day 28. Ten (10) Group 4 animals were bacteremic prior to treatment and 7 of these animals (70%) survived. Statistical analysis of survival results that include only the animals bacteremic prior to treatment confirmed that when comparing Group 2 (p-value =0.0451), Group 3 (p-value = 0.0170), and Group 4 (p-value = 0.0010) to the IGIV treatment group, all AIGIV treatment groups had significantly higher percentage of survival.

Table 7 summarizes the survival rates and one-sided Fisher's exact test comparisons of survival rates when only the animals bacteremic prior to treatment are included.

Table 7: Survival rates and p-values of One-sided Fisher's Exact Test, excluding animals not bacteremic prior to treatment

Group	Number bacteremic animals Survived/Total	Survival rate (95% CI)	Unadjusted p-value	Bonferroni-Holm adjusted p-value
1	0/11	0.00 (0.00, 0.28)	-	-
2	4/11	0.36 (0.11, 0.69)	0.0451	0.0451
3	6/14	0.43 (0.18, 0.71)	0.0170	0.0339
4	7/10	0.70 (0.35, 0.93)	0.0010	0.0031

The study results support that a single dose of AIGIV administered as monotherapy following the onset of clinical disease was highly efficacious in enhancing the survival of cynomolgus monkeys with symptomatic inhalational anthrax. In addition to increasing survival, treatment with AIGIV also appeared to resolve bacteremia.

Reviewer Comment: This statistical reviewer verified the survival rate and p-value calculations in Table 6 and Table 7. The applicant claims significance based on a one-sided test with alpha level of 0.05. However, conventionally for one-sided hypothesis testing, the alpha level should be set as 0.025. Using unadjusted p-values summarized in Table 5, the survival rates in the groups with the two highest treatment doses (Groups 3 and 4) were significantly greater than that in the control group since both p-values are less than 0.025. However, after the Bonferroni-Holm adjustment, only the difference between the Group 4 and the control group remained significant, i.e., the p-value is less than 0.025. The p-value of Group 3 is slightly higher than 0.025. Similar results were also observed in Table 6. The point estimates, as well as the 95% CIs, show that Group 4 achieves a much higher survival rate compared with Group 3.

It is also observed that the survival rate increases as a linear function of the dose in this study. The applicant chose the medium dose (15 U/kg) in the following two rabbit studies (b) (4)-1182 and (b) (4)-1207) and had its efficacy confirmed. However, the benefit in

the survival rate from the high dose for cynomolgus macaques is noticeable. This statistical reviewer defers to the product officer and/or the clinical reviewer to select the optimal dose for human beings.

6.2 Trial #2: (b) (4)1182

6.2.1 Objective

The objective of this study was to assess the therapeutic efficacy of the combination treatment of AIGIV and levofloxacin over that of Immune globulin intravenous (IGIV) and levofloxacin when either treatment was initiated at 96 h after aerosol exposure to a lethal dose (200 x LD_{50}) of *Bacillus anthracis* spores in rabbits.

6.1.2 Design Overview

This was a two-stage group sequential study with an information-based sample size re-estimation. An Independent Reviewer determined whether the second stage should be added. In the first stage (Stage I) 336 rabbits were exposed to a target aerosol dose of 200 x LD_{50} *Bacillus anthracis* (Ames strain) spores. All the animals surviving to 96 h post exposure received oral levofloxacin treatment. Approximately half of these animals were treated with the control article (IGIV), while the other half received the test article (AIGIV).

Rabbits were selected based on body weight and they were randomized to 14 cohorts each containing 24 animals. An equal number of male and female rabbits were randomized to each cohort. Secondly, animals that were randomized to the cohorts were further randomized for the order in which they were to be exposed to spores on a given aerosol exposure day (A to N). The final randomization (for treatment group assignment) was done manually at the treatment time of 96 h post exposure for the surviving animals. There were 23 animals replaced. Animals L43175 was replaced due to having been exposed to an incorrect aerosol challenge dose, and L52940 was replaced due to exhibiting contaminants in the pre-challenge blood sample. All the other 21 animals were replaced prior to challenge due to nonfunctional venous access ports (VAP).

Randomization of IGIV and/or AIGIV was performed with Stata® statistical software.

6.2.3 Population

Specific pathogen-free New Zealand White Rabbits (Oryctolagus cuniculus) with surgically-implanted VAPs were purchased from (b) (4)
Rabbits used in the study weighed between 2.58 and 3.57 kg on the day of exposure to *Bacillus anthracis* spores. Age was not a criterion for placement of animals on the study.

6.2.4 Study Treatments or Agents Mandated by the Protocol

A pre-formulated solution of AIGIV in frozen form was provided by the applicant at a concentration of approximately 3.19 U/mL of AIGIV (potency confirmed by TNA). The test article was provided in 50mL glass containers with an extractable drug volume of 23.85mL (actual fill volume of 24.65mL).

The control was normal human IGIV.

Levaquin® Oral Solution (levofloxacin 25 mg/mL; lot number CDB3QOO; expiration date Mar 2014), commercially-available from Ortho-McNeil, was used. It was stored at room temperature (between 15°C and 30°C, inclusive). It is a multi-use self-preserving aqueous solution of levofloxacin with pH ranging from 5.0 to 6.0.

All aerosol exposures occurred with a well-characterized, single lot of *Bacillus anthracis* spores (Ames strain; Lot B-37) prepared according to (b) (4) SOP Number (b) (4). X-072.

6.2.6 Sites and Centers

This study was performed by (b) (4) located in (b) (4) . Histopathology was performed by (b) (4) . Serum specimen testing for pharmacokinetics and immunogenicity of the test article was conducted by Bio/Immuno-Assay Development, Cangene Corporation located in Winnipeg, Manitoba, Canada. Pharmacokinetic analysis was performed at (b) (4) .

6.2.8 Endpoints and Criteria for Study Success

The primary endpoint is the survival rate at 36 days post-challenge.

Secondary endpoints include:
- Time to death - defined as the number of hours from the end time of that animal's exposure to Bacillus anthracis spores to the time of death.
- Time to treatment - defined as the number of hours from the end time of that animal's exposure to Bacillus anthracis spores to the end of intravenous infusion.
- Time to onset of bacteremia - defined as the number of hours from the end time of an animal's exposure to anthrax spores to the time of that animal's first positive bacteremia result. If an animal does not have a positive bacteremia result before study termination or death, this animal will be censored at the last time point that is assessed.
- Time to recovery from bacteremia - defined as the number of hours between end of intravenous infusion and the first negative result for bacteremia post-treatment that does not have a positive sample preceding it. If an animal is positive for bacteremia and does not recover before study termination or death, this animal will be censored at the last time point assessed.
- Time to onset of toxemia - defined as the number of hours from the end time of an animal's exposure to anthrax spores to the time of that animal's first positive toxemia result. If an animal does not have a positive toxemia result before study termination or death, this animal will be censored at the last time point that is assessed.
- Time to recovery from toxemia - defined as the number of hours between end of intravenous infusion and the first negative result for toxemia post-treatment that does not have a positive sample preceding it. If an animal is positive for toxemia

and does not recover before study termination or death, this animal will be censored at the last time point assessed.

- Toxin levels over time – quantitative toxin results analyzed by electrochemiluminescence assay will be used to assess the treatment effect on toxin levels over time. Values less than the limit of detection will be replaced with one half of the limit of detection for analysis.

6.2.9 Statistical Considerations & Statistical Analysis Plan

Study Design and Sample Size Determination

This was a two-stage group sequential study design. An interim analysis was performed on the MITT set after Stage 1. The total sample size was planned to be re-estimated based on the target maximum Fisher information (I_{max}) of this study. The Fisher information was approximated by the inverse of the estimated variance of the treatment effect δ in this study. The applicant selected I_{max}=95 and provided its justification in SAP.

The sample size calculation for Stage I assumed a 40% absolute treatment effect (survival rates of 30% in the IGIV + levofloxacin group and 70% in the AIGIV + levofloxacin group), 80% power and a 5% probability of type I error which resulted in a sample size of 30 animals per arm. Because about 80% of rabbits would die before the treatment was initiated at 96 h after aerosol exposure to a lethal dose (200 x LD_{50}) of *Bacillus anthracis* spores and about 10% of rabbits who are alive at 96 h would not be both toxemic and bacteremic prior to treatment, the sample size was adjusted upwardly to enroll 168 animals per treatment group (336 animals total) for Stage I in order to ensure that the required number of animals will survive to be treated.

Populations
Two analysis sets were used; the Intent-to-Treat (ITT) set and the Modified Intent-to-Treat (MITT) set. The MITT set was the set used for the primary analysis and was used to generate the study conclusions. The ITT set was used to assess the robustness of the conclusions generated by the MITT set.

The ITT set includes animals that have been successfully exposed to anthrax and survive to receive full intravenous infusion of the assigned treatment (AIGIV or IGIV) following established toxemia. An animal that dies only due to reasons completely unrelated to anthrax exposure or treatment related toxicity will be excluded based on the pathologist and/or Study Director's recommendation.

The MITT set includes all animals which satisfy the criteria for inclusion in ITT set and were positive for both toxemia and bacteremia at least once prior to treatment and survived to receive full infusion dose (either IGIV or AIGIV). The MITT set excludes animals that were not bacteremic at least once prior to the treatment.

Hypotheses Testing Procedure
This is two-sided hypotheses testing procedure. The null hypothesis is that there is a similar survival rate in the AIGIV + levofloxacin treated animals and the IGIV +

levofloxacin treated animals. The alternative hypothesis is that the survival rate in AIGIV + levofloxacin is either higher or lower than the survival rate in the IGIV + levofloxacin treated group.

Statistical Methodologies

For the primary efficacy analysis, the proportions of surviving animals and Clopper-Pearson 95% CIs (based on the exact binomial distribution) were calculated for each group. A two-sided Z-test comparing two proportions was performed to determine if the proportions of surviving animals in each group significantly differed from each other.

The applicant proposed to examine the primary endpoint by 2-sided Fishers' exact test at the 5% significance level at the final analysis stage based on the MITT population dataset in order to confirm the robustness of the test result and conclusions based on the primary analysis using the normal approximation. In addition, the primary endpoint and time-to-death endpoint were examined in ITT population.

As the loss-to-follow-up is expected to be minimal (animals are held in a secure location and will be accounted for at the end of study), missing values were not imputed for the primary endpoint analysis. The missing values were treated as censored observations. When performing summary statistics, all missing values were excluded.

Interim Analysis
An interim analysis was executed after Stage 1 to determine if Stage 2 was required and, if so, how many animals would be challenged in Stage 2. The sample size was re-estimated as 93 and the total number of animal needed was 482. The interim statistical analysis concluded that the study should continue to Stage 2 with 146 additional animals (482 total animals – 336 Stage 1 animals). However, the study was stopped after Stage 1 at the direction of the applicant following discussion with the FDA because there may not be sufficient power to demonstrate added benefit resulting from the AIGIV treatment. A much larger sample size would be required in order to adequately power the study based on the observed effect size from the interim analysis. Therefore, in order to avoid sacrificing more animals, CBER suggested the applicant not to proceed to Stage 2. (See the meeting minutes of CRMTS 9053).

6.2.10 Study Population and Disposition

6.2.10.1   Populations Enrolled/Analyzed

6.2.10.1.1 Demographics
Rabbits used on the study weighed between 2.58 to 3.57 kg on the day of exposure to *Bacillus anthracis*. Three hundred and thirty six (336) rabbits (168 male, 168 female) were used in this study (exposed to spores).

6.2.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

6.2.10.1.3 Subject Disposition

Eighty-four (84) animals survived to receive at least a single dose of levofloxacin, comprising 25% of the animals exposed to *Bacillus anthracis* via the inhalational route. Of these 84 animals, three rabbits died prior to start of IV infusion; therefore, 81 animals were included in the ITT analysis set. A total of 14 animals in the ITT analysis set died during infusion or did not complete a full infusion. In addition, three animals in the ITT analysis set were not bacteremic and toxemic at least once prior to treatment. Thus, 64 animals were included in the MITT analysis set, in which 31 animals received AIGIV and 33 animals received IGIV.

6.2.11 Efficacy Analyses

6.2.11.1 Analyses of Primary Endpoint(s)

Of the 33 animals in the MITT analysis set who received IGIV, 13 animals survived through 36 days post-exposure (39% survival). Of the 31 animals in the MITT analysis set who received AIGIV, 18 animals survived through Day 36 (58% survival). The Z-test indicated that there was no significant difference in the survival proportions between the IGIV and AIGIV groups (Table 9). A similar conclusion was drawn based on Fisher's Exact test.

Table 9: Summary of survival rate in (b) (4)-1182 (MITT)

Group	Number animals survived/total	Survival rate (95% CI)	Z-test p-value	Fisher's Exact Test p-value
IGIV	13/33	0.39 (0.23, 0.58)	-	-
AIGIV	18/31	0.58 (0.39, 0.75)	0.1353	0.2106

6.2.11.2 Analyses of Secondary Endpoints

The median time to death for IGIV treated animals was estimated to be 175.1 hours post-exposure. As less than half of the animals administered AIGIV succumbed to disease, a median time to death could not be estimated.

6.3 Trial #3 : (b) (4)-1207

6.3.1 Objectives

The primary objective of this study was to determine the efficacy of AIGIV in comparison to IGIV when treatments were administered after the first detection of PA in serum.

The secondary objective of this study was to determine the pharmacokinetics and immunogenicity of AIGIV in the rabbits exposed to inhalation anthrax.

6.3.2 Design Overview

One hundred and ten (55 male and 55 female) rabbits were challenged with Bacillus anthracis (Ames strain) spores via aerosol exposure. The exposed animals were treated with either a single intravenous infusion of 15 U/kg of AIGIV or an equivalent volume of IGIV (50 rabbits per treatment group) at the onset of toxemia (PA detection). The remaining 10 animals served as process controls.

Randomization of study rabbits is described in Table 10:

Table 10: Randomization of Study Rabbits

Experimental group	No. of Animals per Cohort					Total per Group
	Cohort A	Cohort B	Cohort C	Cohort D	Cohort E	
Untreated control	2	2	2	2	2	10
AIGIV	10	10	10	10	10	50
IGIV	9	10	10	11	10	50
Total per cohort	21	22	22	23	22	110

This study was conducted in a blinded fashion such that the study director, technicians performing the dosing, technicians observing the animals, and microbiologists did not know the treatment group identity of animals during the in-life phase of the study. The study pathologist was blinded until all histopathology slides were read. The study was unblinded after the study pathologist had read all of the slides and the pathology findings were peer reviewed.

6.3.3 Population

Specific pathogen-free New Zealand White Rabbits (Oryctolagus cuniculus) with surgically-implanted VAPs were purchased from (b) (4) Rabbits weighing > 2.5 and < 3.5 kg were included in the study.

6.3.4 Study Treatments or Agents Mandated by the Protocol

The average challenge dose received by the animals was 194 ± 33 x LD$_{50}$. The investigated article is AIGIV (Lot 11007147). The control article is normal human IGIV (Immune Globulin Intravenous; Lot 10703403).

6.3.6 Sites and Centers

This study was performed by (b) (4) . Histopathology was performed by (b) (4) . Pharmacokinetic and immunogenicity sample analysis were conducted by Cangene Corporation in Winnipeg, Manitoba, Canada.

6.3.8 Endpoints and Criteria for Study Success

The proportion of animals that survived to day 36 was the primary endpoint used to determine the efficacy of AIGIV over IGIV.

Secondary endpoints include time to death, time to treatment, bacteremia, and toxemia.

6.3.9 Statistical Considerations & Statistical Analysis Plan

Populations
Two analysis sets were used: the ITT set and the MITT set. Primary efficacy analyses of this study were based on the MITT population.

The ITT set included rabbits that were successfully exposed to *Bacillus anthracis* spores and survived to receive either full or partial intravenous infusion of the assigned treatment (AIGIV or IGIV) following established toxemia.

The MITT set included all animals who satisfy the criteria for inclusion in the ITT set, however it excludes animals that a) did not receive full dose of either AIGIV or IGIV, b) were not bacteremic at least once prior to the treatment and c) any animal that died due to reasons completely unrelated to anthrax exposure or treatment related toxicity based on the pathologist/study director recommendation.

Hypothesis Testing Procedure
The null hypothesis is that there is a similar survival rate in the AIGIV treated animals and the IGIV treated animals. The alternative hypothesis is that the survival rate in AIGIV is either higher or lower than the survival rate in the IGIV treated group.

Sample Size Determination
Based on results from previous studies conducted by the applicant (Study 677 and 1079), it was assumed that 20% of the anthrax exposed animals may not be bacteremic prior to treatment, and none of the animals survive in the IGIV control group once they develop bacteremia. Based on these assumptions, a sample size of 50 animals per group will result in at least 40 animals per group being bacteremic prior to treatment. This sample size allows more than 80% power to detect a difference between the group survival rates of 0% and 20% in IGIV and AIGIV groups, respectively at a significance level of 5%. The test statistic used is the two-sided Fisher's exact test.

Statistical Methodology
For the primary analysis, the proportion of animals that survive in each group was calculated along with a two-sided 95% confidence interval using the exact binomial distribution. The proportion of animals that survive in each treatment group was compared using a two-sided Fisher's Exact Test.

For secondary endpoints, the medians were determined along with a two-sided 95% confidence interval for each study group using the product-limit method, and survival curves for each treatment group were provided on a Kaplan-Meier plot. The survival curves were compared using a two-sided log-rank test.

6.3.10 Study Population and Disposition

6.3.10.1   Populations Enrolled/Analyzed

6.3.10.1.1 Demographics

The rabbits weighed between 2.3 and 3.5 kg on the day of challenge (Rabbit L49347 was below the protocol directed weight limit of 2.5 kg on the day of challenge). Among the 110 rabbits used in this study, 55 were male and 55 were female.

6.3.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

6.3.10.1.3 Subject Disposition

One hundred and ten rabbits were challenged and only 100 rabbits were treated. Additional animals (30 extra) were purchased in case at any time up to the initiation of the study (aerosol exposure to anthrax spores), rabbits needed to be replaced. Rabbits were identified by ear tags and cage cards.

One animal was replaced after initiation of the study. During the infusion process Rabbit L49372 chewed through the infusion line in such a way that the line could not be replaced. The study director determined that the animal should be taken off study and replaced in a subsequent challenge cohort resulting in a deviation, DR-12550.

Two animals in the IGIV treatment group were never bacteremic prior to treatment (Animals L49304 [Cohort A] and L43313 [Cohort D]). The MITT population includes 50 subjects in the AIGIV group and 48 subjects in the IGIV group.

6.3.11 Efficacy Analyses
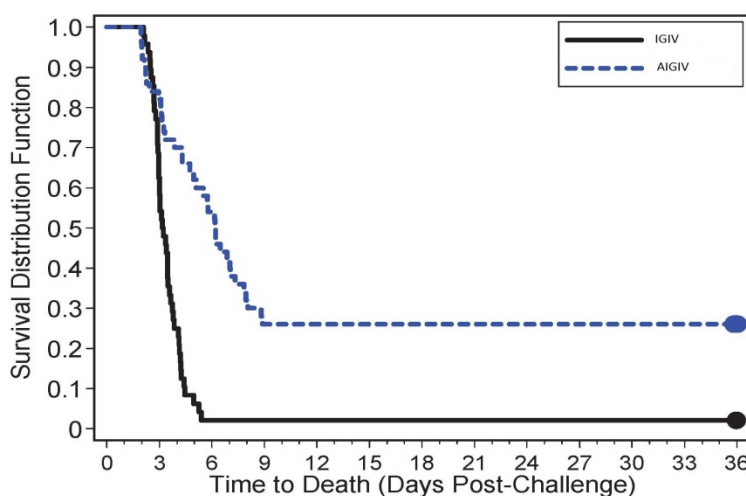
6.3.11.1 Analyses of Primary Endpoint(s)

All rabbits (50/50) in the AIGIV group and 96% (48/50) in the IGIV group were bacteremic prior to treatment. Of the animals that were bacteremic prior to treatment, 26% (13/50) that received AIGIV survived to the end of the study while only 2% (1/48) rabbits that received IGIV survived (Table 11). The two-sided p-value of Fisher's exact test is 0.0008612, indicating that the proportion of survivors in the AIGIV group was significantly greater than that in the IGIV group.

Table 11: Summary of survival rate in (b) (4)-1207 (MITT)

Group	Number animals survived/total	Survival rate (95% CI)	2-sided Fisher's exact test p-value	2-sided log-rank test for time to death p-value
IGIV	1/48	0.02(0.00, 0.12)	-	-
AIGIV	13/50	0.26 (0.16, 0.40)	0.0008612	<0.0001

Figure 1 displays the Kaplan-Meier curves associated with time to death for each treatment group (IGIV and AIGIV), when considering treated animals that were received a full dose of either IGIV or AIGIV and bacteremic at least once prior to treatment (MITT data set).

Figure 1: Kaplan-Meier curves of Time-to-Death (MITT)

6.3.11.2 Analyses of Secondary Endpoints

The median times to death were 75.8 and 148.5 h post-challenge for IGIV and AIGIV-treated animals, respectively. The log-rank test shows that overall survival and time to death were significantly greater in the AIGIV group.

If the animal that received a partial dose of IGIV (L49372) and the two animals that were not bacteremic prior to infusion (both from the IGIV group) are included in the analysis (ITT data set), the proportion of survivors (p<0.0009, two-sided Fisher's exact test) and time to death (p<0.0001, two-sided log-rank test) in the AIGIV group were still significantly greater than those in the IGIV group.

There was no significant difference between the treatment groups in the median time from challenge until toxemia (first instance of PA detected in the serum post-challenge; 24.1 h for IGIV group and 24.3 h for AIGIV group) or from challenge until bacteremia (24.3 h for IGIV group and 24.7 h for AIGIV group). There was also no significant difference in the PA levels just prior to treatment (23.02 ng/mL for IGIV group and 26.29 for AIGIV group; geometric means). However, after treatment the proportion of rabbits that were toxemic or bacteremic and the levels of circulating PA were significantly decreased in the AIGIV group. The time to toxemia or bacteremia resolution was also significantly shorter in rabbits that received AIGIV.

Individual elimination half-life values ranged from 0.190 to 2.40 days with a mean of 0.897 days for the males and from 0.160 to 2.27 days with a mean of 1.01 days for the females with the (b) (4) data. The individual elimination half-life values ranged from 0.242 to 2.83 days with a mean of 1.1 days for the males and from 0.235 to 2.30 days with a mean of 1.26 days for the females with the TNA data.

6.4 Trial #4: AX-001

6.4.1 Objectives

The primary objective of the study was to assess the pharmacokinetics of three doses of AIGIV (210 U, 420 U and 840 U by TNA) in healthy volunteers.

Secondary objectives of the study were to evaluate the safety of AIGIV as well as to determine the pharmacokinetic dose proportionality relation of three different doses of AIGIV.

An additional objective was to assess differences in blood glucose levels to determine whether the maltose content in AIGIV interferes with accurate measurement of blood glucose by GNS-POC glucose monitoring devices.

6.4.2 Design Overview

This study consisted of two stages. The first stage was a sequential, phase 1, randomized, double-blinded, placebo controlled dose-ranging study designed to assess the pharmacokinetics and safety of three doses of AIGIV after intravenous administration to healthy volunteers. The second stage was a randomized, open-label study assessing the safety of two additional lots of AIGIV at the highest dose.

In the first stage, 72 healthy adult male and female subjects were recruited in three cohorts of 24. In each cohort, 18 subjects were randomized to receive a single dose of AIGIV, namely 210 U (cohort 1), 420 U (cohort 2) or 840 U (cohort 3) and 6 subjects from each cohort were randomized to receive equal volume of saline placebo. Subjects were monitored over 28 days with safety laboratory tests and AE monitoring, and blood samples were taken for PK measurements. However the PK portion of this study is evaluated by the PK reviewer.

The second stage of the study was a randomized, open-label study in 20 healthy adult male and female volunteers (cohort 4). Subjects in this cohort were randomized to receive a dose of 840 U by TNA from one of two additional product lots (10 subjects per lot). There was no placebo group. The second stage was to proceed only if no safety concerns were raised by the Data Safety and Monitoring Committee (DSMB) following review of the safety data from the first stage. Safety data only (hematology, blood chemistry and urinalysis) was collected for 28 days after dosing for this stage of the study.

The following table (Table 11) describes the treatment administered in 4 cohorts:

Table 11: Treatment Groups in AX-001

Cohort	Treatment	Drug Administration
1	A	AIGIV (210U) (18 subjects) or saline placebo (6 subjects)
2	B	AIGIV (420U) (18 subjects) or saline placebo (6 subjects)
3	C	AIGIV (840U) (18 subjects) or saline placebo (6 subjects)
4	D	AIGIV (840U by TNA, lot Number 10804812) (10 subjects)
	E	AIGIV (840U by TNA, lot Number 10804816)(10 subjects)

Source: Section 5.3.5.1: AX-001-study-report.pdf, pages 44.

6.4.3 Population

To recruit healthy volunteers, subjects were included in the study only if they met all of the following inclusion criteria, unless otherwise specified:
Male or female

- Age 19 – 55 years.
- Body mass index of 19 to 29.
- For female subjects that were not surgically sterilized, willingness to use an effective method of contraception throughout the trial including:
- Use of hormonal contraception (oral, injectable or implant) continuously for 3 months prior to the start of the trial and willing to continue to use hormonal contraception throughout the entire trial.
- IUD inserted at least 3 months prior to dosing.
- For female subjects who were postmenopausal < 2 years, an FSH $\geqslant 40$ mIU/mL must be obtained. If the FSH was < 40 mIU/mL, the subject must agree to use an acceptable form of contraception (see above).
- For males that did not have a vasectomy, willingness to use a condom with spermicide for the duration of the study. Also, male subjects must not donate sperm for the duration of the study.
- Normal and healthy as determined by medical history, physical exam, ECG, vital signs and laboratory tests of liver, kidney and hematological functions.
- Provide a written informed consent.

Subjects were excluded from the study if there was evidence of any of the following criteria at screening or anytime during the study:

- Heavy smokers (>10 cigarettes/day) or individuals who used smokeless tobacco or nicotine containing products.
- Use of any investigational product within the past 30 days.
- Recipient of any blood product within the past 12 months.
- Plasma donation within 7 days or blood donation within 56 days of baseline.
- Females with a hemoglobin level < 12 g/dL.
- Males with a hemoglobin level < 13 g/dL.
- History of hypersensitivity to blood products.
- History of allergy to latex or rubber.
- History of IgA deficiency.
- Pregnancy or lactation.

- Positive serology test for HIV or HCV, positive test for HBV as determined by HBsAg.
- History of, or suspected substance abuse problem (including alcohol).
- Failure of drug test at screening or baseline.
- Failure of alcohol test at baseline or consumption of alcoholic beverages within 48 hours of baseline.
- History of anthrax vaccination with AVA or any other anthrax vaccine.
- Individuals with planned medical procedures that were to occur during the study.
- Use of prescription medications within 7 days prior to baseline, or anticipated use during the study (with the exception of hormonal contraceptives for females).
- Use of over-the-counter or herbal medications within 7 days of study admission.
- An opinion of the investigator that it would have been unwise to allow participation of the subject in the study.

6.4.4 Study Treatments or Agents Mandated by the Protocol

AIGIV in three doses (210U, 420U and 840U) or equal volumes of saline placebo.

6.4.6 Sites and Centers

This study was a single center study.

6.4.7 Surveillance/Monitoring

An independent DSMB reviewed blinded safety data for the first three cohorts. A safety dataset consisting of up to at least day 14 post-dosing was provided to the DSMB for each cohort. A blinded analysis was prepared for the committee and the DSMB voting members provided their recommendation on whether to proceed to the next higher dosage level prior to the start of dosing for the next cohort. The decision to proceed was based on the safety and risk to the subjects.

6.4.8 Endpoints and Criteria for Study Success

All PK endpoints are reviewed by the PK reviewer.
The safety endpoints are adverse events, laboratory values and blood glucose levels measured using GS-POC and GNS-POC glucose monitoring devices.

6.4.9 Statistical Considerations & Statistical Analysis Plan

Population Defined

Two populations were defined in this study: Safety population and PK population.

Safety Population: all subjects who received treatment and provided safety information will be included in safety summaries for vital signs, ECG, clinical laboratory values and adverse events.

This memo focuses on safety results only. Therefore only safety population is discussed.

Sample Size Determination

No formal sample size calculation was performed for this study.

Statistical Methodology

Differences in the incidence of adverse events, including those temporally related to infusion, were tested across the three AIGIV cohorts and the saline placebo groups for the most frequent related adverse events. The placebo group was analyzed both as a single group of saline controls, and as three cohorts of placebo subjects, receiving different volumes of saline. Generalized Fisher's exact test was used to determine if the number of subjects who experienced a particular event in each treatment group was statistically different across the groups. The overall probability of a type I error was set at 0.05. If a difference was determined to be statistically significant, then multiple comparison methods were used, when appropriate, to determine where the significant differences lay.

Interim Analyses

Blinded safety data was examined on an interim basis throughout the study by the DSMB. A blinded interim safety report was generated after completion of all patient visits for cohorts 1-3 and was a planned event for the study. It was submitted to the FDA on August 28, 2008 (IND 11982/77).

An interim PK analysis was also performed and a report generated. This interim PK analysis was not planned and was performed at the request of the FDA (telephone contact on July 30, 2008 and submitted on September 8, 2008 (IND 11982/80)).

6.4.10 Study Population and Disposition

A total of 529 subjects were screened for this study. Ninety-two (92) subjects satisfied the screening evaluation and successfully completed the baseline visit. Seventy-two (72) subjects were randomized to cohorts 1-3 (54 subjects in the three AIGIV arms and 18 subjects in the saline control arm) and 20 subjects to cohort 4.

Please refer section 6.4.2 for definition of cohort 1 to 4.

6.4.10.1   Populations Enrolled/Analyzed

All 92 subjects were included in the safety population and used for all safety analyses.

We refer the reader to the PK review for details on the PK study but note that about half or 48 subjects were enrolled in the PK portion of the study.

6.4.10.1.1 Demographics

Table 13 summarizes the basic characteristics of subjects enrolled in this study.

Table 13: Summary of Subject Demographics in AX-001

|	Cohort 1 (N=18) Treatment A	Cohort 2 (N=18) Treatment B	Cohort 3 (N=18) Treatment C	Cohort 4 Group 1 (N=10) Treatment D	Cohort 4 Group 2 (N=10) Treatment E	All placebo (N=18)
Gender						
Male	10	9	9	5	4	11
Female	8	9	9	5	6	7
Age (years)						
Mean	30	29	32	29	34	32
SD	10	10	13	12	15	11
Race						
White	12	13	13	9	9	13
Black or African American	3	4	3	0	1	2
Asian	3	1	0	1	0	2
American Indian / Alaska native	0	0	2	0	0	1
Ethnicity						
Hispanic or Latino	1	1	1	0	0	0
Not Hispanic or Latino	17	17	17	10	10	18
Weight (kg)						
Mean	73.2	71.5	74.6	71.0	68.1	75.0
SD	7.6	12.1	12.1	11.6	10.6	11.0
Height (cm)						
Mean	171.4	170.6	173.3	170.6	166.0	173.6
SD	8.7	11.6	8.5	10.6	12.9	9.2

6.4.10.1.3 Subject Disposition

All 92 subjects received at least partial doses. Eighty-seven (87) subjects completed the trial. Eighty-eight (88) received the entire planned infusion volume and four subjects received partial infusions.

- Subjects No. (b) (6) (cohort 1) and (b) (6) (cohort 1) had their infusion stopped 50.5 min and 5 min, respectively, after the start of the infusion due to infiltration and pain distal to IV site, respectively. These two subjects did not receive the full dose of AIGIV and were withdrawn from the study.
- Subject No (b) (6) (cohort 2) had his/her infusion stopped after 23.25 minutes due to adverse events judged by the investigator to be related to study treatment. Subject (b) (4) completed study procedures, but did not have serum product concentration drawn.
- Subject No (b) (6) (cohort 3) had his/her infusion stopped after 2.72 minutes due to adverse events judged by the investigator to be related to study treatment. Subject (b) (6) was removed from the study by the investigator due to chest discomfort, flushing, tachycardia, and throat tightness.

Two additional subjects were discontinued during the follow-up observation period and were withdrawn from the study due to non-compliance:

- Subject No. (b) (6) (cohort 1) did not return for visits starting on day 28
- Subject No. (b) (6) (cohort 3) did not return for visits starting on day 42.

Figure 2: Flow Chart of Disposition of Subjects (cohorts 1-3)
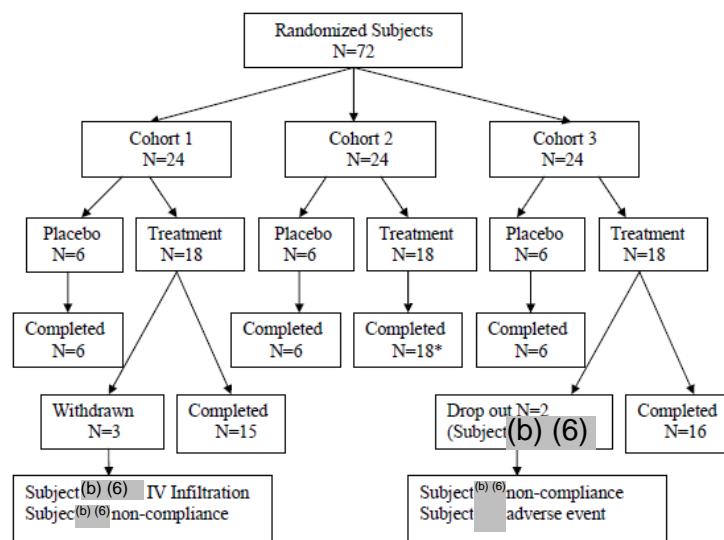
* No serum concentration data was collected for Subject 42.

Source: Section 5.3.5.1: AX-001-study-report.pdf, pages 49.

6.4.11 Efficacy Analyses

No efficacy analyses were conducted for this PK/safety study.

6.4.12 Safety Analyses

Comparisons of AEs and laboratory values between the four arms of the placebo-controlled part of the trial were planned. Differences in the incidence of adverse events, including those temporally related to infusion, were tested across the three AIGIV treatment groups and the saline placebo groups for the most frequent related adverse events. Generalized Fisher's exact test was used to determine if the number of subjects who experienced a particular event in each treatment group was statistically different across the groups. The overall probability of a type I error was set at 0.05.

6.4.12.1 Methods

Descriptive statistics were applied in the safety analyses.

6.4.12.3 Deaths

There were no deaths in this study.

6.4.12.4 Nonfatal Serious Adverse Events

All adverse events (AEs) were classified according to MedDRA Version 10.0. The number of subjects presenting with AEs in each treatment group was reported by the applicant as shown in Table 14:

Table 14: Summary of Overall Adverse Events

	Cohort 1 AIGIV N=18 Treatment A	Cohort 2 AIGIV N=18 Treatment B	Cohort 3 AIGIV N=18 Treatment C	Cohort 4 group 1 N=10 Treatment D	Cohort 4 group 2 N=10 Treatment E	All Active N=74	All Placebo N=18	Total N=92
Number of subjects	14 (78%)	12 (47%)	15 (83%)	8 (80%)	7 (70%)	56 (76%)	9 (50%)	65 (71%)
Number of AEs	46	47	83	24	23	223	28	251

No serious AEs were reported during the study. A total of 65 subjects (71%) reported 251 AEs in this study. Four (4) AEs were severe, 36 AEs were moderate and 211 were mild. Of the moderate AEs, 8 were reported in subjects receiving placebo. One hundred and thirty (130) AEs were treatment-related, and 121 AEs were not treatment-related. Of the treatment related AEs, 12 were reported in subjects receiving placebo.

The most frequently reported AEs were headache, pharyngolaryngeal pain and nausea (Table 15).

Table 15: Summary of Adverse Events Most Frequently Reported

	Cohort 1 AIGIV N=18 Treatment A	Cohort 2 AIGIV N=18 Treatment B	Cohort 3 AIGIV N=18 Treatment C	Cohort 4 group 1 AIGIV N=10 Treatment D	Cohort 4 group 2 AIGIV N=10 Treatment E	All Placebo N=18	Total N=92
Nervous system disorders: Headache	3 (17%)	9 (50%)	8 (44%)	5 (50%)	3 (30%)	3 (17%)	31 (34%)
Respiratory, thoracic and mediastinal disorders: Pharyngo-laryngeal pain	2 (11%)	3 (17%)	3 (17%)	1 (10%)	1 (10%)	1 (6%)	11 (12%)
Gastrointestinal disorders: Nausea	4 (22%)	2 (11%)	1 (6%)	1 (10%)	1 (10%)	1 (6%)	10 (11%)

The incidence of infusion site pain, regardless of PI assessment, for the first three cohorts with placebo groups combined was found to be significantly different across treatment groups (p=0.0483), where four (22.2%) subjects in Treatment A and two subjects (11.1%) in Treatment C experienced the event. Multiple comparisons between the treatment

groups revealed no significant differences between individual groups. No other incidence rates differed significantly, but headaches were common across all of the treatment groups, especially Treatment B (33.3% of subjects) and Treatment C (27.8%). Collapsing preferred terms into their respective system organ classes, while maintaining separate placebo groups, revealed an overall statistical difference in incidence rates of adverse events in the General disorders and administration site conditions system organ class across the treatment groups (p=0.0334) . Multiple comparisons between the treatment groups did not reveal any significant differences when comparing two groups at a time.

When cohorts 3 and 4 were considered together (Treatments C, D, and E pooled since all 3 received the same dose of AIGIV), and all the placebo subjects were pooled, the incidence of infusion site pain was again significantly different (p=0.0423) where a total of four (10.5%) subjects in the combined C/D/E treatment group experienced the event. Multiple comparisons between the treatment groups revealed no significant differences when groups were subsequently compared two at a time. No other incidence rates differed significantly, but headaches were again common across all of the treatment groups, especially Treatment B (33.3% of subjects) and Treatments C/D/E (28.9%). An overall statistical difference in incidence rates of adverse events was found in the General disorders and administration site conditions system organ class across the treatment groups (p=0.0389). However, when comparing groups two at a time using a multiple comparison procedure did not result in significant differences.

Adverse events considered by the PI to be related to the study drug showed a similar pattern for cohorts 1-3 with overall incidence of infusion site pain different (p=0.0497, no significant differences between individual groups), and headaches more frequent in Treatments B and C (~25%) versus Treatment A and placebo groups (~6%). No significant differences between incidence rates were observed when Treatments D and E were pooled with Treatment C. Overall statistical difference in incidence rates of adverse events were found in the General disorders and administration site conditions system organ class across the treatment groups with cohort 3 alone (p=0.0219) and cohorts 3 and 4 pooled (p=0.0183). Multiple comparisons between the treatment groups did not reveal any significant differences between individual groups. When cohorts 3 and 4 were pooled, a marginally significant (p=0.0501) difference in incidence rates of adverse events was found in the Nervous system disorders system organ class (most common preferred term of Headache).

10. CONCLUSIONS

10.1 Statistical Issues and Collective Evidence

This BLA original submission includes three animal studies and one health volunteer study. The objective of these studies is to pursue the product for licensure under the Animal Rule.

This statistical reviewer verified the efficacy analyses in animal studies (b) (4)-828, (b) (4) 1182, and (b) (4)-1207. The survival rate with the investigated product AIGIV was increased

in all animal studies, with study results in (b) (4)-828 and (b) (4)-1207 showing a significant improvement.

Study (b) (4)-828 was designed to determine the dose related efficacy of AIGIV in cynomolgus monkeys. In this study both the medium dose group (15U/kg) and high dose group (30U/kg) achieved significant improvement in survival rate over placebo without a multiple comparison adjustment (p-value = 0.0186 and 0.0003 respectively). After a Bonferroni-Holm adjustment, only the high dose group retained its significance (p-value=0.0009) and the p-value of the medium dose group is slightly higher than 0.025 (p-value=0.0372), the alpha level for one-sided hypothesis testing.

The applicant evaluated the medium dose (15U/kg) in studies (b) (4)-1182 and (b) (4)-1207, both of which were designed to determine the efficacy of AIGIV in rabbits. Study (b) (4) 1182 was designed to assess the therapeutic efficacy of the combination treatment of AIGIV and levofloxacin over that of Immune globulin intravenous (IGIV) and levofloxacin when either treatment was initiated at 96 h after aerosol exposure to a lethal dose (200 x LD_{50}) of *Bacillus anthracis* spores in rabbits. The survival rate was 0.58 vs. 0.39 (AIGIV vs. control) after the first stage. Interim analysis showed that continuation to the second stage would provide only approximately 48% power in this study. Therefore, (b) (4)-1182 was terminated after the first stage. Although numerically the survival rate in the AIGIV treated arm is higher compared to the control, the difference is not statistically significant. .

Study (b) (4)-1207 was designed to evaluate the efficacy of AIGIV in comparison to IGIV when treatments were administered after the first detection of PA in serum. The survival rate was 0.26 vs. 0.02 (AIGIV vs. control). This improvement in survival rate was statistically significant (p=0.0008612). Besides evaluating the added benefit of AIGIV over the use of levofloxacin in Study (b) (4)-1182, the other difference in study design between (b) (4)-1182 and (b) (4)-1207 was the time of administration of AIGIV. In (b) (4)-1182, all animals were treated after 96 hours post exposure while in (b) (4)-1207, all animals were treated at the onset of toxemia. The average time to treatment was 32.41 hour post exposure in (b) (4)-1207.

Considering the results from all three studies, it appears AIGIV significantly increases survival rate in animals (cynomolgus monkeys and rabbits) when administered therapeutically at the onset of toxemia.

For safety, no serious concern was detected from study AX-001. The investigated product appeared to be safe and well tolerated at all three doses (210U, 420U and 840U) in the healthy adults.

10.2 Conclusions and Recommendations

The efficacy of AIGIV was substantially supported by the three animal studies.

The safety of the investigated product in was also examined with the healthy volunteer study AX-001. No serious concerns were detected.