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*APPLICATION NUMBER:*

**125509Orig1s000**

**PHARMACOLOGY REVIEW(S)**

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH**

**PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION**

Application number: 125509  
Supporting document/s: Original submission  
Applicant's letter date: 3/20/15  
CDER stamp date: 3/20/15  
Product: Obiltoxaximab (Anthim™)  
Indication: For the treatment of adult and pediatric patients with inhalational anthrax due to *Bacillus anthracis* in combination with antibacterial drugs and for prophylaxis of inhalational anthrax when alternative therapies are not available or are not appropriate  
Applicant: Elusys Therapeutics, Inc.  
Review Division: DAIP  
Reviewer: Amy Nostrandt  
Supervisor/Team Leader: Wendelyn Schmidt  
Division Director: Sumathi Nambiar  
Project Manager: Jane Dean

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# 1 Executive Summary

## 1.1 Introduction

ETI-204 is a chimeric human/mouse monoclonal antibody directed against *Bacillus anthracis* (*B. anthracis*) protective antigen (PA). The application states that ETI-204 is of the IgG1κ isotype, consisting of two heavy and two light chains, produced by a murine myeloma NS0 cell line. PA is a pore-forming protein that binds to one of two anthrax toxin receptors (ATRs) on the cell surface, either capillary morphogenesis gene 2 protein (CMG-2) or tumor endothelial marker 8 (TEM-8), which are stated to be widely expressed on the majority of mammalian cells. Receptor-bound PA is proteolytically cleaved and can then facilitate binding of anthrax lethal factor (LF) or anthrax edema factor (EF) and subsequent internalization into the host cell. The application states that, because of PA's role in toxin assembly and intoxication of target cells, PA neutralization may be effective in preventing the establishment and progression of disease subsequent to inhalational exposure to anthrax spores.

Clinical trials of ETI-204 were limited to pharmacokinetics and safety studies in healthy volunteers. Demonstration of efficacy was based on studies in animal models of inhalational anthrax.

## 1.2 Brief Discussion of Nonclinical Findings

Tissue cross-reactivity (TCR) studies were performed in human, rat, and cynomolgus monkey tissues. The test article was said to stain various cell types in most tissues examined in a similar manner in all three species. Staining was reported to be cytoplasmic and was specifically localized to cytoplasmic granules and/or filaments, often associated with cell borders, suggestive of intracellular cytoskeletal elements, and therefore not relevant. One additional TCR study was performed in a limited set of human tissues to compare antibody derived from (b) (4) and (b) (4) cell lines after a manufacturing change from the former to the latter. Staining was reported to be similar for both test articles, and consistent with previous findings for the (b) (4) antibody. In these studies, ETI-204 concentrations of 1-10 µg/mL were used, although it is unclear if these concentrations were high enough to be relevant to clinical exposures.

Two safety pharmacology studies were conducted to evaluate cardiovascular function in cynomolgus monkeys. In the first study, compound-related elevation in blood pressure at 2 and 4 hours after IV and IM administration was seen, as well as apparent increase in QT interval on ECG. The reviewer concluded that a more rigorous study was needed. In the second study, the changes in blood pressure were not seen; blood pressures were in the normal range for this species. No QT prolongation was reported.

General toxicology studies were performed in rats and cynomolgus monkeys. The initial study no. ARR002 ( (b) (4) study no. 03553) was performed in 10-13 week old male Fischer 344 rats at repeated IV and IM doses up to 2.91 mg/rat (approximately 10.6 mg/kg) of the (b) (4) antibody. Doses were administered on Days 1, 4, and 7, with sacrifice on Day 10/11. No test article-related findings were reported for

mortality, clinical signs, hematology, clinical chemistry, organ weights, or gross or microscopic pathology. The NOAEL was determined to be the high dose, 10.6 mg/kg.

The second safety pharmacology study in cynomolgus monkeys included limited general toxicology evaluation of test article generated in the (b) (4) cell line. The NOAEL was 30 mg/kg, which resulted in an AUC of approximately 150,000 µg·hr/mL.

More recently, pilot and definitive toxicology studies were performed in Sprague-Dawley rats. In the pilot study, doses of vehicle (saline), 10, 30, or 100 mg/kg were administered by slow IV bolus injection on Days 1, 4, and 7 to five male rats per group. No effects were noted on survival, clinical observations, body weights, clinical pathology or gross pathology. The study concluded that the maximum tolerated dose had not yet been reached. In the definitive study, 8 rats/sex/group were administered vehicle (saline), 3, 10, or 30 mg/kg by IV injection on Days 1, 4, and 7. The test article was the new material manufactured by Lonza, and this study included an additional high dose (30 mg/kg) group utilizing material manufactured by Baxter in order to compare material made by the two manufacturers. Additional satellite groups were used for pharmacokinetics. No test article-related findings were reported in clinical observations, body weights, food consumption, clinical pathology, organ weights, or macroscopic or microscopic pathology. While no differences between materials made by the two manufacturers were reported in the toxicologic or toxicokinetic profiles, the report did note increased variability in Cmax with the Lonza material.

An assessment for neuropathological changes was performed on tissue from studies in infected monkeys and infected and non-infected rabbits. In primates and rabbits exposed to inhalational anthrax *that did not survive* (found dead or moribund sacrificed animals), administration of ETI-204 at doses at and above 4 mg/kg was associated with an increased incidence (frequency) of histological findings, consistent with a severe acute inflammatory reaction. The changes in the non-survivors, including those treated with only saline, with ETI-204, or with levofloxacin, were stated to be consistent with morphologic lesions/hemorrhagic meningoencephalitis previously reported in monkeys and rabbits with inhalation anthrax. Biologically significant reactions (hemorrhage, inflammation, necrosis) in non-survivors were associated with the presence of extravascular bacteria in all dose groups, including saline controls. The occurrence of an acute inflammatory response in the ETI-204 treated non-survivors did not exhibit a dose response relationship (i.e., changes were not more pronounced at higher doses). The administration of the ETI-204 was not associated with any biologically significant morphologic reactions in surviving animals exposed to inhaled *B. anthracis*. Similarly, no significant neuropathological lesions were reported in rabbits not exposed to anthrax spores and given up to 32 mg/kg intravenous ETI-204 (EFT001) in a reproductive toxicology study.

In a developmental toxicity study in rabbits, two treatment groups of 30 time-mated female New Zealand White Hra:(NZW)SPF rabbits/group were administered ETI-204 at doses of 16 or 32 mg/kg/dose, respectively. A vehicle control group of 22 time-mated females were administered sterile 0.9% Sodium Chloride for Injection, USP. The vehicle or ETI-204 was administered to all groups via intravenous (IV) injection on Gestation Day (GD) 6, 10, 13, and 17, at a dose volume of 1 mL/kg/dose. All but one

high dose animal that received the full dosing regimen survived to terminal euthanasia on GD 29. That animal exhibited inappetance and body weight loss and delivered early on GD 28, similar to one animal in the pilot study that received 32 mg/kg on GD 13 and 17. Since this was an isolated occurrence, it was not considered to be test article-related. The No-Observed-Effect Level (NOEL) of ETI-204 for maternal and developmental toxicity was determined to be 32 mg/kg/dose, the highest dose tested. The report states that systemic exposure associated with this dose in the pilot study was approximately four times higher than that of healthy human subjects administered an IV dose of 8 mg/kg. Since the clinical dose is more likely to be 16 mg/kg, this may represent a 2-fold safety margin. Results from immunogenicity analysis confirmed two animals to be positive for antibodies to ETI-204.

In general, a toxic dose was not reached in GLP-compliant toxicology studies in rats and monkeys at doses up to 30 mg/kg. An MTD was not reached in a pilot study in rats at doses up to 100 mg/kg. ETI-204 does not appear to be neurotoxic in anthrax-infected or in non-infected animals, but does not appear to always protect against anthrax-related meningitis. No adverse reproductive or developmental effects were noted in an embryo-fetal toxicity study in rabbits at doses up to 32 mg/kg, nor was maternal toxicity observed.

### 1.3 Recommendations

#### 1.3.1 Approvability

This application is approvable from a pharmacology/toxicology standpoint.

#### 1.3.2 Additional Non Clinical Recommendations

None at this time

#### 1.3.3 Labeling

In the Highlights section, under Indications and Usage, obiltoxaximab is described as (b) (4). This is not entirely accurate, since PA (b) (4), but is a component of both anthrax lethal toxin and anthrax edema toxin. There currently is no established pharmaceutical class (EPC) for (b) (4) in CDER, and this category is not adequately specific. The word (b) (4) should be removed. An alternative EPC, “anthrax protective antigen-directed antibody” is suggested.



Pregnancy letter categories will no longer be used, and should be removed. The proposed text describing the embryo-fetal toxicology study is accurate. Reference to clinical patients should precede animal data.

## 8 USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

#### Pregnancy Category B

No adequate and well controlled studies in pregnant women were conducted. Because animal reproduction studies are not always predictive of human response, Anthim should be used during pregnancy only if clearly needed.

A single embryonic-fetal development study was conducted in pregnant, healthy New Zealand White rabbits administered 4 intravenous doses of Anthim up to 32 mg/kg (2 times the human dose on a mg/kg basis) on gestation days 6, 10, 13, and 17. No evidence of harm to the pregnant dam or the fetuses due to Anthim was observed. Cumulative exposures in rabbits (10,000 mcg·day/mL) at the NOAEL of 32 mg/kg/dose (n=4 doses) based on AUC<sub>0-15 days</sub> were approximately two-fold the human male and female combined mean AUC at the clinical IV dose of 16 mg/kg. C<sub>max</sub> values following a 32 mg/kg/dose were 1180 mcg·day/mL. ~~No adequate and well controlled studies in pregnant women were conducted. Because animal reproduction studies are not always predictive of human response, Anthim should be used during pregnancy only if clearly needed.~~

The first two paragraphs of section 13.2 describe (b) (4)

Both paragraphs should be removed.

The description of data from (b) (4)

should be removed. The reference to the (b) (4)

should be removed. The reference to (b) (4) is removed and replaced with “disease confirmation” (b) (4) because some nonclinical efficacy studies used significant increase in body temperature and time after exposure as triggers to treat.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity, genotoxicity, and fertility studies have not been conducted with obiltoximab.

### 13.2 Animal Toxicology and/or Pharmacology

(b) (4)

(b) (4)

Central nervous system (CNS) lesions (bacteria, inflammation, hemorrhage and occasionally necrosis) were seen in Anthrax infected non-surviving rabbits and (b) (4) administered IV of obiltoxaximab ( $\geq 4$  mg/kg) or control at the time of (b) (4) disease confirmation. Microscopic changes in the non-surviving animals that received obiltoxaximab were due to the presence of extravascular bacteria and not the effect of obiltoxaximab. No dose response relationship for brain histopathology was identified. (b) (4)

(b) (4) No treatment-related brain lesions (b) (4) were shown (b) (4)

(b) (4) in anthrax-treated surviving rabbits (at day 28) or (b) (4) (up to day 56) after a single administration of obiltoxaximab at doses up to 16 mg/kg and up to 32 mg/kg/dose, respectively. No obiltoxaximab-related neurobehavioral effects were observed in surviving anthrax infected monkeys following treatment with obiltoxaximab.

## 2 Drug Information

### 2.1 Drug

Generic Name

obiltoxaximab.

Code Name

ETI-204

Molecular Formula/Molecular Weight

The molecular formula of ETI-204 (b) (4) is:

(b) (4)

The relative molecular masses from two representative lots of ETI-204 produced by the commercial manufacturing process at Lonza are presented in the Sponsor's table below:

**Table 3: Relative Molecular Mass of ETI-204 Whole Molecule**

Lot Number	Molecular Mass (Da)	Predominant (Da)
250241	(b) (4)	(b) (4)
268779	(b) (4)	(b) (4)

**Structure or Biochemical Description**

ETI-204 is described as an affinity enhanced deimmunized anti-anthrax protective antigen (PA) monoclonal antibody (mAb) of the IgG1κ isotype (~148 kDa) produced via cultures of (b) (4) glutamine synthetase (GS)-NS0 myeloma cells. The complete molecular structure is comprised of two heavy chains and two light kappa (κ) chains. (b) (4) The molecular sizes of the heavy chain and light chain from two bulk drug substance (BDS) lots representative of the commercial manufacturing process at Lonza Biologics, Inc are presented in the Sponsor’s tables below:

**Table 1: Molecular Size and Subunit Structure of Heavy Chain**

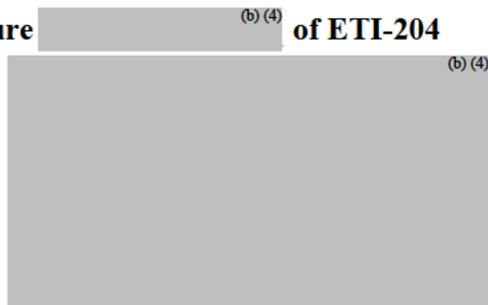
Lot Number	Molecular Mass (Da)	Predominant (Da)
250241	(b) (4)	(b) (4)
268779	(b) (4)	(b) (4)

**Table 2: Molecular Size and Subunit Structure of κ Light Chain**

Lot Number	Molecular Mass (Da)
250241	(b) (4)
268779	(b) (4)

ETI-204 is produced by cultures of (b) (4) GS-NS0 myeloma cells. ETI-204 contains human constant region sequences and deimmunized murine variable region sequences generated from the murine mAb clone 14B7. The chemical structure (b) (4) of ETI-204 is shown in the Applicant’s figure below:

**Chemical Structure** (b) (4) **of ETI-204**



**Pharmacologic Class**

Chimeric (human-murine) monoclonal antibody

**2.2 Relevant INDs, NDAs, BLAs and DMFs**

IND 12,285

**2.3 Drug Formulation**

The application states that ETI-204 drug product is a sterile, liquid formulation in single-use vials. Each vial contains 100 mg/mL ETI-204 in histidine (6.2 mg/mL), sorbitol (36 mg/mL), polysorbate 80 (0.10 mg/mL [w/v]) with a pH of 5.5. Each vial contains a target fill of (b) (4) mL into a (b) (4) mL vial (to allow delivery of 600mg/6 mL). Composition is shown in the Applicant’s table below:

**Components of the ETI-204 Drug Product**

Component	Function	Quality Standard	Concentration
ETI-204	Active ingredient	Elusys (see (b) (4))	100 mg/mL
L-Histidine	(b) (4)	USP/NF	40 mM
Sorbitol	(b) (4)	USP/NF	200 mM
Polysorbate 80	(b) (4)	USP/NF	0.01%

(b) (4)

**2.4 Comments on Novel Excipients**

The application states that the specified BDS process-related impurities of ETI-204 are limited to (b) (4). It states that these impurities were qualified in the nonclinical toxicology program for ETI-204.

**2.5 Comments on Impurities/Degradants of Concern**

The application states that no drug product process-related impurities or degradation products have been detected.

## 2.6 Proposed Clinical Population and Dosing Regimen

The proposed label states that the proposed clinical use will be a single administration of ETI-204 is at a dose of 16 mg/kg IV over 1 hour 30 minutes after dilution in 0.9% Sodium Chloride Injection, USP (normal saline) to a final volume of 250 mL to adult patients. Tables are provided for adjusted doses and infusions for pediatric patients. The label states that (b) (4) diphenhydramine should be administered (b) (4) prior to Anthim infusion.

## 2.7 Regulatory Background

According to the nonclinical overview, Elusys has utilized two different cell lines, designated (b) (4) and (b) (4), for the manufacture of ETI-204 over the course of development. The (b) (4) cell line was utilized to manufacture ETI-204 at (b) (4) and (b) (4) was utilized to manufacture ETI-204 at Baxter Healthcare and Lonza EFT001 Phase B (definitive) (b) (4) study no. 1984-005)Biologics. The application states that both cell lines use the same mouse myeloma host cell, NS0, and that the specific immunoglobulin genes are identical between the two cell lines. The (b) (4) cell line was the early research cell line while (b) (4) is the final manufacturing cell line. It further states that all of the pivotal toxicology studies utilized ETI-204 derived from the (b) (4) cell line manufactured at either Baxter Healthcare or Lonza Biologics. Lonza Biologics manufactures the to-be-marketed product.

## 3 Studies Submitted

### 3.1 Studies Reviewed

#### Safety Pharmacology studies

1. **Study no. AP106: Single Dose Intravenous and Intramuscular Pharmacokinetics Study of ETI 204 (Anthim™) in Cynomolgus Monkeys.**
2. **Study no. AP-106 PK ( (b) (4) Study no. 1180-04527): A single-dose intravenous and intramuscular pharmacokinetics study of ETI-204 (Anthim™) in cynomolgus monkeys**
3. **Study no. AP-115: A Cardiovascular and Toxicology Evaluation Study Following Intravenous Infusion of Anthim (b) (4) in Cynomolgus Monkeys**

#### General Toxicology studies

1. **Study no. ARR002 ( (b) (4) study no. 03553): Repeat dose IV and IM toxicity of antibody (b) (4) in male Fischer 344 rats**
2. **Study no. TOX001 pilot: A 14-day pilot IV toxicity study of ETI-204 in Sprague-Dawley rats**
3. **Study no. TOX001 definitive: A 14-day definitive IV toxicity study of ETI-204 in Sprague-Dawley rats**

### Developmental and reproductive toxicology studies

1. Study no. EFT001 pilot ( (b) (4) study no. 1984-002): ETI-204: An intravenous range-finding developmental toxicity study in rabbits with toxicokinetic evaluation
2. Study no. EFT001 Phase B (definitive) ( (b) (4) study no. 1984-005): A definitive IV dose study for effects of ETI-204 on embryo-fetal development in rabbits

### Other Toxicology studies

1. Study no. AH001 ( (b) (4) study IM1082): Cross-reactivity study of Anthim™ with normal human tissues
2. Study no. ARR003 ( (b) (4) study IM1083): Cross-reactivity study of Anthim™ with normal rat tissues
3. (b) (4) study no. IM1215: Cross reactivity study of AnthIM™ with normal human, rat and cynomolgus monkey tissues.
4. (b) (4) study no. IM1219: Processing and detection of test article in support of (b) (4) . study no 03553 entitled “Repeat-dose intravenous and intramuscular toxicity of antibody (b) (4) in male Fischer 344 rats”
5. (b) (4) study no. IM1368: Limited human tissue cross-reactivity study with (b) (4) and (b) (4)
6. Report no 358-0006: Combined review of neuropathology data to determine the potential brain toxicity of eti-204: review of multiple studies in an inhalational *Bacillus anthracis* exposure therapeutic model and a single study using intravenous dosing (without *B. anthracis* exposure)

## 3.2 Studies Not Reviewed

Primary pharmacology and pharmacokinetics studies are not reviewed in full. Summaries based on the Sponsor’s summaries are provided in the appropriate sections below. For selected pivotal efficacy studies, review is limited to methods of the animal disease model and nonclinical pathology.

## 3.3 Previous Reviews Referenced

Nonclinical reviews of submissions to IND 12,285

# 4 Pharmacology

## 4.1 Primary Pharmacology

Thirty-two reports were submitted, including studies of efficacy in animal models of inhalation anthrax. The following is based on the Sponsor’s summaries. Efficacy studies in animal models are reviewed in greater detail in clinical and clinical microbiology reviews. Under “Trigger-to treat studies” below, aspects of the conduct of the animal model and animal pathology for the pivotal efficacy studies are reviewed.

ETI-204 is described as an affinity enhanced deimmunized mAb of the IgG1k isotype [~148 kilodaltons (kDa)], and its target is *B. anthracis* protective antigen (PA),

the cell-binding component of the anthrax tripartite toxin. Evaluation of the pharmacodynamics (PD) of ETI-204 consisted of mechanistic binding studies, in vitro characterization of ETI-204's activity via the lethal toxin neutralization assay (LNA), and in vivo measurements of free PA and anti-PA antibodies subsequent to anthrax challenge and administration of test article.

#### In vitro studies

ETI-204 is described as containing human constant region sequences and de-immunized murine variable region sequences generated from the murine mAb clone 14B7. The mAb 14B7 is said to bind to domain 4 of PA (PAD4), which the application states is the domain responsible for the binding of PA to cell surface receptors. The application states that ETI-204 has a high affinity for PA with a dissociation constant ( $K_D$ ) of 0.33 nM, and that the dissociation constant is similar to that of the the PA receptor  $K_D$  (i.e., 0.17 to 1 nM). The application claims greater affinity for PA than the approved anti-PA mAb, Raxibacumab.

The application states that Western blot analysis indicated that ETI-204 can bind both PA<sub>83</sub> and the cleavage product PA<sub>63</sub> from three strains of *B. anthracis* (Ames, Sterne, and Vollum) while not binding to the non-specific control protein, ovalbumin. The application goes on to state that ETI-204 dose-dependently blocked PA<sub>83</sub> and PA<sub>63</sub> binding to a soluble form of the cell surface receptor CMG-2, and that CMG-2 is the anthrax toxin receptor (ATR) predominantly responsible for the effects of anthrax toxin.

The ability of ETI-204 to neutralize LT-mediated toxicity was tested in an in vitro Lethal Toxin Neutralization assay (LNA). ETI-204 and its parent mAb 14B7 were said to have increased survival in murine macrophage-like RAW 264.7 cells when co-incubated with lethal toxin (LT). The half maximal effective concentration ( $EC_{50}$ ) was achieved at a 4-fold lower concentration of ETI-204 compared to the parent mAb, 14B7.

The application states that the ability of rabbits to mount an adaptive immune response to LT subsequent to *B. anthracis* exposure and ETI-204 administration was demonstrated in the LNA. Rabbit sera collected after exposure to *B. anthracis* spores and post-administration of ETI-204 were able to neutralize LT in the LNA with  $EC_{50}$  values which correlated with the observed anti-PA titers ( $R_2=0.95$ ).

#### In vivo studies

The concentration of free PA was measured in the serum of rabbits and monkeys via a quantitative enzyme-linked-immunosorbent-assay (ELISA) method after exposure to *B. anthracis* (Ames strain) spores, prior to treatment with ETI-204 or vehicle, and post-dosing to demonstrate the pharmacological effect of ETI-204, which binds PA and prevents binding of PA to ATRs such as CMG-2.

Following a single IV bolus administration of ETI-204 at 1, 4, 8, or 16 mg/kg in New Zealand White (NZW) rabbits in a trigger-to-treat study (Study No. AR033), the saline control group had significantly ( $p < 0.0001$ ) more animals with free PA above the lower limit of quantitation (LLOQ) compared to the ETI-204-treated groups at 8, 24, and 48 hours post-treatment. At the last (terminal) time point, there were significantly more rabbits with measureable free PA levels in the saline control and 1 mg/kg treatment groups compared to rabbits administered  $\geq 4$  mg/kg ETI-204 ( $p < 0.0001$ ).

In an ETI-204 and levofloxacin combination study in NZW rabbits (Study No. AR028), 72 hours post-challenge (PC) to *B. anthracis* spores, a single IV bolus administration of ETI-204 at 16 mg/kg plus levofloxacin orally (PO) at 6.5 mg/kg/day for 3 days resulted in free PA levels which were below the limit of quantitation (BLQ) at all post-treatment time points through terminal sacrifice on Day 28.

Following a single IV administration of ETI-204 in three trigger-to-treat studies in monkeys, PA levels dropped below the LLOQ by the first post-dose time point in the majority of animals (93%) following administration of 4 and 16 mg/kg (Study No. AP204) or 8 and 32 mg/kg (Study No. AP203). In the third trigger-to-treat study (Study No. AP201), which utilized a different analytical method than AP203 and AP204, free PA levels in monkeys declined steadily following a single IV administration of 4 or 8 mg/kg but did not drop below the LLOQ until Day 14 PC. When ETI-204 was administered IM to monkeys at 16 mg/kg 24 to 48 hours PC (Study No. AP307), free PA levels dropped below the LLOQ in 89% of monkeys administered ETI-204 at the 24-hour post-treatment time point. In contrast, the majority of vehicle-treated rabbits and monkeys in these studies demonstrated a continual rise in free PA levels post-challenge to *B. anthracis* spores until succumbing to anthrax infection prior to scheduled sacrifice.

#### Adaptive immunity

Endogenous anti-PA antibodies were measured in several studies in rabbits administered ETI-204 IV or IM following challenge with *B. anthracis* (Ames strain) spores, in order to determine whether adaptive immunity against anthrax develops in ETI-204-treated surviving animals and to confirm that administration of ETI-204 does not interfere with the development of adaptive immunity.

A single IV dose of ETI-204 of 10 mg (Study No. PCRPT0003) or 16 mg/kg (Study No. AR034) resulted in positive anti-PA antibody titers by Day 7 to 10 post-treatment or at Day 28 post-challenge (PC), respectively. In the former study, at Day 7 to 10 post-treatment, ETI-204 was said to be nearly undetectable in sera of surviving rabbits. In the latter study, anti-PA antibody persisted up to 6 months PC. In Study No. AR028, anti-PA antibody response post-treatment with levofloxacin (6.5 mg/kg/day for 3 days) was not inhibited by a concomitant administration of a single IV dose of 16 mg/kg ETI-204.

When ETI-204 was administered IM to rabbits at 4 or 16 mg/kg at 18 or 24 hours PC (Study No. AR0315) or at 16 mg/kg at 18 to 30 hours PC (Study No. AR035), all surviving animals on Day 28 PC were positive for anti-PA IgG, indicating an immune response to *B. anthracis*. In Study No. AR037, administration of ETI-204 to rabbits via IM injection at 8, 16, or 32 mg/kg at 24 hours PC similarly resulted in positive anti-PA IgG values in most survivors (14/15) on Day 28 PC.

#### Animal models of inhalation anthrax

Studies of efficacy in animal models of inhalation exposure to anthrax spores included trigger-to-treat (TtT) studies of ETI-204 alone or in combination with antibiotic, post-exposure prophylaxis (PEP) studies, and pre-exposure prophylaxis (PreEP) studies in rabbits and cynomolgus monkeys. While these species have been used before as disease models of inhalational anthrax, no natural history studies to define the

course of the disease in these species were provided in the application. Published studies are referenced:

1. Zaucha, GM; Pitt, LM; Estep, J; Ivins, BE; and Friedlander, AM (1998). The pathology of experimental anthrax in rabbits exposed by inhalation and subcutaneous inoculation. *Arch. Pathol. Lab. Med.* 122: 982-992.
2. Vasconcelos, D; Barnewall, R; Babin, M; Hunt, R; Estep, J; Nielsen, C; Carnes, R; and Carney, J (2003). Pathology of inhalation anthrax in cynomolgus monkeys (*Macaca fascicularis*). *Lab. Invest.* 83:1201-1209.

The models are described below as described in the reports of the submitted efficacy studies. Pathology data are reviewed below.

### **Trigger to treat (TtT) studies**

#### **1. Study no. AR021 ( (b) (4) Study no. 832-G924202): Evaluating the efficacy of ETI-204 when administered therapeutically in the New Zealand White rabbit inhalational anthrax model**

This GLP-compliant study was conducted at (b) (4). Histopathology was performed by (b) (4).

Sixty-four (32/sex) SPF-New Zealand White rabbits weighing 2.8-3.5 kg on arrival were randomized by body weight to one of five dose groups and to one of three challenge days following a quarantine period. Rabbits were surgically implanted with vascular access ports (VAP). Study design is described in the Applicant's table below:

Group	Number of Animals	ETI-204 Dose mg/kg	Levofloxacin Dose <sup>x</sup>
1	10	Saline <sup>^</sup>	Control*
2	10	1.0	Control*
3	17	4.0	Control*
4	17	16.0	Control*
5	10	Saline <sup>^</sup>	50 mg/kg

\* Water For Injection (WFI) was administered as a control (at 2 ml/kg) for levofloxacin.

<sup>^</sup> Saline was administered as a control (at 0.5ml/kg) for ETI-204

<sup>x</sup> Levofloxacin or control material was administered in three doses: upon meeting treatment intervention criteria and at 24 (±1) and 48 (±3) hours after the initial treatment.

<sup>o</sup> Trigger for treatment intervention was either first positive PA result (via ECL assay) or three consecutive critical temperature readings or when an animal had exhibited two consecutive critical temperature readings twice.

Three animals were subsequently replaced due to problems not related to treatment.

The animals were challenged via the inhalation route with *B. anthracis* (Ames strain) spores on Study Day 0. Rabbits were placed individually into a plethysmography chamber and a Class III biological safety cabinet system and were aerosol challenged with a targeted 200 LD<sub>50</sub> inhaled dose of *B. anthracis* spores (spore lot Ames B31) that were aerosolized by a three-jet Collison nebulizer and delivered via a nose-only inhalation exposure chamber. Aerosol challenge duration was based on estimated aerosol challenge concentration and cumulative minute volume data gathered during exposure. Aerosol concentrations of *B. anthracis* spores were quantified by determination of cfu in effluent streams collected directly from an animal exposure port by an in-line all-glass impinger. Serial dilutions of the impinger samples were then plated onto tryptic soy agar (TSA) plates and enumerated. Aerosol particle size was determined using an aerosol particle sizer (APS) spectrometer which drew an atmospheric sample from the exposure chamber. Whole body plethysmography was performed in real-time during each animal challenge to calculate tidal volume, total accumulated tidal volume, and minute volume; these parameters were used to determine the inhaled dose.

The average ( $\pm$  standard deviation) aerosol exposure dose for all animals was 180 ( $\pm$ 56) *Bacillus anthracis* (Ames strain) LD<sub>50</sub> equivalents (range 79-343) as shown in the Sponsor's table below. The average mass median aerodynamic diameter (MMAD) was 1.12, 1.10 and 1.09 $\mu$ m for challenge days A, B and C respectively. The MMAD of the aerosol particles generated for this study were stated to be consistent with lower respiratory tract deposition.

#### Average Challenge Doses (LD<sub>50</sub> Equivalents)

Challenge Day	Average Dose (SD)
A	134 ( $\pm$ 58)
B	212 ( $\pm$ 38)
C	196 ( $\pm$ 34)
Group	Average Dose (SD)
1	185 ( $\pm$ 72)
2	168 ( $\pm$ 41)
3	200 ( $\pm$ 52)
4	175 ( $\pm$ 61)
5	165 ( $\pm$ 48)

Animals were monitored for up to 28 days post-challenge for clinical signs. Monitoring was performed every 6 hours between approximately 18-72 hours post-challenge, then twice daily thereafter until Day 28.

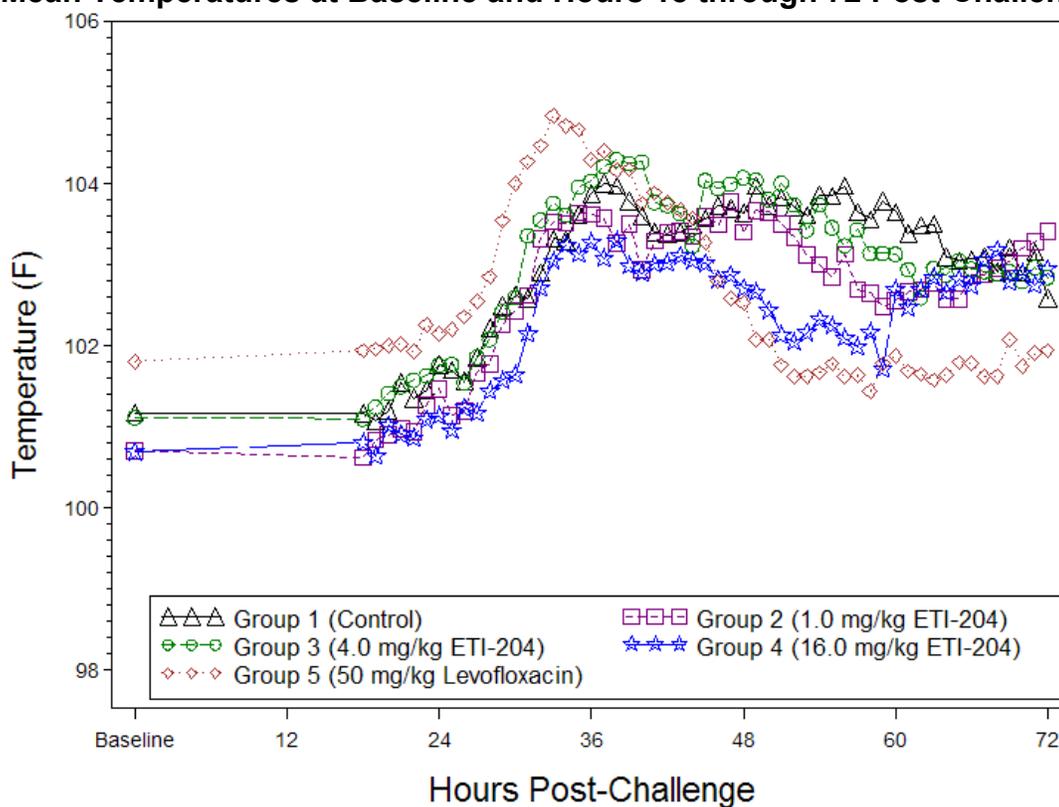
Temperatures ( $^{\circ}$ F) were measured hourly from approximately 18 to 72 hours post-challenge and twice daily for the remainder of the study. Body temperatures were recorded via an implantable programmable temperature transponder (IPTT-300, BMDS, Seaford, DE implanted on or before Day -7. Each rabbit had two transponders injected subcutaneously (one chip at shoulder blade level and one at rump level). Data from the

transponder with the least variable baseline readings was used for each animal, with the second providing a backup in case of failure of the primary transponder. A baseline average temperature was calculated from pre-challenge measurements for each rabbit.

A significant increase in body temperature (SIBT) was defined as either three consecutive critical temperature readings or two consecutive critical temperature readings exhibited on two separate occasions. Critical temperature was defined as a reading equal to or greater than a two-standard deviation increase from each individual rabbit's average baseline body temperature.

The variability in the temperatures recorded by the shoulder transponders was significantly less than that recorded from the rump transponders ( $p$ -value=0.0044) for Challenge Day A, so shoulder temperatures were used for all animals in the analyses that followed for all three challenge days (with the exception of Animal K99423, for which the shoulder transponder stopped working). There were no significant group effects for time from challenge until significant increase in body temperature (SIBT). The average time (per group) to SIBT from challenge was between 26.76 and 31.98 hours post-challenge. The average group temperatures are shown in the Applicant's graph below.

#### Group Mean Temperatures at Baseline and Hours 18 through 72 Post-Challenge



Blood samples were collected from each rabbit from the VAP or from the marginal ear vein relative to the median challenge time. Samples were used for determination of serum PA using the ECL screening assay, for complete and differential

blood counts, for C-reactive protein (CRP) analysis, for culture to determine the presence of bacteremia, and for pharmacokinetic analysis. The schedule for blood sampling and tests are shown in the Applicant’s table below:

**Blood Draw Schedule<sup>a</sup>**

Approximate Time Point	Blood Tube type/ Approximate	Bacteremia (Culture)	CBC/ CRP	Serum PA level (via ECL assay)	Serum for ETI-204 assay	Plasma for Levofloxacin Assay
Day -7	EDTA ~1.5 ml *EDTA ~1.0ml SST ~2.0ml	X	X	X	X	X
^18hr PC	EDTA ~1.5 ml SST ~1.0 ml	X	X	X		
^24hr PC	EDTA ~1.5 ml SST ~1.0 ml	X	X	X		
^30hr PC	EDTA ~1.5 ml SST ~1.0 ml	X	X	X		
^36hr PC	EDTA ~1.5 ml SST ~1.0 ml	X	X	X		
^42hr PC	EDTA ~1.5 ml SST ~1.0 ml	X	X	X		
^48hr PC	EDTA ~1.5 ml SST ~1.0 ml	X	X	X		
PTT	EDTA ~1.5 ml SST ~1.0ml *EDTA ~1.0ml SPS ~1.0	X <sup>#</sup>	X	X <sup>f</sup>		X
1hr PT	*EDTA ~1.0ml					X
24hr PT (prior to Trt2)	EDTA ~1.5 ml *EDTA ~1.0ml SST ~1.0ml	X	X		X	X
49hr PT (1hr PTrt3)	*EDTA ~1.0ml					X
72hr PT (24hr)	EDTA ~1.5 ml *EDTA ~1.0ml	X	X			X
7 days PC <sup>b</sup>	EDTA ~2.0 ml	X	X			
14 days PC <sup>b</sup>	EDTA ~2.0 ml	X	X			
21 days PC <sup>b</sup>	EDTA ~2.0 ml	X	X			
28 days PC <sup>b</sup>	EDTA ~2.0 ml	X	X			
Terminal <sup>c,d</sup>	EDTA ~2.0 ml *EDTA ~1.0ml	X	CRP only			X <sup>e</sup>

PC = Post-Challenge

Trt2 = Treatment 2

PTT = Prior to Treatment

Trt3 = Treatment 3

PT = Post-Treatment

<sup>a</sup> Post-challenge pre-treatment bleed time points were relative to a median challenge time for a challenge cohort.

Post-treatment bleed times were calculated from the time each animal’s IV treatment ended. Blood samples occurred within ±60 minutes of

the calculated time, except for the 1hr PT and 49hr PT samples which occurred within 15 minutes of the calculated time. The

Day -7, Day 7, Day 14, Day 21 and Day 28 post-challenge bloods were relative to the day of challenge.

<sup>b</sup> Blood samples 7, 14, 21 and 28 days post-challenge were not collected from the VAP.

<sup>c</sup> The terminal sample, if occurring >7 days post-challenge, was not collected from the VAP.

<sup>d</sup> If collection was possible.

<sup>e</sup> Plasma for Levofloxacin analysis was only collected from terminal samples occurring ≤48 hours post treatment #3.

<sup>f</sup> This sample for Serum PA level (via ECL assay) was not be run immediately onsite (relative to collection time).

\* Chilled EDTA for Levofloxacin Analysis

<sup>^</sup> Post-Challenge, pre-treatment sampling stopped once decision to treat was made.

# PTT Bacteremia performed on sample collected in SPS tube

Eighty two percent (51/62) of the rabbits on study had a positive ECL result at some time point prior to treatment (excludes animals K99373 and K99383, which were assigned to control Group 1, but were inadvertently administered levofloxacin). The report states that there were no significant group effects for time from challenge until a positive ECL result was observed. The average time (for a group) to an abnormal (positive) ECL result from challenge was between 25.26 and 30.36 hours post-challenge. The Applicant’s table below summarizes the proportion of abnormal ECL values (by group) at the various study time points.

Study Time	Group 1		Group 2		Group 3		Group 4		Group 5	
	P	Proportion Abnormal (95% Confidence Interval)								
Baseline	0/9	0.00 (0.00, 0.34)	0/9	0.00 (0.00, 0.34)	0/17	0.00 (0.00, 0.20)	0/17	0.00 (0.00, 0.20)	0/10	0.00 (0.00, 0.31)
Hour 18 PC	0/9	0.00 (0.00, 0.34)	0/9	0.00 (0.00, 0.34)	0/17	0.00 (0.00, 0.20)	0/17	0.00 (0.00, 0.20)	0/9	0.00 (0.00, 0.34)
Hour 24 PC	2/8	0.25 (0.03, 0.65)	4/8	0.50 (0.16, 0.84)	6/15	0.40 (0.16, 0.68)	6/16	0.38 (0.15, 0.65)	5/8	0.63 (0.24, 0.91)
Hour 30 PC	4/6	0.67 (0.22, 0.96)	1/3	0.33 (0.01, 0.91)	3/6	0.50 (0.12, 0.88)	5/8	0.63 (0.24, 0.91)	1/2	0.50 (0.01, 0.99)
Hour 36 PC	0/2	0.00 (0.00, 0.84)	1/1	1.00 (0.03, 1.00)	0/0	NA	2/3	0.67 (0.09, 0.99)	0/0	NA
Hour 42 PC	2/2	1.00 (0.16, 1.00)	0/0	NA	0/0	NA	0/0	NA	0/0	NA
Hour 48 PC	0/0	NA								
PTT	8/9	0.89 (0.52, 1.00)	7/9	0.78 (0.40, 0.97)	14/17	0.82 (0.57, 0.96)	13/17	0.76 (0.50, 0.93)	8/10	0.80 (0.44, 0.97)

P Number of animals with positive PA-ECL/number of animals alive in the group.

NA No blood was drawn from these animals at this study time due to animal already being abnormal.

Test articles (ETI-204 or levofloxacin) or control material (saline or water for injection, respectively) were administered based on either a positive serum PA result via the electrochemiluminescence (ECL) assay or a significant increase in body temperature (SIBT).

Fifty percent (31/62) of the animals were treated based on SIBT and 50% (31/62) were treated based on a positive ECL result (excluding animals K99373 and K99383). Once one of the above criteria had been met, levofloxacin or water for injection (WFI) was administered by gastric intubation for three doses; initially once one of the above criteria for treatment are met and then at 24hrs ( $\pm$  1 hour) and 48hrs ( $\pm$  3 hours) after the initial treatment. Following levofloxacin/WFI dose administration, approximately 3 ml of water was administered to flush the gavage tube. Subsequently, a single intravenous bolus of ETI-204 or saline was administered, via the VAP, immediately (within 10 minutes) after administration of the 1st levofloxacin or WFI dose. One to two ml of sterile saline was administered to flush the contents of the port after administration of the test/control article.

Complete gross necropsies were conducted on all rabbits found dead or euthanized in extremis in order to confirm *B. anthracis* infection. Tissues collected for histopathology included gross lesions, brain, heart, lungs, spleen, liver, kidney, and mediastinal or bronchial lymph nodes. A representative sample of spleen and mediastinal or bronchial lymph nodes were collected during gross necropsy, homogenized individually in approximately 2.0mL phosphate buffered saline (PBS) and plated on blood agar plates for qualitative assessment of bacterial presence. On Study Day 28 or Day 29, all surviving animals underwent complete gross necropsies and the same list of tissues were collected and preserved in 10% neutral buffered formalin. Histopathology was conducted on the 11 collected tissues from all rabbits that died on study and from six rabbits surviving to study termination (two randomly selected animals, one animal per sex, per group in groups 2, 3 and 4), with the exception of a few tissues that were not successfully processed to slides and were coded as "missing." Tissues were processed to 5-micron sections for routine hematoxylin and eosin staining and were examined microscopically by a board-certified veterinary pathologist. The purpose of the microscopic examination was described as a search for evidence of anthrax.

All of the (100%, 9/9) of rabbits in the saline control (Group 1) died following challenge with *B. anthracis*. Mortality decreased in a dose-dependent manner in ETI-204-treated groups to 67% (6/9), 24% (4/17) and 6% (1/ 17) in groups receiving 1, 4 or 16 mg/kg ETI-204, respectively. In the levofloxacin-only treatment Group 5, 10% (1/10) of rabbits died following challenge. In all groups, more males than females died following challenge, although the cause and significance of this finding were not apparent. Gross lesions in rabbits dying post-anthrax challenge included discolorations and/or foci in the appendix, brain, lung and large intestine, enlargement of multiple lymph nodes, and fluid in multiple body cavities (effusion) and the ventral skin (edema). These gross lesions were described as typical of anthrax in rabbits (Zaucha et al., 1998), and correlated histologically with hemorrhage, necrosis, edema and acute inflammation. In the six surviving animals that were examined at study completion, no gross lesions were reported.

Microscopic findings were present in all rabbits that died or became moribund during the study that were considered to be consistent with anthrax. Those lesions included acute fibrinous to heterophilic inflammation, necrosis, hemorrhage, edema, and the presence

of large rod-shaped bacteria in the brain (meninges), heart, kidney, liver, lung, spleen, cecum/appendix and multiple lymph nodes. Slightly more bacteria were evident in organs from the saline control Group 1 animals as compared to other groups. There were no other qualitative differences in lesions of anthrax among the control and experimental groups. No significant microscopic lesions were reported in the six rabbits that survived to euthanasia at study termination.

(Reviewer's comment: These data would have been more meaningful if all survivors had been evaluated.)

## 2. Study no. AR033 ( (b) (4) Study No. 1185-100003006): Evaluating the Efficacy of ETI-204 when Administered Therapeutically in New Zealand White Rabbits

This GLP-compliant study was conducted at (b) (4)

(b) (4). Histopathology was performed by (b) (4)

(b) (4). Neuropathology was performed by (b) (4)

Seventy New Zealand White rabbits (Hra:[NZW] SPF; 14 per dose group, half male and half female) plus 8 replacement animals (3.16-4.00 kg, 6 to 15 months of age on arrival) were placed on the study. Rabbits were surgically implanted with vascular access ports (VAP) prior to shipment to (b) (4)

The animals were challenged via the inhalation route with *B. anthracis* (Ames strain) spores on Study Day 0. Prior to challenge the rabbits were placed individually into a plethysmography chamber and a Class III cabinet system. The inhalation exposure report indicates that they were anesthetized, but the details are not provided. The rabbits were aerosol challenged with a targeted 200 LD<sub>50</sub> [2.1 x 10<sup>7</sup> spores] inhaled dose of *B. anthracis* spores. The Ames LD<sub>50</sub> value (105,000 colony forming units [cfu]) published from USAMRIID (Zaucha, 1998) was used for the dose calculations. The spore concentration and Spray Factor characterization for the spore lot used on this study (Spore Lot B37) were used to calculate the estimated aerosol concentration. Spores were aerosolized by a three-jet Collison nebulizer (designed to generate aerosols with approximate mean diameter of 1-2 μm) and delivered via a muzzle-only inhalation exposure chamber. After the aerosol concentration was estimated, the volume (total accumulated tidal volume [TATV]) of atmosphere that an animal needs to inhale to reach the target LD<sub>50</sub> dose was determined. The length of time of the aerosol exposure was determined by the rate at which the animal inhaled the calculated TATV, which was measured in real-time during each animal's challenge. Aerosol concentrations of *B. anthracis* spores were quantified by determination of cfu in effluent streams collected directly from an animal exposure port by an in-line all-glass impinger. Serial dilutions of the impinger samples were then plated onto tryptic soy agar (TSA) plates and enumerated. Particle size was determined using the Aerodynamic Particle Sizer (APS) spectrometer at 5 minutes into the exposure to allow the system to reach near steady state conditions. Mass median aerodynamic diameter (MMAD) for each of three exposure days ranged from 1.23-1.27 μm, falling within the targeted 1-5 μm expected to reach the alveoli. Aerosol exposure data are summarized in the Sponsor's tables below:

**Table 1 Target Parameters and Summary Data for Aerosol Exposures**

Target Parameter		Target Value	Actual Values		
			CDA	CDB	CDC
Target Exposure Parameters	1	105000	N		
	Spray Factor	1.00E-06	1.06E-	9.37E-	8.79E-
	Exposure Level	200	21	19	18
	Neb. Suspension	1.56E+09	1.60E+09	1.66E+09	1.17E+0
	Aerosol Particle	1.1 - 13 µm	1.2	1.2	1.2
	Aerosol	1.56E+06	1.66E+06	1.46E+06	1.37E+0
Animal Exposure Parameters	Animal Weight	3.0 - 4.5 kg	3.5	3.5	3.4
	Animals/Exposure	2	2	2	2
	Exposure Time	10 min	8.2	8.0	7.9
	Total Inhaled	13.46 L	13.85	14.31	14.34
	Total Inhaled Dos	2.10E+07 cfu	2.30E+07	2.08E+07	1.95E+0

**Table 6 Average Challenge Doses (LD<sub>50</sub> Equivalents)**

Challenge	Average LD <sub>50</sub> Equivalent Dose (SD)
A	219 (± 35)
B	198 (± 32)
C	186 (± 33)
Group	Average LD <sub>50</sub> Equivalent Dose (SD)
4 (Saline)	201 (± 34)
3 (1 mg/kg ETI-	209 (± 28)
1 (4 mg/kg ETI-	209 (± 45)
5 (8 mg/kg ETI-	189 (± 38)
2 (16 mg/kg ETI-204)	196 (± 30)

Animals were monitored for up to 28 days post-challenge for outward clinical signs of disease, hematological abnormalities, abnormal C-reactive protein (CRP) levels, quantitative bacteremia, and circulating levels of *B. anthracis* free PA as assessed quantitatively by a PA-ELISA. The sample collection schedule is outlined in the Applicant's table below:

**Blood Collection and Assay Schedule<sup>a</sup>**

Time-point	Collection Window	Collection Tube and Approximate Volume	Bacteremia (Qualitative & Quantitative)	CBC/CRP	Serum PA level (via ECL assay)	Serum for PA ELISAs	Serum for ETI-204 Levels	Serum for Anti-drug antibodies	CSF for bacterial assessment
Day -7	-	EDTA ~1.5 mL SST ~2.5 mL	X (Qual. only)	X	X	X	X	X	
<sup>^</sup> 24hr PC	± 60 min	EDTA ~0.7 mL SST ~2.0 mL	X		X	X			
<sup>^</sup> 30hr PC	± 60 min	EDTA ~0.7 mL SST ~2.0 mL	X		X	X			
<sup>^</sup> 36hr PC	± 60 min	EDTA ~0.7 mL SST ~2.0 mL	X		X	X			
<sup>^</sup> 42hr PC	± 60 min	EDTA ~0.7 mL SST ~2.0 mL	X		X	X			
# PTT	-	EDTA ~1.5 mL SST ~2.0 mL mL SPS	X	X	X	X			
15 min PT	± 5 min	SST ~2.0 mL				X	X		
4hr PT	± 15 min	EDTA ~0.7 mL SST ~2.0 mL	X			X	X		
8 hr PT	± 15 min	SST ~2.0 mL				X	X		
24hr PT	± 60 min	EDTA ~1.5 mL SST ~2.0 mL	X	X		X	X		
48 hr PT	± 60 min	EDTA ~0.7 mL SST ~2.0 mL	X			X	X		
72 hr PT	± 60 min	EDTA ~1.5 mL	X	X					
*8 days PC	-	EDTA ~1.5 mL SST ~2.0 mL	X	X		X	X		
*12 days	-	SST ~2.0 mL				X	X		
*16 days PC	-	EDTA ~2.0 mL SST ~4.0 mL	X	X		X	X		
*20 days PC	-	EDTA ~2.0 mL SST ~4.0 mL	X	X		X	X		
*28 days PC	-	EDTA ~1.5 mL SST ~4.0 mL	X	X		X	X	X	X
Terminal <sup>b</sup>	-	EDTA ~1.5 mL SST ~4.0 mL	X	CRP only		X	X		X

PC = Post-Challenge, PTT = Prior to Treatment, PT = Post-Treatment, - = not applicable

<sup>a</sup> Post-challenge pre-treatment bleed time points were relative to a median challenge time for a challenge cohort. Post-treatment bleed times were calculated from the time each animal's IV treatment ends.

<sup>b</sup> If collection was possible (performed by cardiac stick if required).

\* Collected via ear.

<sup>^</sup> Post-Challenge, pre-treatment sampling stopped once decision to treat has been made.

# PTT Bacteremia enrichment performed on sample collected in SPS tube (see section 4.10)

Initiation of intravenous (IV) ETI-204 or saline (control) treatment for each rabbit was based on a positive result for PA or a significant increase in body temperature (SIBT). PA was measured with a qualitative, electrochemiluminescence PA (PA-ECL) assay. The Rapid Protective Antigen Screening Assay, technology produced by MesoScale Discovery (MSD, Gaithersburg, MD), is a 96-well ECL assay designed to

detect the presence of *B. anthracis* PA. Test samples were considered positive for PA when the mean ECL value was greater than or equal to the mean electrochemiluminescence (ECL) value of the 1 ng/mL positive control (1 ng/mL was used as threshold for treatment). Body temperatures were measured using an implanted programmable temperature transponder (IPTT-300, BMDS, Seaford, DE). Body temperatures (°F) were measured twice daily with the exception of the period from ~18 to ~54 hours post-challenge, when temperatures were measured once every hour. SIBT was defined as either three consecutive critical temperature readings or when an animal had exhibited two consecutive critical temperature readings twice. Critical temperature was defined as a reading equal to or greater than a two-standard deviation increase from each individual rabbit's average baseline body temperature.

All animals survived to treatment. All summary statistics were relatively similar between the groups indicating that the animals were at a similar disease stage when saline or treatment was administered. Seventy-six percent (53/70) of the animals were treated based on increased body temperature. The mean group time from challenge until a positive SIBT result was between 26.07 and 28.42 hours. There were no statistically significant mean group differences in the proportion of animals with a SIBT prior to treatment or for time from challenge until a SIBT. Data are shown in the Applicant's table below:

#### Summary Statistics for Time from Challenge until SIBT

Treatment	n/N#	Geometric Mean (Hours) (95%)	Hours (Min, Max)
Saline	14/14	26.07 (23.08, 29.44)	(18.35 - 37.07)
1 mg/kg ETI-204	14/14	27.58 (25.19, 30.20)	(20.53 - 33.60)
4 mg/kg ETI-204	14/14	28.42 (25.40, 31.80)	(19.75 - 42.87)
8 mg/kg ETI-204	14/14	27.06 (24.26, 30.18)	(19.97 - 37.82)
16 mg/kg ETI-204	14/14	27.60 (24.50, 31.09)	(17.70 - 38.37)

# Number of animals that exhibited a SIBT / number of animals in the group

The mean time for all groups from challenge until a positive PA-ECL result was between 26.77 and 28.90 hours. Three to four animals in each group were not positive for PA-ECL at any time point before treatment. As many as two animals in a group were not positive for bacteremia before treatment. Only one or two animals from each group had PA-ELISA results greater than the LLOQ before treatment. Summary data are shown in the Applicant's table below:

**Pre-Treatment Summary Statistics**

Parameter	ETI-204 Dose				
	Saline	1	4	8	16
<b>Prior To</b>					
	N = 14	N = 14	N = 14	N = 14	N = 14
Sex n (%)					
Male	7 (50%)	7 (50%)	7 (50%)	7 (50%)	7 (50%)
Female	7 (50%)	7 (50%)	7 (50%)	7 (50%)	7 (50%)
Weight (kg) Mean (SD)	3.56 (0.18)	3.51 (0.18)	3.55 (0.16)	3.50 (0.15)	3.55 (0.21)
Temperature (F) Mean (SD)	100.5 (1.2)	100.1 (1.2)	100.0 (1.1)	100.7 (0.9)	100.9 (1.8)
<b>Prior To Treatment</b>					
	N = 14	N = 14	N = 14	N = 14	N = 14
Sex n (%)					
Male	7 (50%)	7 (50%)	7 (50%)	7 (50%)	7 (50%)
Female	7 (50%)	7 (50%)	7 (50%)	7 (50%)	7 (50%)
Temperature (F) Mean (SD)	102.8 (1.5)	102.9 (2.8)	102.2 (1.7)	102.6 (1.8)	102.9 (2.2)
PA-ELISA n (%)					
Positive	1 (7.14%)	1 (7.14%)	1 (7.14%)	2 (14.29%)	1 (7.14%)
Negative	13 (92.86%)	13 (92.86%)	13 (92.86%)	12 (85.71%)	13 (92.86%)
Mean Time to Positive PA-	24.90 (--)	29.93 (--)	27.65 (--)	30.42 (3.16)	26.63 (--)
PA-ELISA Geometric Mean	<LLOQ (38.42)	<LLOQ (47.22)	<LLOQ (68.36)	<LLOQ (44.49)	<LLOQ (80.14)
PA-ECL n (%)					
Positive	10 (71.43%)	11 (78.57%)	10 (71.43%)	11 (78.57%)	11 (78.57%)
Negative	4 (28.57%)	3 (21.43%)	4 (28.57%)	3 (21.43%)	3 (21.43%)
Mean Time (hr) To Positive PA-	27.53 (3.92)	27.03 (3.86)	26.99 (3.58)	27.48 (4.34)	29.20 (4.48)
Bacteremia <sup>#</sup> n (%)					
Positive	13 (92.86%)	12 (85.71%)	12 (85.71%)	13 (92.86%)	13 (92.86%)
Negative	1 (7.14%)	2 (14.29%)	2 (14.29%)	1 (7.14%)	1 (7.14%)
Mean Time (hr) To Positive	24.79 (4.69)	25.44 (2.99)	25.32 (2.82)	24.85 (4.41)	26.37 (4.26)
Quantitative Bacteremia (cfu/mL) Geometric	706 (1.07x10 <sup>5</sup> )	1.31x10 <sup>3</sup> (2.82x10 <sup>5</sup> )	1.94x10 <sup>3</sup> (5.60x10 <sup>4</sup> )	2.05x10 <sup>3</sup> (3.75x10 <sup>4</sup> )	1.36x10 <sup>3</sup> (3.01x10 <sup>4</sup> )

SD = Standard deviation.

F = Degrees Fahrenheit.

-- = Standard deviation could not be calculated since only one animal from this group was positive for PA-ELISA prior to treatment. LLOQ = Lower limit of quantification (9.68 ng/mL).

CV = Coefficient of variation.

# = Calculations included quantitative, qualitative, and enriched bacteremia measurements.

An IV bolus of the test article (ETI-204) or control material (saline) was administered based on either a positive serum PA result in the ECL assay or on a SIBT. Once one of these criteria was met, a single IV bolus of ETI-204 or saline was administered via the VAP. The port was then flushed ~ 2 mL sterile saline.

**Pathology**

All surviving rabbits were euthanized at the end of the in-life phase on Study Day 28. No animals in the control group survived; all died within one week of challenge.

Gross necropsy was performed on all rabbits that were found dead or euthanized (including survivors euthanized on Study Day 28). Target tissues (brain/meninges, lungs, liver, spleen, spinal cord, kidney and mediastinal or bronchial lymph nodes, and gross lesions) were collected and preserved in 10% neutral buffered formalin. Each rabbit brain was cut sagittally into 2 halves. The left hemisphere was placed in 10% formalin for subsequent neuropathology examination. The right hemisphere was fixed and processed to paraffin-embedded blocks to be stored for potential future immunohistochemical analyses of ETI-204, PA, and/or *B. anthracis*. All rabbit tissues collected, with the exception of brains, were stained with H&E and evaluated microscopically (b) (4) by a board-certified veterinary pathologist. Prior to fixation, a representative sample of the right cerebellar brain hemisphere, spleen, lung, liver, kidney, and mediastinal or bronchial lymph node were collected from all animals found dead or euthanized (including survivors) during gross necropsy for culture and qualitative assessment of bacterial presence in the target tissues.

Selected left brain hemispheres fixed in 10% formalin were shipped to (b) (4). The specimen selection included three non-survivors from each dose group (including control) and three survivors from each ETI-204-treated group. The left brain hemispheres were cut into ~8 sections, stained with H & E (hematoxylin and eosin), and examined microscopically by a separate board certified veterinary pathologist.

Gross lesions in non-surviving animals included discoloration or foci in the brain and large intestines (appendix), enlargement of mediastinal and bronchial lymph nodes, and fluid (effusion) in the pericardial cavity, thymus, and thoracic cavity (fluid in body cavities was not examined microscopically). These gross lesions were considered typical of anthrax presentation in rabbits and correlated in general with hemorrhage, edema, and acute inflammation. The only gross lesion present in any of the animals surviving to study termination on Study Day 28 was renal pelvis dilatation in one 8 mg/kg male, which was considered to be an incidental finding.

Microscopic findings that were considered to be consistent with anthrax (based on Zaucha *et al.*, 1998) were present in all animals examined that died or became moribund prior to the scheduled study termination on Day 28. Those lesions included acute inflammation, necrosis, hemorrhage, fibrin exudation, and lymphoid depletion/necrosis. In nearly all early decedents, large rod-shaped bacteria consistent with *B. anthracis* were present in one or more organs. The report states that, in animals without microscopic evidence of bacteria, terminal blood and/or organ culture were positive for *B. anthracis*. In control animals, the highest incidence of microscopic lesions and bacteria were noted. Most control animals had widely disseminated bacteria throughout all organs, and particularly in lymphoid organs. These tissues exhibited the greatest consistency and severity of necrosis (particularly of lymphocytes), inflammation, fibrin exudation, and hemorrhage, while similar findings were more sporadic in other examined organs. Another consistent finding in most control animals was sinusoidal leukocytosis in the liver. These lesions were only slightly less common in early decedent animals treated with 1 mg/kg ETI-204, and bacteria were still present in multiple organs in this group. In non-surviving animals treated with ETI-204 doses higher than 1 mg/kg, infection-related findings and bacteria appeared to be more

sporadic and/or of lower average severity. Notably, there were fewer animals in these groups that died prior to study termination.

The only microscopic lesions present in any of the animals surviving to study termination on Study Day 28 were: nephropathy in an 8 mg/kg female, nephropathy and alveolar histiocytosis in a 16 mg/kg female, lung inflammation in a 16 mg/kg male, and renal infarct and renal pelvis dilatation in an 8 mg/kg male. These were considered to be incidental findings with no relation to *B. anthracis* challenge or treatment.

#### Neuropathology

The brains (left hemispheres) of 12 surviving animals (3 survivors per treated group) and 15 animals that were found dead (between Study Days 2 and 8; 3 non-survivors per group, including controls) were microscopically evaluated. Approximately 25% of the animals were triggered for treatment by PA-ECL, consistent with the proportion of animals in the study that were triggered by PA-ECL.

<b>ETI-204 Dose</b>	<b>Number of survivors analyzed at (b) (4)</b>	<b>Number of non-survivors analyzed at (b) (4) (Total)</b>
0 mg/kg	0 (0)	3 (14)
1 mg/kg	3 (4)	3 (10)
4 mg/kg	3 (6)	3 (8)
8 mg/kg	3 (10)	3 (4)
16 mg/kg	3 (9)	3 (5)

The study neuropathologist included the following sites in or around the brain in his evaluation: meninges, ventricular system, cerebral cortex (including numerous sections through the cerebral cortex/cerebrum from the rostral (prefrontal) to the caudal (occipital) cerebrum), basal nuclei (ganglia), thalamus/hypothalamus, midbrain, pons region/pontine nuclei, hippocampus, cerebellum, and medulla oblongata. The pathologist was blinded to group assignment until after the initial neuropathology review.

Gross pathological findings were limited to three annotations of dark or red meninges in two non-survivors treated with 16 mg/kg and one non-survivor treated with 8 mg/kg ETI-204. There was no microscopic correlation to two (L48796; L48772) of those observations; they were considered likely to be related to post-mortem changes (congestion/pooling of blood in the meninges). In one animal (L48781, 16 mg/kg), the observation of diffuse red meninges correlated microscopically to moderate extravascular bacteria in the meninges and severe hemorrhage in the cerebral cortex.

Ten of the 12 treated animals surviving to study termination had no evidence of bacteria (intravascular or extravascular) or signs of reaction to bacteria. Two treated and surviving animals did exhibit lesions. One animal treated with 1 mg/kg ETI-204 had minimal hemorrhage in or near the cerebellum. This finding was considered to be either a residual effect of resolved infection or a handling artifact. The second animal, treated with 8 mg/kg ETI-204 had minimal neuronal loss/necrosis in the hippocampus,

considered by the pathologist to be most likely due to infection, but possibly incidental or due to the test article (although similar changes were not seen in any other animal).

Of the animals found dead, six had no biologically significant findings in the brain. One in each of the control, 1 mg/kg, and 16 mg/kg groups and all three of the 8 mg/kg subsets were considered to have died before being able to mount any inflammatory response and before microscopically-detectable bacteremia. Three of the animals found dead included two controls exhibiting hemorrhage and one 1 mg/kg animal with intravascular bacteria; these animals were also considered to have died of “septicemia without any notable morphologic response.” The remaining six nonsurvivors exhibited extravascular bacteria and/or hemorrhage, and were considered to have survived long enough to mount a variable inflammatory reaction to the infection and/or had sufficient vascular damage to have hemorrhage associated with extravascular bacteria. This group included one animal treated with 1 mg/kg, all three animals treated with 4 mg/kg, and two animals treated with 16 mg/kg.

None of these changes in any group were considered to have any relationship to the test article. There was no apparent dose-response in the presence or severity of findings in the brain and no apparent correlation between severity of findings and time of death.

The Applicant’s table below summarizes the findings for non-surviving animals examined:

Group	Animal Number	Survival Outcome	Time to Death (days)	Presence of Lesions <sup>1</sup>		
				Bacteria (Intravascular)	Bacteria (Extravascular)	Inflammation
<b>SALINE CONTROL (Group 4)</b>	<b>Males</b>					
	L48765	FD	2	-	-	-
	L48795	FD	5	-	-	-
	<b>Females</b>					
	L48747	FD	5	-	-	-
<b>1 mg/kg (Group 3)</b>	<b>Males</b>					
	L48768	FD	7	X	-	-
	L48778	FD	3	-	-	X
	<b>Females</b>					
	L48744	FD	2	-	-	-
<b>4 mg/kg (Group 1)</b>	<b>Males</b>					
	L48764	FD	7	-	X	X
	L48782	FD	4	-	X	-
	L48788	FD	6	X	X	X
<b>8 mg/kg (Group 5)</b>	<b>Males</b>					
	L48772	FD	2	-	-	-
	<b>Females</b>					
	L48732	FD	8	-	-	-
	L48748	FD	4	-	-	-
<b>16 mg/kg (Group 2)</b>	<b>Males</b>					
	L48781	FD	3	-	X	-
	L48796	FD	2	-	-	-
	<b>Females</b>					
	L48756	FD	7	-	X	X

<sup>1</sup> = An “X” denotes the microscopic change was present.

The report concludes that this study lacked a group where only the test article was administered in the absence of infection and notes that, in an embryo-fetal toxicity study, doses up to 32 mg/kg were administered with no microscopic changes found in the brain related to the test article at the end of the study. This pathologist took this as evidence that administration of ETI-204 had no adverse morphologic effects in the brain, and that all the morphologic lesions encountered in this study were either related to the bacteria or were spontaneous/incidental changes. He also noted that the variability seen in the study is not unexpected following exposure to an infectious agent. In general, in the opinion of the study neuropathologist, animals that received the test article were more likely to mount some sort of an inflammatory response (and thus to have more evidence of inflammation) as compared to the animals that were given saline alone.

### 3. Study no. AP201 ( (b) (4) study no. 834-G924202): Evaluating the Efficacy of ETI-204 when Administered Therapeutically in the Cynomolgus Macaque Inhalational Anthrax Model

This GLP-compliant study was performed at (b) (4) (final report date 8/25/11).

Forty-four juvenile (2.6-5.1 years of age and 2.5-5.3 kg at randomization) cynomolgus macaques (*Macaca fascicularis*) were randomized by weight into two groups of 15 animals and one group of 14 animals (with each group containing ~50% male, ~50% female) according to the Applicant's table below:

#### Study Design

Number of	Treatment	Blinded Group
15	0 (Saline)	Y
14	4 mg/kg ETI-	X
15	8 mg/kg ETI-	Z

APPEARS THIS WAY ON ORIGINAL

All animals were verified negative for tuberculosis and were pre-screened to verify that they were seronegative for Simian Immunodeficiency Virus (SIV), Simian T-Lymphotropic Virus-1 (STLV-1), and *Cercopithecine herpesvirus 1* (Herpes B virus), as well as negative for Simian Retrovirus (SRV1 & SRV2) by polymerase chain reaction (PCR). During the pre-test quarantine, they had TA-D70 telemetry transmitters [Data Sciences International (DSI), St. Paul, MN] surgically implanted.

One animal (C39111) died prior to telemetry implantation surgery, and another animal (C39076, randomized to Group Yin Challenge Day C) was removed from study prior to challenge, leaving a total of 43 monkeys that were challenged. The Applicant's table below summarizes the treatment group assignments:

**Table 4 Study Group Assignments**

Group	Treatment	Dose Volume (mL/kg)	Number of Animals (Males , Females)
X	4 mg/kg ETI-204	0.5	14 (7,7)
Y	Saline	0.5	14 (6,8)
Z	8 mg/kg ETI-204	0.5	15 (8,7)

The monkeys were randomized to one of three aerosol challenge days. The staff treating and assessing the animals were blinded to the respective treatment group. Study Day 0 was the day of aerosol challenge for each challenge cohort. Monkeys were challenged via the inhalation route with *B. anthracis* (Ames strain), (b) (4) Spore Lot B35. Prior to challenge, monkeys were anesthetized with Telazol (1-6 mg/kg, IM) and placed into a plethysmography chamber in a Class III biosafety cabinet system.

The target aerosol challenge dose was 200 LD<sub>50</sub> (1.24 x 10<sup>7</sup> spores) of *B. anthracis* (Ames strain) spores, as determined by Vasconcelos et al. (2003). The spore concentration and Spray Factor characterization were used to calculate the estimated aerosol concentration. Spores were aerosolized by a Collison nebulizer and delivered via head-only inhalation. After the aerosol concentration was estimated, the volume (total accumulated tidal volume [TATV]) of atmosphere that each animal needed to inhale to reach the target LD<sub>50</sub> dose was calculated. The length of time of the aerosol exposure for each animal was determined by plethysmography data measured in real-time during each animal's challenge. To quantify actual exposures, aerosol concentrations of *B. anthracis* were quantified by determination of colony forming units (cfu) in effluent streams collected directly from an animal exposure port by an in-line all-glass impinger. Particle size was determined using an Aerodynamic Particle Sizer; mass median aerodynamic diameters and geometric means and standard deviations were calculated.

Aerosol challenge days were 8/24/09 (Challenge Day A), 8/31/09 (Challenge Day B) and 9/08/09 (Challenge Day C). The average (± standard deviation) aerosol exposure dose for all animals on study was 199 (± 60) *B. anthracis* (Ames strain,) LD<sub>50</sub> equivalents. Challenge doses by day and treatment group are shown in the Applicant's table below:

**Table 5 Average Challenge Doses (LD<sub>50</sub> Equivalents)**

Challenge Day	Average Dose (SD)
A	251 (± 54)
B	175 (± 57)
C	168 (± 18)
Group	Average Dose (SD)
X (4 mg/kg ETI-204)	201 (± 52)
Y (Saline)	199 (± 66)
Z (8 mg/kg ETI-204)	199 (± 65)

The average mass median aerodynamic diameter (MMAD) was 1.11 µm, 1.11 µm and 1.12 µm on Challenge Days A, B, and C, respectively.

Monkeys were observed for clinical signs every six hours (± 1 hour) beginning approximately 24 hours post-median challenge time and ending approximately 192 hours (8 days) post median challenge time. Appetite was monitored twice daily. Monkeys were also monitored for abnormal body temperature by telemetry, and blood was sampled according to the Sponsor's schedule below to monitor for hematological abnormalities, abnormal C-reactive protein (CRP) levels, qualitative and quantitative

bacteremias, and circulating levels of *B. anthracis* PA (protective antigen) as assessed qualitatively by an electrochemiluminescence (ECL) screening (trigger to treat) assay or quantitatively by a PA-ELISA assay.

**Table 2 Blood Collection and Assay Schedule<sup>a</sup>**

Approximate Time Point	Blood Tube type/ Approximate Blood volume	Bacteremia (Culture)	CBC/ CRP	Serum PA levels (via ECL and PA-ELISA)	Serum for ETI-204 dose confirmation	* Retention Serum for Potential Future Use
Day -7	EDTA ~1.5ml SST ~2.0ml	X	X	X	X	X
^24hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^30hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^36hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^42hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^48hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^54hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
PTT <sup>#</sup>	EDTA ~1.5 ml SST ~2.0ml SPS ~1.0 ml	X <sup>#</sup>	X	X		X
5 min PT	SST ~1.0ml					X
6hr PT	SST ~1.0ml					X
24hr PT	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>	X	X
96hr PT	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
7 days PC	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
14 days PC	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
21 days PC	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
30 days PC	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
Terminal <sup>b, d</sup>	EDTA ~1.5 ml SST ~2.0ml	X	CRP only	X <sup>c</sup>		X

PC = Post-Challenge PTT = Prior to Treatment PT = Post-Treatment

<sup>a</sup> Post-challenge pre-treatment bleed time points were relative to a median challenge time for a challenge cohort. Post-treatment bleed times were calculated from the time each animal's IV treatment ended. Blood sample collection occurred within ±60 minutes of the calculated time, except for the 5 min PT, 6hr PT and 96hr PT samples which occurred within 2 min, 15 min and 3 hours of their calculated times, respectively. The Day -7, Day 7 PC, Day 14 PC, Day 21 PC and Day 30 PC bleeds were relative to the day of challenge.

<sup>b</sup> If collection was possible

<sup>c</sup> Samples tested via PA-ELISA only

<sup>d</sup> Terminal samples were not collected on animals euthanized at the end of study as day 30 PC samples were already scheduled

\* If remaining serum was sufficient after aliquots for other analyses were made. Samples will be stored for potential future testing

<sup>#</sup> Post-Challenge, pre-treatment sampling stopped once decision to treat was made

<sup>#</sup> PTT Bacteremia enrichment performed on sample collected in SPS tube

Blood samples were taken from a femoral artery or vein, saphenous vein, or other appropriate vein. All post-challenge, pre-treatment blood samples were collected relative to the median challenge time at the time points stated  $\pm$  1 hr. Median challenge time was calculated from the end time of the first and last animals challenged within a single challenge day. Post-challenge, pretreatment blood sampling was discontinued once a decision to treat was made. Post-treatment bleed times were calculated from the time each animal's IV treatment ended. Collected blood was processed to serum. At applicable time points, a portion of the serum from all animals was used in the electrochemiluminescence (ECL) assay, run on-site immediately as a screening assay for the presence of PA. When the mean ECL value was greater than or equal to the mean ECL value of the 2 ng/mL positive control (2 ng/mL was used as the threshold for treatment), the test sample was considered positive for PA, and thus triggered an animal to receive treatment.

In addition to evaluation of bacteremia by blood culture, qualitative evaluation of sections of spleen and mediastinal or bronchial lymph nodes collected during gross necropsies was performed.

A portion of the serum samples at the time points indicated in the above table were collected and stored at  $-70^{\circ}\text{C}$ . A quantitative evaluation of circulating PA levels was performed by ELISA in order to retrospectively quantify circulating PA levels.

Serum for ETI-204 dose confirmation was collected at 24 hours post-dose and assayed using the PAA3 ELISA. In the saline control group, no quantifiable levels of ETI-204 were found except for a sample labeled C37736 24-hour PTX-C (post-treatment, Challenge Day C), which measured 133.22  $\mu\text{g/mL}$ . The 24-hour serum samples from the 4 mg/kg ETI-204 group contained 38-83  $\mu\text{g/mL}$  ETI-204, with the exception of one animal (C39063), which was below the lower limit of detection. The 24-hour samples from the 8 mg/kg ETI-204 group were in the range of 87-168.95  $\mu\text{g/mL}$  ETI-204 except for 2 anomalous results: sample C39065 measured 1.6  $\mu\text{g/mL}$  and C39089 was below detection. The exact cause of the 4 unexpected values was not determined; it was hypothesized that samples may have been switched or mis-labeled when processed to serum.

*Reviewer's comment: This introduces a degree of uncertainty in the accuracy of the findings for the whole sample set.*

The report states that the majority of animals exhibited abnormal outward clinical signs consistent with anthrax following challenge, including lethargy, hunched posture, inappetance, and stool abnormalities. In animals that survived, signs generally returned to normal between 8-10 days post-challenge, but occasional inappetance and/or soft stools, which were not considered to be abnormal in laboratory NHPs, were evident. In animals that eventually succumbed, signs progressed through lethargy, inappetance, respiratory abnormalities, and finally moribundity.

Body temperature and activity were monitored and stored electronically beginning at least 7 days prior to challenge until the end of the in-life animal phase (30 days post-challenge) using an implantable TA-D70 DSI telemetry device. Activity data were taken as counts per minute. Prior to challenge, the mean baseline temperature and activity values were computed, and average hour differences were referred to as the "baseline-adjusted" values in the analysis. The standard deviation of all of the pre-challenge baseline-adjusted temperatures was calculated for each animal, and two

times this standard deviation was used as the threshold for an elevated temperature. A post-challenge temperature was considered elevated if the baseline-adjusted temperature for the time point was greater than this threshold. Animals were determined to have a significant increase in body temperature (SIBT) if six consecutive measurements were above the threshold. For calculation of time until SIBT, the time of the sixth consecutive elevated temperature that caused the criteria to be met was defined as the time of abnormal temperature for SIBT.

**Table 8 Summary Statistics for Time from Challenge until SIBT**

Dose	N	Geometric Mean (Hours) (95% Confidence Interval)	(Min, Max)
Saline	14	37.58 (33.40, 42.28)	(27.00, 57.00)
4 mg/kg ETI-204	14	37.66 (30.51, 46.48)	(15.00, 64.00)
8 mg/kg ETI-204	15	34.83 (27.13, 44.72)	(8.00, 61.00)

**Table 9 Proportion of Animals Reaching SIBT Prior to Treatment**

Dose	Proportion Abnormal	Abnormal Rate (95% Confidence Interval)
Saline	9/14	0.64 (0.35, 0.87)
4 mg/kg ETI-204	6/14	0.43 (0.18, 0.71)
8 mg/kg ETI-204	11/15	0.73 (0.45, 0.92)

Baseline body weights were determined prior to challenge and on Day 0. Day 0 body weights were used for treatment calculations. Post-challenge body weights were not collected as no prolonged inappetence (for several consecutive days) was observed, and therefore weight loss was not expected.

Treatment for each monkey with either ETI-204 or saline (control) was to be initiated based on a positive result in a qualitative electrochemiluminescence (ECL) protective antigen (PA) assay or at the discretion of the Study Director. If a PA positive result was not available after 54-hours post-challenge, animals were administered either test article or control as appropriate. The mean time (per group) from challenge until a positive PA-ECL result was between 36.75 and 38.76 hours (see the Applicant's table below). There were no significant group differences in the proportion of animals positive for PA-ECL prior to treatment. Additionally, there were no significant group effects for time from challenge until a positive PA-ECL result.

Eighty-eight percent (38/43) of the animals were treated based on a positive PA-ECL result. Three animals from the 4 mg/kg ETI-204 group (C38261, C37686, and C36338) and 1 animal from the 8 mg/kg ETI-204 group (C37072) were treated as directed by the Study Director; they were described as "failed ECL plates," but were considered to have values above those for positive controls. One additional animal from the saline group (C38277) was treated after the 54-hour post-challenge time point despite a negative ECL result. One-hundred percent of the monkeys had a positive bacteremia result prior to treatment.

**Table 10 Summary Statistics for Time from Challenge until a Positive PA-ECL Result**

Treatment	N	Geometric Mean (Hours) (95% Confidence Interval)	(Min, Max)
Saline	13	38.76 (34.34, 43.74)	(28.58, 52.57)
4 mg/kg ETI-204	13	36.75 (31.33, 43.10)	(25.53, 55.92)
8 mg/kg ETI-204	15	37.93 (33.99, 42.33)	(25.43, 54.83)

**Table 11 Proportion of Monkeys Positive by ECL Prior to Treatment**

Treatment	Proportion Abnormal	Abnormal Rate (95% Confidence Interval)
Saline	13/14	0.93 (0.66, 1.00)
4 mg/kg ETI-204	13/14	0.93 (0.66, 1.00)
8 mg/kg ETI-204	15/15	1.00 (0.78, 1.00)

The average time (by group) from challenge until treatment was 44.5, 41.3 and 42.5 hours for animals treated with saline, 4 mg/kg, and 8 mg/kg ETI-204 respectively. Test article was ETI-204 (Baxter 103B20-X109-TR06, Elusys Lot number ET 472-084; purity – 98% monomer). The control was 0.9% saline. An intravenous bolus of the test article (ETI-204 at 4 mg/kg or 8 mg/kg) or control was administered at a volume of 0.5 mL/kg (usually into the saphenous vein).

Dose formulation analysis was performed on aliquots of control and test article formulations using PAA3 ELISA assay and an optical density assay measuring absorbance at 280 nm. Analyses on the dilutions of ETI-204 prepared by (b) (4), for all three challenge days are shown in the Applicant's table below:

**Table 3 Dose formulation Analysis Results**

Vial Group ID	Preparation Date	Expected Concentration (mg/mL)	Elusys Determined Protein Concentration by A280 (mg/mL)	<span style="background-color: #cccccc;">(b) (4)</span> Determined Concentration (mg/mL)
X	8/24/09	8.0	7.20	6.69
	8/31/09	8.0	8.27	7.19
	9/8/09	8.0	8.26	7.15
Y	8/24/09	0.0	BLQ	<0.00000156
	8/31/09	0.0	BLQ	<0.00000156
	9/8/09	0.0	BLQ	<0.00000156
Z	8/24/09	16.0	15.33	14.25
	8/31/09	16.0	16.73	15.67
	9/8/09	16.0	16.19	15.96

BLQ = Below Limit of Quantitation

Survival in the control group was 14% (2/14), while 79% (11/14) and 73% (11/15) of the animals treated with ETI-204 at 4 or 8 mg/kg, respectively, survived. The overall Fisher's exact test was significant (p-value = 0.0008). The survival rates were significantly greater for both treatment groups relative to the saline control group, but there was no significant difference in survival between the two dose groups treated with the test article. The report states that higher quantitative bacteremia levels and higher PA levels prior to treatment were associated with lower probability of survival.

The report noted that 100% of the animals were bacteremic prior to treatment, indicating that all animals had a systemic infection prior to treatment. It also notes that abnormal PA appears to be one of the later indicators to be confirmed. Complete resolution of bacteremia (by the quantitative method) occurred by Day 14 PC for all surviving monkeys.

Gross necropsy was performed on all monkeys that were found dead or were euthanized prior to the end of the study. Samples of anthrax target tissues (brain/meninges, lungs, liver, spleen, kidney and mediastinal lymph nodes) and all gross lesions were preserved in 10% neutral buffered formalin. Histopathology was performed by a board-certified veterinary pathologist on all animals found dead or euthanized due to illness on study to confirm death or illness due to anthrax. All survivors were euthanized at termination on Study Day 30 and samples of the tissues identified above were collected and preserved for histopathology evaluation. A representative sample of spleen and mediastinal or bronchial lymph node was collected from all animals found dead or euthanized (including those euthanized at termination) during gross necropsy, homogenized, and plated on blood agar plates for qualitative assessment of bacteria in these target tissues.

Animals that were found dead or euthanized in a moribund condition prior to Day 30 all had gross and/or microscopic findings consistent with anthrax. All of these gross lesions, with the exception of the finding of a small right kidney in control female monkey C37736, were stated to be consistent with those described previously in inhalation anthrax in cynomolgus macaques (Vasconcelos et al., 2003). When examined microscopically, the small size of the right kidney in monkey C37736 was found to correspond with infarction, which the pathology narrative states is an incidental finding in the cynomolgus macaque. Based on the degree of fibrosis and absence of recent necrosis, this infarction was interpreted to have been present before the study began. The incidence of microscopic findings in kidney, spleen, liver and lymph node was higher in control animals compared to ETI-204 treated animals.

Six gross findings were noted in five of the monkeys that survived until Day 30. None of these gross findings corresponded with any indication of anthrax, nor were the findings interpreted to be due to ETI-204 administration. These findings included: 1) grossly evident mottled discoloration of the lungs in animal C38261 from the 4 mg/kg treatment group that had no microscopic correlate (lungs within normal limits histologically, no anthrax-like bacteria), 2) red discoloration in the cecum of animal C34985 from the 8 mg/kg treatment group (microscopically hemorrhage of the cecal muscular wall and submucosa with no associated anthrax-like bacteria, rather than the ulceration and necrosis of gut-associated lymphoid tissue that is classically found in some monkeys with anthrax), 3) a single white focus on the lungs of animal C36423 in the 4 mg/kg treatment group had, which corresponded microscopically with fibrosis and was considered to be incidental, 4) diffuse mottled discoloration of all lung lobes in monkey C38621 without microscopic abnormalities, and 5) enlarged mediastinal and bronchial lymph nodes in animal C37247 in the 4 mg/kg treatment group, and 6) enlarged bronchial lymph nodes in animal C36382 from the 4 mg/kg treatment group, both corresponding microscopically with lymphoid hyperplasia, and considered an

incidental finding in the absence of anthrax-like bacteria and associated lymphoid depletion or atrophy.

In general, monkeys that were found dead or were euthanized in moribund condition all had one or more microscopic lesions consistent with anthrax. Monkeys that survived to Day 30 demonstrated no gross or microscopic findings indicative of anthrax infection.

### Neuropathology

Additional microscopic evaluations of the brain were performed to characterize potential test article-related changes. These evaluations were performed blinded. The following materials were sent to [REDACTED] (b) (4)

[REDACTED]: all formalin-fixed blocks of brain tissue prepared at the in-life testing facility (all animals), all H&E stained sections of brain prepared by the in-life testing facility (all animals), and all remaining wet tissue (formalin fixed) from the brain from all monkeys. The remaining brain tissues were trimmed into 2 to 3 mm coronal slices to produce a total of approximately 15 sections of brain per animal (the 15 sections included the 3 sections previously trimmed and embedded by the testing facility). The slices were arranged from rostral to caudal for orientation. The areas trimmed were placed into a cassette, embedded in paraffin, and stained with hematoxylin and eosin (H&E) prior to examination.

Microscopic changes were said to be essentially absent in animals that survived until scheduled sacrifice at Day 30, with the exception of occasional background changes, including slight mononuclear cell infiltrates in the meninges (a change stated to be unrelated to anthrax or treatment). In animals that died or were euthanized prior to the end of the study, there was a spectrum of relatively consistent morphologic changes that included the following:

- Bacteria, hemorrhage and inflammation in the meninges (bacterial meningitis) o In some animals, bacteria were noted in the absence of an appreciable response by the host animal.
- Vasculitis (inflammation/destruction of blood vessel walls) in the meninges
- Bacteria, hemorrhage and inflammation in the brain (bacterial encephalitis) o In some animals, bacteria were noted in the absence of an appreciable response by the host animal.
- Vasculitis in the brain.

In animals (saline- and test article-treated) with an appreciable response, the changes were said to predominate in the meninges, where inflammation/vasculitis and hemorrhage were the predominant morphologic findings. The report states that responses in the brain tended to mimic the changes in the meninges but were generally less pronounced. The report states that the most pronounced brain changes were noted usually immediately adjacent to the most pronounced meningeal changes, leading the pathologist to speculate that because of the increased surface area exposure to the meninges, microscopic changes in the brain were more numerous in the cerebrum and cerebellum. The report goes on to state that, in animals that died prior to the end of the study or were sacrificed in a moribund condition, there was an overall difference between the control- and test article-treated animals in the number of animals that had a significant inflammatory reaction. It states that the data indicate that

in those animals that were treated with the test article and died (or were sacrificed in a moribund condition), there was more likely to be a significant inflammatory response by the host to the bacteria as compared to controls. The report states that there was no discernible difference between the two treated groups.

*Reviewer's comment: It seems reasonable that, if the test article was successful in preventing toxin formation in treated animals that would be expected to impair the immune response, that evidence of immune response would be seen, but may have been resolved in survivors prior to necropsy.*

The report states that, in these same animals, microscopic changes in the brain indicating a response by the host animal (principally inflammation, including vasculitis and hemorrhage) were, in general, more pronounced in the test article treated animals as compared to the saline treated controls. As noted earlier, the areas of the brain that were most affected tended to be those with the greatest surface area (cerebrum and cerebellum) and therefore with the most exposure to the meninges. Additionally, among the animals that died (or were sacrificed moribund), bacteria were more commonly observed in the brain of control animals as compared to the test article-treated animals (both treatment levels). Monkeys that survived to Day 30 did not have lesions characteristic of anthrax or lesions that indicated prior anthrax disease.

The following table from the neuropathologist's report compares study findings to published findings in anthrax-infected monkeys:

Table 3. Comparison of Published Morphologic Changes in the Brain of Anthrax Infected (Inhalation) Monkeys with those in the Current Study

Diagnosis	Percentage Published Study	Percentage Current Study Controls (Males and Females combined)	Percentage Current Study, 4.0 mg/kg ETI-204 (Males and Females Combined)	Percentage Current Study, 8.0 mg/kg ETI-204 (Males and Females Combined)
Bacteria, extravascular (Meninges, Bacteria)	71%	50%	21%	27%
Necrotizing vasculitis (Meninges, Vasculitis)	14%	7%	14%	20%
Hemorrhage, meningeal (Meninges, Hemorrhage)	57%	7%	14%	27%
Hemorrhage, parenchymal (Hemorrhage, brain, any area)	29%	7%	14%	20%
Meningitis, suppurative (Meninges, Inflammation)	21%	7%	14%	20%
Leukomalacia (Molecular Layer, Vacuoles, brain, any area)	14%	0%	0%	7%
(Brain, Perivascular Inflammation)*	--	21%	14%	33%
(Brain, Vasculitis)*	--	0%	14%	20%
Overall mortality	Not provided	86%	21%	27%

\*Diagnosis not used in the published study (Reference 3)

\*\*Mortality figures taken from the SP's draft report (21% = Group X [4.0 mg/kg ETI-204], 27% = Group Z [8.0 mg/kg ETI-204])

For the remaining pivotal trigger to treat studies in cynomolgus monkeys, the methods of infection and monitoring were similar to the established protocols used in the preceding and other studies of inhalation anthrax in this species. Nonclinical review for the remaining studies was limited to review of the pathology appendices. In general, findings were limited to those associated with anthrax, and tissues examined microscopically were those considered to be target organs of the disease, as well as any other gross lesions.

**4. Study no. AP202 ( (b) (4) study no. 2826-100020847): Three armed trigger-to-treat efficacy study of intravenously administered ETI-204 in cynomolgus monkeys with inhalational anthrax**

In this GLP-compliant study, treatment consisted of a single IV dose of ETI-204. The study was designed to compare the Lonza product to saline control and to the Baxter product. Fifty-one (25 male, 26 female) experimentally naïve cynomolgus monkeys were aerosol challenged with a targeted dose of 200 LD<sub>50</sub> (1.24x 10<sup>7</sup> spores) *Bacillus anthracis* (Ames strain) via head-only inhalation exposure. The trigger to treat was a positive serum PA result using the ECL assay. Study design is shown in the Applicant's table below:

**Study Design**

Group	Dose Level <sup>a</sup> (mg/kg)	Number of Animals	Description
1	0	17	Placebo (control)
2	16	17	ETI-204 (Lonza manufactured)
3	16	17	ETI-204 (Baxter manufactured)

a. All animals on study received the same dose volume (1 mL/kg) regardless of treatment.

All animals found dead or euthanized in moribund condition, as well as all animals surviving to termination at Day 28, were subjected to a complete necropsy. Protocol-required tissues were collected and preserved and were stored for possible future examination.

Mortality was 100% in the saline control group, and was comparable between the two treatment groups (69% and 65% in the Lonza and Baxter groups, respectively). One male monkey in the group treated with the Lonza product died before treatment and was excluded from the mortality calculations.

Gross lesions in monkeys found dead or euthanized prior to the end of the study included discoloration of the brain and/or lung, enlarged bronchial and/or renal lymph nodes, and fluid in the thoracic or abdominal cavities. These findings were consistent with those described in the literature (Vasconcelos et al, 2003) as typical of acute fulminant anthrax in this species.

No histopathology was performed.

**5. Study no. AP203 ( (b) (4) study no. 1219-100005989): Evaluating the efficacy of intravenous ETI-204 when administered therapeutically in the cynomolgus macaque inhalational anthrax model**

In this GLP-compliant study, 48 cynomolgus monkeys were aerosol challenged with a targeted dose of 200 LD<sub>50</sub> (1.24x 10<sup>7</sup> spores) *Bacillus anthracis* (Ames strain) spores via head-only inhalation exposure. Treatment with a single IV bolus injection of ETI-204 at a dose of 8 mg/kg or 32 mg/kg, or of sterile saline control was administered at the time of PA detection in the blood or at 54 hours post-challenge if PA was never detected. The study design is shown in the Applicant's table below:

**Table 1. Study Design**

Group	Number of Animals	ETI-204 Dose <sup>a</sup>	Formulation Concentration
1	16	0 (Saline)	0 mg/mL
2	16	8 mg/kg	8 mg/mL
3	16	32 mg/kg	32 mg/mL

a. All animals on study received the same IV dose volume (1 mL/kg) regardless of treatment in order to maintain blinding.

All animals found dead or euthanized in moribund condition, as well as all animals surviving to termination at Day 28, were subjected to a complete necropsy. Protocol-required tissues (brain/meninges, lungs, liver, spleen, spinal cord, kidney, mediastinal and bronchial lymph nodes, and gross lesions) were collected and preserved. Hematoxylin and eosin stained sections of most collected tissues were examined by a board-certified veterinary pathologist who was blinded to study group until after the initial examination. Brain and spinal cord were not to be examined, but two samples of spinal cord were evaluated due to the presence of gross lesions

The report states that all left brain hemispheres from surviving animals were shipped to (b) (4) for microscopic evaluation, and five non-surviving animals from each group were randomly selected for neuropathic examination (excluding animals that died less than 12 hours after treatment administration). A total of twenty four left brain hemispheres in formalin were processed to slides by (b) (4) and H & E (hematoxylin and eosin) sections were examined. Approximately 15 sections were made per brain hemisphere, and evaluation included meninges, cerebrum, neocortex, ventricular system, paleocortex, basal nuclei, striatum, limbic system, thalamus/hypothalamus, midbrain, cerebellum, pons, and medulla oblongata. Spinal cord samples were not examined.

Mortality in the saline control group was 88%, while mortality in the 8 mg/kg and 32 mg/kg groups was 94% and 63%, respectively. It is notable that mortality was lower than it should have been in controls and that test article efficacy was lower than expected and was not dose-related.

**Table 2. Mortality Following *B. anthracis* Challenge, by Group**

<b>Treatment</b>	<b>Saline (control)</b>	<b>8 mg/kg ETI-204</b>	<b>32 mg/kg ETI-204</b>
<b>Group Number</b>	<b>1</b>	<b>2</b>	<b>3</b>
<b>Mortality (% overall)</b>	14/16 (88%)	15/16 (94%)	10/16 (63%)
<b>Mortality (by sex)</b>	6M, 8F	8M, 7F	4M, 6F

In monkeys that died or were euthanized in moribund condition, gross findings included dark, red, or mottled discoloration in the brain, lungs, or spinal cord, enlarged lymph nodes (bronchial and mediastinal), and fluid in the abdominal cavity, pericardial cavity, or skin. These lesions have been described as typical of anthrax in cynomolgus monkeys (Vasconcelos, et al., 2003). Most findings were similar across treatment groups, although brain discoloration or foci were slightly more common among treated monkeys (particularly in the 8 mg/kg group) than saline control monkeys that died on study. The only gross finding in animals that survived to study termination on Day 28 was a deformity of the dura mater in a high dose male animal that was considered to be incidental. A lung adhesion was noted in a low dose female that died on Day 4, but was considered to be too chronic a lesion to be related to anthrax exposure, and was also judged to be incidental.

Microscopically, according to the pathology report, all monkeys that died prior to study termination had at least a few typical anthrax lesions, including suppurative to necrotizing inflammation, hemorrhage, edema, fibrin exudation, necrosis, lymphoid depletion, and/or the presence of bacteria consistent with *B. anthracis* in multiple organs. Of those animals, seven in the low dose group and six in the high dose group had no visible bacteria on microscopic examination, but nearly all of these animals had gross lesions in the brain (not examined microscopically per protocol), leading the report author to consider that the cause of death may have been meningeal anthrax. Lesions in monkeys surviving to study termination on Day 28 exhibited only “trivial inflammation or lymphoid depletion,” and no lesions considered to be significant.

In the separate neuropathology report, evaluation of brains from the nine surviving animals and 5 non-surviving animals from each treatment group were described. Most gross lesions involved discoloration of the meninges and usually correlated to the presence of hemorrhage. Surviving animals included two, one, and six animals in the saline control, 8 mg/kg ETI-204, and 32 mg/kg ETI-204 groups, respectively. None were reported to have any evidence of bacteria. No microscopic changes were seen in one of the controls and three of the high dose animals. In the remaining control monkey, mild hemorrhage and minimal mononuclear cell infiltrate were noted in the meninges. In the remaining low dose and three high dose animals, slight to minimal hemorrhage in the meninges and/or miscellaneous areas of the brain and/or minimal mononuclear infiltrates were considered to be similar to the control animal and due to either handling artifact or to have had some association with the resolved infection.

In the five monkeys per group that were sacrificed early or found dead, all had intravascular and/or extravascular bacteria, suggesting that the bacterial infection was the cause of death. Only the ETI-204-treated animals had evidence of an inflammatory

response (mild to severe), and it was always associated with extravascular bacteria (4 of 5 low dose and 3 of 5 high dose animals). Slight to severe hemorrhage was associated with extravascular bacteria in 3 control animals, all 5 low dose animals, and 4 of 5 high dose animals. The neuropathologist hypothesized that treated animals were more likely to mount an inflammatory response than were control animals. It was notable that two non-surviving control animals and one non-surviving high dose animal died with intravascular bacteria, no reported hemorrhage, and no visible inflammatory reactions. All microscopic changes were attributed to infection or artifact and not to test article.

**6. Study no. AP204 ( (b) (4) study no. 1121-G924204): Evaluating the efficacy of ETI-204 when administered therapeutically in the cynomolgus macaque inhalational anthrax model**

In this GLP-compliant study, cynomolgus monkeys under 5 years of age, 2.0-3.2 kg, were randomized to 3 groups of 16 animals (8/sex) each. Group Y received saline (control), Group X received 4 mg/kg ETI-204, and Group Z received 16 mg/kg ETI-204. Treatment was initiated when a positive PA result was noted 24-54 hours post-challenge or at 54 hours post-challenge without a positive PA result.

Complete necropsies were performed on all animals found dead or euthanized in moribund condition. Terminal necropsies were performed on approximately half of the animals surviving to Day 28 and on the remainder surviving at Day 56. Protocol-specified tissues (brain, kidneys, liver, lungs, mediastinal lymph nodes, spinal cord, spleen, and gross lesions) were collected and preserved. All but brain and spinal cord were processed to slides, stained with hematoxylin and eosin and examined microscopically in a blinded manner by a board-certified veterinary pathologist. Neural tissues were sent to a separate lab for neuropathological examination of brains.

Mortality in treated groups was lower than in the control group. Seventy-five percent of low dose and 50% of high dose animals died, while 94% of control animals died. The average number of days to death was similar between treated and control groups. In general, animals died within 3-6 days post-exposure.

In animals that were found dead or euthanized in moribund condition, gross findings included diffuse discoloration of meninges and/or brain, multiple red foci in liver, and fluid in the skin (ventral), thymus, abdominal or thoracic cavity. Approximately half of the monkeys that were found dead or euthanized in moribund condition had no gross lesions. However, one or more microscopic lesions indicative of anthrax (Vasconcelos *et al.*, 2003) were said to have been found in each of the monkeys that died prior to the end of the study. With the exception of a cyst in the liver of saline-treated (Group Y) female ( (b) (4) Identity No. C42482), all gross lesions identified in these animals were typical of gross findings described previously in inhalation anthrax in monkeys (Vasconcelos *et al.*, 2003). When examined microscopically, that cyst was found to be a granuloma and was considered likely to be due to a parasite. The report cites literature indicating that parasitic granuloma of the liver is a common incidental finding of cynomolgus macaques (Ryan, *et al.*, 1986). The microscopic lesions that were found and considered to be consistent with anthrax included the presence of bacteria consistent in appearance with *B. anthracis*, hemorrhage, necrosis, lymphoid atrophy,

inflammation, edema/exudate, neutrophil infiltration and fibrin deposition. Bacteria were generally seen in the blood vessels in affected organs, but also in the cytoplasm of macrophages, alveoli of lung, perithymic interstitial tissue and subcutis of skin. In lung, hemorrhages in peribronchial vessels, acute bronchial inflammation, and edema/exudate were seen. Multifocal necrosis was seen in the liver, necrosis and atrophy were seen in the spleen and mediastinal lymph nodes, and fibrin deposition was seen in the red pulp of the spleen. Microscopically visible bacteria were said to be found in greater incidence and severity in early decedents in the control group than in the treated groups. Otherwise, incidence and severity of the remaining anthrax-related findings were similar between early decedents in control and treated groups and were not considered to be due to ETI-204 administration.

In animals that survived to scheduled termination on Day 28 or 56, no gross findings were noted at necropsy. Microscopically, there were no findings consistent with anthrax and no findings considered to be related to ETI-204.

*Reviewer's comment: The study report states that all protocol-required tissues were examined microscopically, but a protocol deviation dated 2/13/12 indicates that on a number of dates tissues were left in 70% ethanol for more than 72 hours rather than the < 72 hours specified by the protocol. The report goes on to state that there was no impact on the study because the tissues were never examined by a pathologist. This appears to be in conflict with the reporting of results of microscopic examination.*

#### Neuropathology

Left brain hemispheres were fixed in 10% formalin and sent to (b) (4) for examination. Each was cut into approximately 15 sections, stained with hematoxylin and eosin (H&E) and examined microscopically. Evaluation included the meninges, ventricular system, prefrontal/cingulate/premotor cortex, cerebral cortex, basal nuclei, temporal lobe, thalamus/hypothalamus, midbrain, pons/pontine nuclei, hippocampus, cerebellum, occipital/visual cortex, and medulla oblongata. Initial evaluation was blinded to treatment.

In the control group, most (13 of 16) animals died with intravascular bacteria as the main change observed. Only two had meningeal bacteria with a notable immune reaction. The absence of other morphologic changes was considered by the pathologist to indicate that the animals died before they could mount an immune response or were prevented from mounting an immune response by the bacteria and/or their toxins.

In the ETI-204 treated groups, 12 of 16 in the 4 mg/kg group and 8 of 16 in the 16 mg/kg group were found dead or sacrificed in moribund condition. Of these 20 animals, findings in 3 (2 at 4 mg/kg and 1 at 16 mg/kg) were limited to the presence of intravascular bacteria and/or leukocytosis. In the remaining 17 (10 from the 4 mg/kg group and 7 from the 16 mg/kg group), an appreciable morphologic/immune response was noted, predominantly in the meninges, but also in brain. Morphologically, the changes typically included bacteria, hemorrhage and inflammation in the meninges (bacterial meningitis) and in the brain (bacterial encephalitis), and vasculitis in the meninges and brain. Changes were most pronounced in the meninges and appeared to spread to the brain since the responses in the brain were considered to be similar to those in the meninges, but less pronounced, and were adjacent to the meningeal

changes. Changes in brain were more numerous in cerebrum and cerebellum; this was thought to be due to the increased surface area and exposure to the meninges.

Animals treated with ETI-204 that died or were sacrificed early were more likely than controls to have extravascular bacteria (usually in the meninges) and findings including meningitis, encephalitis, vasculitis, and hemorrhage. The neuropathologist considered these findings to be consistent with an attempt to mount an immune response to the bacteria and/or bacterial products. Meningitis was the main morphologic abnormality reported to be associated with inhalation anthrax in ETI-204-treated animals, and the meninges were the most common and most severely affected area of the brain. The areas of the brain affected tended to be those with the greatest surface area (cerebrum and cerebellum) and the most exposure to the meninges.

Findings consistent with published changes in animal models of inhalation anthrax are compared in the Applicant's table below:

### Comparison of Published Morphologic Changes in the Brain of Anthrax Infected (Inhalation) Monkeys with Those in the Current Study

Diagnosis	Percentage Published Study	Percentage Current Study Controls (Males and Females combined) Group Y	Percentage Current Study, 4.0 mg/kg ETI-204 (Males and Females Combined) Group X	Percentage Current Study, 16.0 mg/kg ETI-204 (Males and Females Combined) Group Z
Bacteria, intravascular/extravascular (Meninges, Bacteria)	71%	94% (15/16)	69% (11/16)	50% (8/16)
Necrotizing vasculitis (Meninges, Vasculitis)	14%	13% (2/16)	50% (8/16)	44% (7/16)
Hemorrhage, meningeal (Meninges, Hemorrhage)	57%	19% (3/16)	69% (11/16)	38% (6/16)
Hemorrhage, parenchymal (Hemorrhage, brain, any area)	29%	13% (2/16)	56% (9/16)	38% (6/16)
Meningitis, suppurative (Meninges, Inflammation)	21%	13% (2/16)	63% (10/16)	44% (7/16)
Leukomalacia (Parenchymal, Necrosis)	14%	0% (0/16)	19% (3/16)	25% (4/16)
(Parenchymal, Vacuolation)*	--	6% (1/16)	38% (6/16)	13% (2/16)
(Brain, Perivascular Inflammation)*	--	13% (2/16)	56% (9/16)	44% (7/16)
(Brain, Vasculitis)*	--	13% (2/16)	50% (8/16)	44% (7/16)
Overall mortality	Not provided	94% (15/16)	75% (12/16)	50% (8/16)

Note: The primary diagnosis is from the referenced publication. The diagnosis in parentheses is the comparable diagnosis in the current study.

\*Diagnosis not used in the published study

\*\*Mortality figures taken from the Study Pathologist's draft report (75% = Group X [4.0 mg/kg ETI-204], 94% = Group Y [saline], 50% = Group Z [16.0 mg/kg ETI-204])

Animals that survived to scheduled sacrifice had no significant microscopic changes. Occasional background changes were reported, including slight mononuclear cell infiltrates in meninges and/or choroid plexus.

### 4.3 Safety Pharmacology

Three safety pharmacology studies were submitted that have been previously reviewed under IND 12,285. Those reviews are reproduced below.

#### Cardiovascular safety pharmacology:

##### **1. Study no. AP106: Single Dose Intravenous and Intramuscular Pharmacokinetics Study of ETI 204 (Anthim™) in Cynomolgus Monkeys.**

(reviewed by Stephen Hundley as submitted in serial no. 013, submitted 8/29/06)

The study was contracted by the sponsor to (b) (4). The study was conducted in accordance with GLP requirements and was audited by a Quality Assurance group. The lot number was ET215-187B with 99 percent analytical purity. Male and female cynomolgus monkeys were purchased from (b) (4), and ranged in age from 3 to 6 years. All monkeys were quarantined and acclimated for approximately 100 days before being released for study. ETI-204 dose levels were 5 and 10 mg/kg intramuscular (*im*) and 5 mg/kg intravenous (*iv*). Each dose group consisted of four male and four female monkeys. The dose volumes were 0.4 ml/kg for *im* administration and 0.8 ml/kg for *iv* administration. The weight range for males was 1.7 to 3.6 kg and 1.8 to 2.4 kg for females. The IV doses were administered as a 3 to 5 minute slow bolus injections via the saphenous vein. The IM doses were administered in the thighs.

All animals were observed twice daily following dose administration for general health and signs of toxicity over a 43-day post-dosing period. Clinical observations were made the day prior to dose administration and on Day 8, 15, 22, 29, 36, and 43 following dosing. Clinical observations included skin and injection site evaluations; eyes and mucous membranes; the respiratory, circulatory, autonomic and central nervous systems; and somatomotor and behavior patterns. Body weights were determined the day prior to dosing and on the same days as clinical observations were made.

Electrocardiograms were generated prior to dose administration and 2, 4, and 24 hours after dosing (nine leads: I, II, III, aVR, aVL, V1, V2, and V3). Blood pressure was also determined at the same time intervals. Monkeys were anesthetized with 0.05 ml/kg of Telazol during the ECG and blood pressure determinations. Blood was collected for pharmacokinetic determinations at the following post-dosing time points: 1, 2, 4, 8, 24, and 32 hours then one sample daily was drawn on the following post-dosing days: 3, 4, 5, 6, 7, 9, 11, 13, 16, 18, 20, 22, 24, 29, 31, 33, 36, 38, 40, and 43. All animals were returned to the stock colony after Day 43.

## Results

No compound-related body weight effects were observed during the post-dosing period. Clinical effects such as intermittent emesis, soft feces, and alopecia were observed during the post-dosing period but were not compound-related. Blood pressure measurements indicated elevated diastolic and systolic blood pressure at the 2- and 4-hour post dosing time intervals after *im* and *iv* doses. Individual animal blood pressure data following the 5 mg/kg *iv* dose and the 10 mg/kg *im* dose are presented in the following tables.

Post-dosing Blood Pressure: 5 mg/kg *iv*

	Diastolic blood pressure (mmHg)			Systolic blood pressure (mmHg)		
	Pre-dose	2 hours	4 hours	Pre-dose	2 hours	4 hours
Males						
1	90	56	62	119	103	134
2	47	<b>146</b>	<b>143</b>	104	<b>229</b>	<b>164</b>
3	21	<b>87</b>	<b>124</b>	62	<b>173</b>	<b>143</b>
4	50	<b>162</b>	<b>124</b>	108	<b>204</b>	143
Females						
1	38	<b>115</b>	<b>83</b>	104	140	110
2	28	<b>136</b>	<b>80</b>	80	<b>212</b>	104
3	30	<b>186</b>	<b>79</b>	80	<b>212</b>	134
4	56	<b>133</b>	37	11	<b>187</b>	58

Post-dosing Blood Pressure: 10 mg/kg *im*

	Diastolic blood pressure (mmHg)			Systolic blood pressure (mmHg)		
	Pre-dose	2 hours	4 hours	Pre-dose	2 hours	4 hours
Males						
1	47	<b>163</b>	<b>116</b>	97	<b>211</b>	145
2	34	<b>139</b>	<b>85</b>	81	<b>263</b>	111
3	71	<b>167</b>	61	92	<b>213</b>	115
4	42	<b>129</b>	<b>101</b>	103	152	115
Females						
1	30	<b>166</b>	<b>99</b>	82	<b>189</b>	139
2	28	<b>188</b>	<b>124</b>	77	<b>211</b>	<b>197</b>
3	30	<b>168</b>	45	102	<b>188</b>	62
4	24	<b>143</b>	64	71	<b>164</b>	84

The values in bold type highlight the elevations in diastolic and systolic blood pressure compared to the pre-dose values. Diastolic pressure was elevated 2- to 6-fold in 3 of 4 males and 4 of 4 females two hours after administration of the 5 mg/kg *iv* dose. Systolic pressure was elevated approximately 2-fold. Similar results were observed following the *im* administration of a 10 mg/kg/dose. Elevated diastolic and systolic pressures were observed four hours after dosing (*iv* and *im*).

Plasma ETI-204 concentrations following the respective 5 mg/kg *iv* and 10 mg/kg *im* doses are presented in the following table.

### Plasma ETI-204 Concentrations

Time points	µg ETI-204/ml Concentrations			
	5 mg/kg <i>iv</i>		10 mg/kg <i>im</i>	
	Males	Females	Males	Females
1 hour	99	93	32	26
2 hours	95	102	58	51
4 hours	96	86	63	46
8 hours	95	87	87	59
24 hours	64	49	74	59
32 hours	58	52	87	74
3 Days	48	46	82	58
4 Days	46	37	78	59
5 Days	32	35	72	55

The ETI-204 plasma concentrations following the *iv* dose to males remained constant over the initial 8 hours following dosing. In females the concentration peaked at 2 hours post-dosing and plateaued between 4 and 8 hours then decreased. The plasma C<sub>max</sub> following *im* administration was observed from 8 hours to 4 days in males (87 to 78 µg/ml) and 32 hours post-dosing in females.

The maximum elevations in blood pressure were observed at C<sub>max</sub> following the *iv* dose but were not maintained at the four-hour time point despite the plateau in plasma concentrations between 2 and 4 hours in males and the minor reduction in plasma concentration in females. Plasma concentrations at 24 hours following the *im* doses were higher than at 2 hours post-dosing, however elevated blood pressure observed at the 2-hour time point had returned to baseline by 24-hours post-dosing (data not shown). These data suggest that the elevation in blood pressure is not exclusively dependent upon maximum ETI-204 plasma levels. The initial introduction of ETI-204 in the plasma may affect blood pressure with the cardiovascular system adapting to the continued presence of ETI-204 in plasma.

ECG data were generated prior to dosing and at the 2- and 4-hour post-dosing intervals. The QT intervals appeared to be prolonged in male and female monkeys receiving the 5 mg/kg *iv* dose. QTc intervals (generated from the QT value using Fridericia's formula) increased in three of four male monkeys by 42 to 65 msec or 17 to 28 percent relative to pre-dose values. The QTc values from all four female monkeys increased by 24 to 31 msec or 9 to 11 percent. The increases in QTc were observed at the 2- and 4-hour postdosing time intervals in males and at only at the 4-hour post-dosing time point in three of four females. QT interval prolongation was not uniformly observed in male and female monkeys receiving the 5 and 10 mg/kg *im* doses. Most of the observed ΔQTc values were five percent or less relative to the pre-dose QTc values and could not be definitively associated with the *im* injection of ETI-204. One male and one female from

the 10 mg/kg *im* dose level at the 4-hour post-dosing time point exhibited  $\Delta$ QTc values of 64 and 53 msec, respectively, or 24 and 20 percent increases relative to the pre-dose QTc. Increases of this magnitude suggest a compound related effect.

## Discussion and Conclusions

The 5 mg/kg IV and 10 mg/kg IM doses of ETI-204 resulted in elevated systolic and diastolic blood pressure. The blood pressure increases ranged from 2- to 6-fold for individual male and female monkeys and was observed at the 2- and 4-hour post-dosing time points. Conclusive assessment of the blood pressure effect was made difficult due to the animal to animal variations in pre-dose blood pressure values. Several systolic and diastolic values were out of the normal range for cynomolgus monkeys as detailed in the following table.

Pre-Dosing BP Outliers

Sex	Animal ID Number	Diastolic or Systolic Blood Pressure
Male	12167	Diastolic BP = 21 mmHg
Male	12170	Diastolic BP = 26 mmHg
Female	12178	Diastolic BP = 28 mmHg
Female	12180	Systolic BP = 11 mmHg
Female	12181	Diastolic BP = 245 mmHg; Systolic BP = 220 mmHg
Female	12182	Diastolic BP = 23
Female	12184	Diastolic BP = 19
Female	12186	Diastolic BP = 28
Female	12188	Diastolic BP = 24

Pre-dosing normal BP range in other studies with cynomolgus monkeys:

Diastolic 60 to 73 mmHg; Systolic 96 to 106

The ECG profiles indicated prolongation of the QTc intervals from most of the male and female monkeys receiving the 5 mg/kg *iv* dose. The QTc interval was prolonged for some individual monkeys at both the 2- and 4-hour post-dosing time points. QTc prolongation was randomly observed in monkeys receiving the 5 and 10 mg/kg *im* doses and was not clearly associated with the administration of ETI-204 although one male and one female from the 10 mg/kg *im* dosing routine exhibited  $\Delta$ QTc values of 64 and 53 msec, respectively. The cardiovascular data from monkeys receiving the 5 mg/kg *iv* dose and the 10 mg/kg *im* dose are sufficient to suggest compound-related effects on blood pressure and QTc intervals.

The study design was inadequate for assessing the general toxicological effects of ETI-204 for the following reasons: no zero-level vehicle control animals were included in the study; no post-dosing blood samples for hematology and serum chemistry were taken; and an insufficient number of post-dosing time points for cardiovascular evaluations were included. Initial post-dosing cardiovascular measurements were not made until 2 hours after the *iv* and *im* doses. One or two time points for cardiovascular and ECG determinations are needed between dose administration and 2 hours post-dosing. The sponsor was notified in a correspondence dated 11/17/06 (in response to an

amendment to the IND received on 8/29/06) of the need to repeat the toxicology/pharmacokinetics study in cynomolgus monkeys. The study needed to include the following: 1) a zero-level vehicle control group; 2) a second or repeat dose 5 to 7 days following the initial dose; 3) hematology and clinical chemistry determinations prior to dose administration and at appropriate time intervals following the first and second doses; and 4) additional early post-dose administration time points for cardiovascular measurements including ECGs. The sponsor also informed the Division that the projected therapeutic route of administration would be by *iv* infusion and that the ETI-204 drug substance would be generated by a different cell line designated (b) (4). Therefore, the new toxicology/pharmacokinetic study in cynomolgus monkeys needs to be an *iv* infusion study with ETI-204 from the (b) (4) cell line. This requested study was discussed with the sponsor in a subsequent face to face meeting held with the Division on 8/7/07.

**2. Study no. AP-106 PK ( (b) (4) Study no. 1180-04527): A single-dose intravenous and intramuscular pharmacokinetics study of ETI-204 (Anthim™) in cynomolgus monkeys**  
(reviewed under SDN 069, submitted 11/29/2011)

This report presents pharmacokinetic data in a study that also evaluated blood pressure and ECG effects of ETI-204 in cynomolgus monkeys. Groups of 4 male and 4 female cynomolgus monkeys (2-3 kg) received either 5 mg/kg IV or IM (0.4 mL/kg) or 10 mg/kg IM (0.8 mg/kg) of ETI-204 (12.5 mg/mL). IV bolus dosing was by slow push over 3-5 minutes, while IM injections were split to deliver half of the dose to the muscle of each thigh. Blood samples were collected from the femoral or cephalic vein pre-dose, at 1, 2, 4, 8, 24, and 32 hours and at 3, 4, 5, 6, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 29, 31, 33, 36, 38, 40, and 43 days post-injection. Serum was separated and frozen at -70°C until analysis by PAA3 ELISA. Serum from all monkeys on Days 0, 13, 26, and 43 were also analyzed for anti-ETI-204 antibody.

Anti-product antibodies were detected in samples from 2 monkeys on Day 13 and an additional 6 monkeys on Day 26, for a total of 8 out of 12 monkeys, or 75%. The reports states that data up to Day 13 would be the most meaningful to understand the disposition of the drug before development of an immune response, so pharmacokinetic analysis and data presented in the report only include serum concentrations through Day 13. Summary pharmacokinetic parameters are presented in the Sponsor's table below.

## Summary of Pharmacokinetic Parameters of ETI-204 in Cynomolgus Monkeys

Males			
Route of Administration	IV	IM	IM
Dose, mg/kg	5	5	10
C <sub>max</sub> , µg/mL	N/A	40.34 ± 4.73	94.91 ± 13.83
T <sub>max</sub> <sup>a</sup> , d	N/A	2.5 (1-6)	0.8 (0.3-1.3)
AUC(0-inf), µg·d/mL	752.47 ± 105.99	614.37 ± 63.54	1284.96 ± 64.18
Clearance, L/d/kg	0.0068 ± 0.0011	N/A	N/A
V <sub>ss</sub> , L/kg	0.0896 ± 0.0507	N/A	N/A
T <sub>1/2</sub> <sup>b</sup> , d	7.9 ± 3.3	6.8 ± 2.9	8.4 ± 2.2
F, %	N/A	81.65	85.38

Females			
Route of Administration	IV	IM	IM
Dose, mg/kg	5	5	10
C <sub>max</sub> , µg/mL	N/A	40.29 ± 7.77	75.64 ± 18.46
T <sub>max</sub> <sup>a</sup> , d	N/A	1.3 (0.08-1.3)	1.3 (1.3-4.0)
AUC(0-inf), µg·d/mL	717.81 ± 153.67	481.79 ± 86.51	1399.46 ± 99.64
Clearance, L/d/kg	0.0072 ± 0.0013	N/A	N/A
V <sub>ss</sub> , L/kg	0.0922 ± 0.0098	N/A	N/A
T <sub>1/2</sub> <sup>b</sup> , d	9.7 ± 1.6	6.0 ± 2.0	12.0 ± 1.9
F, %	N/A	67.12	97.48

<sup>a</sup>Expressed as median and range

<sup>b</sup>Expressed as harmonic mean and pseudo SD based on jackknife variance

N/A - Not Applicable

Clearance was low, and volume of distribution was small at steady state. The terminal half-life was 7.9-9.7 days. Mean bioavailability of IM-administered product was 82-84%. There was no significant difference in pharmacokinetics between males and females, except for AUC<sub>0-inf</sub> after the 5 mg/kg IM dose.

### 3. Study no. AP-115: A Cardiovascular and Toxicology Evaluation Study Following Intravenous Infusion of Anthim<sup>(b) (4)</sup> in Cynomolgus Monkeys (reviewed by Stephen Hundley as submitted in SDN 022, submitted 6/6/08)

The sponsor contracted the study to <sup>(b) (4)</sup>. The study was conducted in accordance with GLP requirements and audited by a Quality Assurance group. The study was conducted with male and female cynomolgus monkeys approximately 3 to 5 years of age (originally supplied by <sup>(b) (4)</sup>). ETI-204 (Anthim<sup>TM</sup>) was generated by the <sup>(b) (4)</sup> mouse myeloma (NSO) cell line and formulated in sterile physiological saline for intravenous infusion. Dose levels of 10 and 30 mg/kg were administered as a single 60-minute *iv* infusion with the 10 mg/kg groups initially serving as the zero-level vehicle controls. The two doses at each dose level were administered eight days apart according to the following schedule: 30

mg/kg groups dosed on Days 1 and 9; 10 mg/kg groups dosed on Days 9 and 17. The monkeys were separated into Cardiovascular and Pharmacokinetic groups with each group consisting of three animals per sex per dose level.

Each animal in the Cardiovascular group was surgically telemeterized for cardiovascular measurements (heart rate, blood pressure, and electrocardiograms). Animals in both the Cardiovascular and Pharmacokinetic groups received surgically inserted Vascular Access Ports (VAP) for intravenous infusion of the drug solutions. Animals were allowed a 2-week post-operative recovery period prior to the initiation of the dosing routines.

The in-life observations consisted of twice daily cageside evaluations and weekly clinical observations. Immediate postdosing observations were also made. Ophthalmologic examinations using indirect ophthalmoscopy and slit-lamp biomicroscopy were made 24 hours after the second dose to the animals in the Pharmacokinetic groups. Food intake was estimated daily. Cardiovascular measurements were continuously collected for approximately 100 minutes prior to dose administration, six hours following dose administration, and from 22 to 24 hours following dose administration. The cardiovascular measurements included heart rate, body temperature, systolic and diastolic blood pressure, mean arterial pressure, and ECG recordings. These data were presented for the 60 minute pre-dosing and 1, 2, 4, 6, and 24 hour post-dosing time intervals. The ECG waveforms provided heart rates and QT, RR, and QRS intervals. QTc values were determined from the QT interval using the Fridericia correction factor.

Pharmacokinetics were determined from blood drawn 5 min., 2, 6, and 24 hr postinfusion (each dose) with additional five and seven day samples following the second dose to the animals in the Pharmacokinetic groups. The Primate anti-Chimeric Antibody Analysis (PACA) analysis was conducted on serum samples collected from the Pharmacokinetic groups seven days following the first and second dose at both dose levels. Clinical pathology was determined on blood samples drawn from animals in the Pharmacokinetic groups prior to initiation of dosing and five days following the first and second dose at both dose levels. The hematology measures are listed in the following table.

Hematology

White Blood Cells	Platelet Volume	Differential Leukocytes
Red Blood Cells	Platelet Count	<i>Neutrophils</i>
Hemoglobin	Red Cell Distribution Width	<i>Lymphocytes</i>
Hematocrit	Absolute Reticulocytes	<i>Monocytes</i>
Mean Corpuscular Volume		<i>Eosionphils</i>
Mean Corpuscular Hemoglobin Concentration		<i>Basophils</i>

Clinical chemistry measurements are listed in the following table:

Alkaline Phosphatase	Chloride
Alanine Aminotransferase	Creatinine
Aspartate Aminotransferase	Carbon Dioxide
Creatine Kinase	Glucose
Gamma Glutamyltransferase	Potassium
Lactate Dehydrogenase	Sodium
Albumin	Phosphorus
Blood Urea Nitrogen	Total Bilirubin
Calcium	Total Protein
Cholesterol	Triglycerides

The animals in the Pharmacokinetics groups were returned to the stock colony on Day 45. The animals in the Cardiovascular groups were sacrificed and necropsied. The male and female monkeys from the 10 mg/kg dose were sacrificed on Day 19, two days after the second dose. Male and female monkeys from the 30 mg/kg dose were sacrificed on Day 12, three days after the second dose. A complete gross pathological examination was conducted on each animal at the terminal sacrifice. All observed gross lesions were preserved for histopathology. Histopathology was not conducted on organs or tissues that exhibited no gross lesions.

## Results

Compound-related clinical effects were not observed at the 10 and 30 mg/kg doses following the first or second dose. Compound-related cardiovascular effects were not observed based upon values generated from individual animals for heart rate, blood pressure, and ECGs (including QTc intervals). Observed hematological effects included elevated white blood cell counts in males at both dose levels, decreased absolute neutrophil count in males following the second 30 mg/kg dose, and 2- to 3-fold increased absolute reticulocyte count in both sexes following the first and second 10 and 30 mg/kg doses. The compound related changes in hematological measurements did not result in adverse health effects. Creatine kinase levels appeared to be depressed in females at the 10 and 30 mg/kg doses compared to the control or pre-dose values. No additional compound-related effects were observed for clinical chemistry measurements.

Compound-related gross pathology was observed in only one monkey. A male monkey from the 30 mg/kg dose exhibited pale discoloration of the medial and caudate lobe of the liver. Histopathology revealed local capsular fibrosis, mild lymphoplasmacytic inflammation, mild biliary hyperplasia, and mild centrilobular hepatocellular vacuolization. The observed hepatic effect was not considered compound-related. The PACA analysis in the serum from monkeys in the Pharmacokinetic groups was conducted by a separate laboratory the results will be presented in a separate report.

Pharmacokinetics indicated protracted plasma half-life for ETI-204. The following table lists estimations for Cmax, AUC, and t1/2:

	10 mg/kg		30 mg/kg	
	1 <sup>st</sup> Dose	2 <sup>nd</sup> Dose	1 <sup>st</sup> Dose	2 <sup>nd</sup> Dose
Cmax (µg/ml)	260	290	780	1060
AUC 0-168 hr	20,100	---	55,000	---
AUC 0-504 hr	---	50,400	---	150,000
t1/2 (hr)	150	200	170	205

AUC = µg hr/ml

The AUC<sub>0-504 hr</sub> more accurately represents the actual AUC value for Anthim in monkeys due to the long terminal plasma half-life (150 to 200 hours).

## Conclusion

The hematological changes in both sexes and hepatic histopathology in one male from the 30 mg/kg dose may have been compound-related, however, adverse clinical consequences were not observed. The apparent blood pressure effects observed in the previously reviewed toxicity study with cynomolgus monkeys (low initial blood pressure and 3-fold or greater increase above normal blood pressure) were not observed in the current study as compound-related cardiovascular effects were not observed. In addition, the blood pressure values for each animal were consistent with the range of values observed in non-treated cynomolgus monkeys.

The sub-clinical results at the 10 and 30 mg/kg dose levels do not present issues for the proposed clinical trial entitled "*Randomized, Placebo-Controlled, Double-Blind, Dose-Escalation Phase I Study of the Safety, Tolerability, and PK of a Single IV Dose of ETI-204 (Protocol AH-102)*". The highest proposed dose in this study is 360 mg. The 10 mg/kg dose to monkeys with an approximate AUC of 50,000 µg·hr/ml provides an appropriate margin of safety based upon the human AUC of 10,000 following a 114 mg dose of Anthim. An extra margin of safety is provided by the 30 mg/kg dose having an approximate AUC of 150,000 µg·hr/ml.

*Current Reviewer's comments: An amended report was submitted in supporting document no. 092 (7/19/12). The cover of that amended report page states that final study report had previously been amended or reissued on 4/14/2010 and 9/29/2011, but there are no documents in DARRTS corresponding to those dates. The original study report was reviewed 1/27/09 in serial no. 022, submitted 6/6/08, although this may be an error, as that supporting document does not appear to contain any nonclinical study reports. What, if any alterations were made in the two intervening amendments are unknown.*

*The amended report in SDN 092 added assessments of anti-drug antibody (ADA, or PAHA, the latter defined in the report as "Anthim-binding antibody) as determined by electrochemiluminescence immunoassay (ECL). After the second dose of Anthim, one Group 1 male (10 mg/kg) tested as positive for ADA. The antibody titers in samples*

from this animal, #16019, were found to increase from 1:80 on Study Day 31 to 1:1280 on Study Day 45. The report notes that, serum concentrations, AUC, terminal half-life, and clearance values for Anthim in this animal were comparable to other animals dosed with 10 mg/kg of the antibody. No toxicological effects or cardiovascular adverse events were reported in this animal. The report concluded that development of detectable Anthim-binding antibodies did not affect pharmacokinetics or safety of the study drug.

#### Respiratory Safety Pharmacology

No separate safety pharmacology studies of the respiratory system were conducted. The Sponsor's nonclinical overview states that no clinical observations indicative of adverse respiratory effects were seen after repeated dosing (2-4 doses) in toxicology studies at doses up to 10.5 mg/kg/dose by IV or IM bolus administration in rats, up to 100 mg/kg/dose by slow IV bolus in rats, up to 32 mg/kg/dose via slow IV bolus in time-mated female rabbits, or up to 30 mg/kg/dose via IV infusion in monkeys. *Reviewer's comment: These observations do not meet the standards of detailed objective evaluations normally performed in respiratory safety pharmacology studies.*

#### CNS Safety Pharmacology

No separate safety pharmacology studies of the central nervous system were conducted. The Sponsor's nonclinical overview states that the potential for neurobehavioral effects associated with administration of ETI-204 was conducted in Study no. AP204, a trigger-to-treat study in monkeys challenged with *B. anthracis* spores and administered ETI-204 at 4 and 16 mg/kg via a single IV bolus dose. Neurobehavioral examinations were conducted by a veterinarian on all monkeys pre-test and on all surviving monkeys at Days 28 and 56 post-challenge, and included behavior, head posture, coordination, oculomotor nerve function, range of motion, extensor-flexor function, deep pain, response to noxious stimuli, Babinski reflex, superficial sensation, anal reflex, and assessment of muscle atrophy. No treatment-related effects were reported for any of these parameters.

An assessment of the CNS was conducted on pathology samples from efficacy studies in animal models of inhalation anthrax (see the neurological assessment report under Special Toxicology studies).

## **5 Pharmacokinetics/ADME/Toxicokinetics**

### **5.1 PK/ADME**

The Pharmacokinetics section included 18 methods validation reports and 10 study reports. These were not reviewed. The following is excerpted from the Applicant's summaries. More information may be found in the Clinical Pharmacology review.

“Nonclinical pharmacokinetic (PK) parameters of ETI-204 were evaluated following single-dose IV and IM administration in normal (i.e., uninfected) NZW rabbits

and cynomolgus monkeys. It has been amply demonstrated that the disposition of mAbs generally involves distribution beyond the vascular space with potential uptake into tissues, and catabolism by proteases to small peptides and amino acids which are subsequently incorporated into the endogenous pool or excreted (Wang et al., 2008). Therefore, formal distribution, metabolism, and excretion studies have not been conducted with ETI-204 to support marketing authorization. Additionally, Elusys validated several ELISA methods to quantify ETI-204 levels, anti-ETI-204 antibodies, anti-PA antibodies, and free PA levels.

*Reviewer's comment: Generally, it is considered that large molecules are limited to the vascular compartment, therefore, interspecies comparisons are made on nominal doses rather than body surface area-normalized doses. It is unclear what difference wider distribution of the antibody would make on the determination of human equivalent doses.*

### Method Validation

Elusys validated several bioanalytical methods during the nonclinical development of ETI-204, including ELISA methods to quantify anti-ETI-204 antibodies, ETI-204, and free PA concentrations in animal serum. Specifically, a Meso-Scale Discovery (MSD) ELISA method was developed and validated to quantify anti-ETI-204 antibodies in rabbit serum (Study No. (b)(4) 11-012) and an ELISA method for monkey serum (Study Nos. 08-164 and 10-068). To quantify levels of ETI-204, ELISA methods were developed and validated in rat (Study No. 1984-009), rabbit (Study Nos. (b)(4) 11-010 and (b)(4) 12-093), and monkey serum (Study No. 11-035). A semi-quantitative ELISA method was also validated for the determination of anti-PA antibodies in rabbit serum (Study No. (b)(4) 11-010) and monkey serum (Study No. 12-117). Additionally, an ELISA method was validated for the determination of free PA in rabbit serum (Study No. VP2012-256) and monkey serum (Study No. VP2012-257). A complete summary of the bioanalytical methods supporting ETI-204 development can be found in Report No. ELR001.

### ADME

The nonclinical absorption of ETI-204 has been evaluated in single-dose studies in healthy (i.e., un-infected) NZW rabbits and cynomolgus monkeys following IV and IM administration. The observed increases in ETI-204 serum concentrations in both species were generally dose-proportional and no gender differences were observed. Preliminary non-GLP single-dose PK studies were conducted in healthy female rabbits administered ETI-204 via IV bolus at 10 mg (Study No. AR002) or 20 mg (Study No. AR008). Serum samples were analyzed for ETI-204 via an early non-validated ELISA assay (Study No. QCRPT0002). In these studies, total exposure was approximately dose proportional, with maximum concentration (C<sub>max</sub>) values of 66.44 µg/mL following a 10 mg dose and 196.23 µg/mL following a 20 mg dose. The area under the concentration versus time curve from time 0 extrapolated to infinity (AUC<sub>0-∞</sub>) was 242.89 and 557.98 µg·d/mL following a 10 and 20 mg dose, respectively. The elimination phase half-life (t<sub>1/2</sub>) in female rabbits was 3.1 to 4.0 days. The steady-state volume of distribution (V<sub>ss</sub>) was small at 199 to 244 mL (~79.6 to 97.6 mL/kg assuming a 2.5 kg

rabbit), which is larger than the total plasma volume in rabbits (i.e., 44 mL/kg). Clearance (CL) was low at 36 to 42 mL/day (~14.4 to 16.56 mL/day/kg).

In a GLP single-dose PK study, healthy male and female rabbits were administered ETI-204 via IV bolus at 3, 10, and 30 mg/kg (Study No. AR010). Serum samples were analyzed for ETI-204 via a validated ELISA assay (Study No. (b) (4) 11-012). There were no gender differences and the observed increases in exposure were dose proportional. The C<sub>max</sub> was 104, 400, and 1000 µg/mL in males and 99.3, 288, and 1060 µg/mL in females at doses of 3, 10, and 30 mg/kg, respectively. Total exposure, estimated by AUC<sub>0-∞</sub>, was 381, 1100, and 3190 µg·d/mL in males and 354, 1300, and 3450 µg·d/mL in females at doses of 3, 10, and 30 mg/kg, respectively. Concentrations of free (unbound) ETI-204 declined slowly when measured in rabbit plasma or serum. The t<sub>1/2</sub> in rabbits following a single IV dose ranged from 2.59 to 5.75 days. The volume of distribution following a single IV administration was low, ranging from 32.1 to 63.2 mL/kg [apparent volume of distribution (V<sub>z</sub>)] and 41.5 to 97.6 mL/kg [volume of distribution at steady state (V<sub>ss</sub>)]. These values extend upwards past the total plasma volume of rabbits, indicating that ETI-204 may have distributed outside of the vascular space (Davies and Morris 1993). CL in rabbits following a single IV administration was low and ranged from 7.97 to 16.56 mL/day/kg, which represents <0.1% of rabbit hepatic and renal plasma flows (Davies and Morris 1993). Preliminary non-GLP single-dose PK studies were also conducted in healthy female rabbits administered ETI-204 IM at doses of 10, 16.82, and 20 mg (Study Nos. AR002 and AR008). Serum samples were analyzed for ETI-204 via an early non-validated ELISA assay (Study No. QCRPT0002). Following a single IM dose, the C<sub>max</sub> ranged from 18.39 to 31.86 µg/mL following a 10 mg dose and 43.01 to 65.81 µg/mL following a 20 mg dose. The time to the maximum concentration (t<sub>max</sub>) was 1.3 days following a 10 mg dose and ranged from 1.7 to 2.0 days following a 20 mg dose. The t<sub>1/2</sub> ranged from 1.4 to 1.69 days at 10 mg and 1.4 to 5.73 days at 20 mg. Total exposure, estimated by AUC<sub>0-∞</sub> ranged from 122.01 to 265.60 µg·d/mL following a 10 mg dose and 300.85 to 606.61 µg·d/mL following a 20 mg dose. The absolute bioavailability ranged from 50.23 to 100% following a 10 mg dose and 61.93 to 100% following a 20 mg dose. Administration of ETI-204 in a more concentrated buffer solution at 16.82 mg (IM) resulted in slightly lower than dose proportional exposures, with a C<sub>max</sub> of 47.95 µg/mL, t<sub>max</sub> of 2.0 days, t<sub>1/2</sub> of 1.36 days, and an AUC<sub>0-∞</sub> of 307.88 µg·d/mL, which corresponded to an absolute bioavailability of 65.61%.

Single-dose GLP PK studies were conducted in healthy male and female rabbits administered ETI-204 via a single IM dose at 3, 10, and 30 mg/kg (Study Nos. AR010 and AR014). Serum samples were analyzed for ETI-204 via a validated ELISA assay (Study No. (b) (4) 11-010). There were no significant gender differences in exposure and C<sub>max</sub> was 25.0, 98.9, and 346 µg/mL in males and 22.6, 77.4, and 293 µg/mL in females at 3, 10 and 30 mg/kg, respectively (Study No. AR014). In Study No. AR010, there were similarly no significant gender differences in exposure following a 10 mg/kg dose and C<sub>max</sub> was 104 and 119 µg/mL in males and females, respectively. Mean t<sub>max</sub> was similar across all dose groups in both studies and ranged from 2.00 to 3.61 days. Total exposure, estimated by AUC<sub>0-∞</sub>, was 309, 1320, and 3330 µg·d/mL in males and 363, 1090, and 4330 µg·d/mL in females at 3, 10, and 30 mg/kg, respectively (Study No. AR014). In a separate 30 mg/kg dosing group in AR014, animals were

administered ETI-204 at 15 mg/kg/site at 2 total dosing sites and the results were comparable to the single site 30 mg/kg group with C<sub>max</sub> values of 273 and 291 µg/mL and AUC<sub>0-∞</sub> values of 3590 and 3530 µg·d/mL in males and females, respectively. Additionally, AUC<sub>0-∞</sub> was calculated in AR010 following a 10 mg/kg IM dose at 1060 and 1520 µg·d/mL in males and females, respectively.”

## 6 General Toxicology

### 6.2 Repeat-Dose Toxicity

Three toxicology studies in rats were submitted. The first was reviewed by Dr. Alexandra Worobec as submitted in the original IND 12285. Her review is reproduced below.

#### 1. Study no. ARR002 ( (b) (4) ) study no. 03553): Repeat dose IV and IM toxicity of antibody (b) (4) in male Fischer 344 rats

**Key Study Findings:** A GLP-compliant, repeat-dose IV and IM toxicity study of the (b) (4) antibody (the same antibody as ETI-204 but without Tween 0.01%) was performed in 120 male Fischer 344 rats (Study No. 03553, (b) (4) ) 10-13 weeks of age, weighing between 245-311 g (15 rats/group) that were randomized into 8 different dose groups, that either received IV or IM Ab (b) (4). Histopathologic evaluation of organs at necropsy overall failed to reveal any significant changes or trends in test article treated animals as compared to the controls (IND # 12285, Vol. 1.22, pages 22-283-22-369). Noted were microscopic findings in the heart (minimal ventricular mononuclear cell infiltrate), liver (minimal-mild mononuclear cell infiltration), kidneys (minimal tubular mineralization and/or regeneration, minimal nephropathy, minimal lymphocytic infiltrate), stomach (minimal submucosal eosinophilic infiltrate), lung (minimal perivascular/peribronchial infiltrate and/or hemorrhage, minimal alveolar hyperplasia), thymus (minimal-mild hemorrhage), Harderian glands (minimal-mild unilateral pigmentation, minimal ductular metaplasia, minimal unilateral chronic inflammation, and minimal atrophy), and lymphoid tissues (minimal histiocytic pigmentation, mild sinus congestion, and mild sinus histiocytosis)—findings which have been described in other toxicology studies of the Fischer 344 rat (background findings) and which were also noted in control group animals (IND # 12285, Vol. 1.22, pages 22-288, 22-294, 22-296-22-299, 22-301-22-306, 22-308). Hemorrhage and mild inflammation was infrequently noted at the injection sites of the tail vein and hindlimb muscles and were considered to be related to injection trauma ((IND # 12285, Vol. 1.22, pages 22-022, 22-295, 22-307). One group 4 rat (1/14) was noted to have mild chronic perineural inflammation of the optic nerve (IND # 12285, Vol. 1.22, page 22-305), 2, Group 4 rats (2/14) were noted to have minimal periductular lymphocytic infiltrates of the pancreas and minimal lymphocytic infiltrates of the thyroid gland, respectively. (IND # 12285, Vol. 1.22, pages 22-305, 22-308). Based on results of this toxicology study, the **NOAEL for this study was hence determined to be 10.6 mg/kg (2.91 mg/0.275 kg/rat = 10.6 mg/kg).**

**GLP, Repeat Dose IV and IM Toxicity Study of Ab <sup>(b) (4)</sup> in Male Fischer 344 Rats**  
 (Study No. 03553, <sup>(b) (4)</sup> conducted in 120 male Fischer 344 rats 10-13 weeks of age, weighing between 245-311 g (15 rats/group) that were randomized into 8 groups (n=15 male rats/group), as delineated below (IND # <sup>(b) (4)</sup> Vol 1.22, page 22-105).

Study Group (n=15)	Treatment	Route	Dose Level (mg/rat)	Dose Concentration (mg/mL)	Dosing Schedule (Days)
1	PBS	IV	0	0	Day 1, 4, and 7, sacrifice day 10/11
2	Ab <sup>(b) (4)</sup>	IV	0.09	0.18	Day 1, 4, and 7, sacrifice day 10/11
3	Ab <sup>(b) (4)</sup>	IV	0.52	1.04	Day 1, 4, and 7, sacrifice day 10/11
4	Ab <sup>(b) (4)</sup>	IV	2.91	5.82	Day 1, 4, and 7, sacrifice day 10/11
5	PBS	IM	0	0	Day 1, 4, and 7, sacrifice day 10/11
6	Ab <sup>(b) (4)</sup>	IM	0.09	0.18	Day 1, 4, and 7, sacrifice day 10/11
7	Ab <sup>(b) (4)</sup>	IM	0.52	1.04	Day 1, 4, and 7, sacrifice day 10/11
8	Ab <sup>(b) (4)</sup>	IM	2.91	5.82	Day 1, 4, and 7, sacrifice day 10/11

All animals in study groups 1-8 received injections (either IV or IM) of either test article in the tail vein or hindlimb muscle (formulation #'s ET-285-052, ET-285-051, ET-285-050) or placebo (PBS, formulation # 092K2323) on Days 1, 4, and 7 and underwent necropsy on Day 10 (1<sup>st</sup> 10 animals in each group) or Day 11 (last 5 animals/group).

Animals underwent cageside observations for mortality, moribundity, general health, and clinical signs of toxicity. Clinical observations included evaluation of body weight and food consumption, evaluation of skin and fur characteristics, injection sites, eye and mucous membranes, respiratory, circulatory, autonomic, CNS, somatomotor and behaviour patterns (IND # 12285, Vol. 1.22, page 22-106).

Laboratory testing comprised evaluation of the CBC with diff, chemistry and LFTs and toxicokinetics and was collected pre-dose (Day 1) and on Day 4, 7, and prior to necropsy (IND # 12285, Vol. 1.22, page 22-106). Hematology testing was performed on the 1<sup>st</sup> 5 animals/group, chemistry was performed on the 2<sup>nd</sup> 5 animals/group, and toxicokinetics was performed on the 3<sup>rd</sup> 5 animals/group (IND # 12285, Vol. 1.22, pages 22-106). Upon necropsy, tissues underwent gross pathologic evaluation and were processed for histopathologic evaluation. The complete panel of tissues underwent histopathologic evaluation (IND # 12285, Vol. 1.22, pages 22-283-22-369). Statistical analysis was performed on body weight, body weight change, food consumption, organ weights and body weight ratios, and clinical laboratory testing using 1-way ANOVA, the Kolmogorov-Smirnov test for normality, and the Levene Median test for equal variance (IND # 12285, Vol. 1.22, page 22-107).

Results of the study revealed no mortality with the exception of one (1) control group animal (Animal No. 13707, Group 4) on Study Day 4 that was attributed to the stress during dosing (necropsy did not reveal any organ pathology (IND # 12285, Vol. 1.22, page 22-020). With respect to morbidity, no test article related findings were noted—a thin appearance was observed in 1 control group animal (Group 1), in Group 6 (0.09 mg dose) animal, and 1 Group 8 (2.91 mg dose) animal (IND # 12285, Vol. 1.22, page 22-020). There were no significant test article related changes in weight or food consumption (IND # 12285, Vol. 1.22, page 22-020). No significant ophthalmologic changes were noted in any of the treatment groups that could be attributed to treatment (IND # 12285, Vol. 1.22, pages 22-100-101), though several animals manifested the following changes (no trend noted per treatment group): focal opacity on the surface of the cornea (animal # 113675), irritated conjunctiva with significant peripheral hemorrhage (animal # 13727), abrasions along the lateral surface of the conjunctiva and slight opacity of the peripheral edges of the cornea of the right eye (animal # 13753), and hemorrhage with consolidation within the anterior chamber of the right eye (animal # 13773) (IND # 12285, Vol. 1.22, pages 22-100-101).

With regard to laboratory parameters, changes that were seen, such as a statistically significant higher sodium concentration in the Group 3 and 4 rats (compared to Group 1 controls) on Study day 4, statistically significant lower globulin in Group 4 rats (compared to Group 1 controls) on Study day 7, and statistically significant lower total protein in Group 7 rats on Study day 4 (compared to Group 5 controls) were attributed to significant individual animal variation (IND # 12285, Vol. 1.22, pages 22-021, 22-119-22-166).

With regard to gross pathology on necropsy, the only finding noted was the presence of a discolored thymus in a small # of animals and a reduced size of testis (left) in a Group 5 rat (animal # 13727) (IND # 12285, Vol. 1.22, pages, 22-032-22-038, 22-076-22-083). The thymus findings appeared to be ~ equally distributed amongst the 8 treatment groups, with no trend noted by dose. There were no

significant changes in organ weights across treatment groups (IND # 12285, Vol. 1.22, pages, 22-032-22-039-22-041).

Histopathologic evaluation of organs at necropsy overall failed to reveal any significant changes or trends in test article treated animals as compared to the controls (IND # 12285, Vol. 1.22, pages 22-283-22-369). Noted were microscopic findings in the heart (minimal ventricular mononuclear cell infiltrate), liver (minimal-mild mononuclear cell infiltration), kidneys (minimal tubular mineralization and/or regeneration, minimal nephropathy, minimal lymphocytic infiltrate), stomach (minimal submucosal eosinophilic infiltrate), lung (minimal perivascular/peribronchial infiltrate and/or hemorrhage, minimal alveolar hyperplasia), thymus (minimal-mild hemorrhage), Harderian glands (minimal-mild unilateral pigmentation, minimal ductular metaplasia, minimal unilateral chronic inflammation, and minimal atrophy), and lymphoid tissues (minimal histiocytic pigmentation, mild sinus congestion, and mild sinus histiocytosis)—findings which have been described in other toxicology studies of the Fischer 344 rat (background findings) and which were also noted in control group animals (IND # 12285, Vol. 1.22, pages 22-288, 22-294, 22-296-22-299, 22-301-22-306, 22-308). Hemorrhage and mild inflammation was infrequently noted at the injection sites of the tail vein and hindlimb muscles and were considered to be related to injection trauma ((IND # 12285, Vol. 1.22, pages 22-022, 22-295, 22-307). One group 4 rat (1/14) was noted to have mild chronic perineural inflammation of the optic nerve (IND # 12285, Vol. 1.22, page 22-305), 2, Group 4 rats (2/14) were noted to have minimal periductular lymphocytic infiltrates of the pancreas and minimal lymphocytic infiltrates of the thyroid gland, respectively. (IND # 12285, Vol. 1.22, pages 22-305, 22-308).

The toxicokinetics section of this study showed no detectable Ab in any of the Group 1 animals (control) at any time point tested (IND # 12285, Vol. 1.22, page 22-106-22-107), as were also (-) the Day 1 pre-dose samples for Groups 2, 3, and 4. For Group 2, the Day 4 <sup>(b) (4)</sup> concentration was 2.7 µg/mL, which increased to 5.4 µg/mL on Day 7 and increased to 6.9 µg/mL on Day 11. The Group 3 animals followed the same trend, with the Day 4 <sup>(b) (4)</sup> concentration being 15.6 µg/mL, the Day 7 concentration being 30.4 µg/mL, and the Day 11 concentration being 37.3 µg/mL. The same trend was observed for the Group 4 animals, with the Day 4 <sup>(b) (4)</sup> concentration being 79.3 µg/mL, the Day 7 concentration being 158.4 µg/mL, and the Day 11 concentration being 186.7 µg/mL (IND # 12285, Vol. 1.22, page 22-106-22-107). For the IM dosed animals (Groups 5-8), no detectable level of <sup>(b) (4)</sup> was present in any of the Group 5 animals (PBS control) at any of the time points tested (IND # 12285, Vol. 1.22, page 22-106, 22-108). All the Day 1 pre-dose samples for Groups 6, 7, and 8 were also (-) for <sup>(b) (4)</sup> activity. For Group 6, the Day 4 <sup>(b) (4)</sup> concentration was 1.9 µg/mL, which increased to 3.8 µg/mL on Day 7 and decreased to 2.7 µg/mL on Day 11. The decrease in concentration and large standard deviation that was noted on Day 11, was due to 2/5 animals having undetectable levels of <sup>(b) (4)</sup>. Group 7 animals had a similar trend as Group 6 animals, with Day 4 <sup>(b) (4)</sup> concentration being 11.8 µg/mL, the Day 7 concentration being 30.0 µg/mL, and the Day 11 concentration being 23.3 µg/mL (IND # 12285,

Vol. 1.22, page 22-106, 22-108). The decrease in concentration and large standard deviation that was noted on Day 11, was due to a decreased concentration in of (b) (4) in 3/5 animals and increasing concentration in 2/5 rats. Group 8 rats followed the same trend as the previous 2 groups, with the Day 4 (b) (4) concentration being 66.8 µg/mL, the Day 7 concentration being 132.0 µg/mL, and the Day 11 concentration being 173.8 µg/mL.

**The NOAEL for this study was hence determined to be 10.6 mg/kg (2.91 mg/0.275 kg/rat = 10.6 mg/kg).**

## 2. Study title: A 14-day pilot IV toxicity study of ETI-204 in Sprague-Dawley rats – amended report

Study no.:	TOX001 pilot (b) (4) Study no. 1984-007
Study report location:	Electronic submission of SDN 1, section 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/20/2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ETI-204, Lot Number 250241, 99..1% monomer

### Key Study Findings

This GLP study was conducted to evaluate the toxicity of the test article, ETI-204, when administered to Sprague-Dawley male rats on Days 1, 4, and 7 to aid in the selection of doses for a potential subsequent toxicity study. The secondary objective was to identify the maximal tolerated dose (MTD) in rats. Four treatment groups of five male CD[CrI:CD®(SD)] rats were administered vehicle, 0.9% Sodium Chloride for Injection, USP, or ETI-204 at dose levels of 10, 30, or 100 mg/kg/dose. Test article was administered to all groups via slow bolus intravenous (IV) injection, on Days 1, 4, and 7, at a dose volume of 1 mL/kg/dose. No effect on survival, clinical observations, body weights, clinical pathology, and macroscopic findings was observed in the ETI-204-treated groups.

Systemic exposure to ETI-204, as estimated by  $AUC_{inf}$ ,  $AUC_{0-3}$ ,  $AUC_{0-14}$  and  $C_{max}$ , increased in approximate proportion to dose between 10 and 100 mg/kg/dose. Mean  $T_{1/2}$  was similar among dose levels, ranging from 2.26 to 2.79 days. Mean  $T_{max}$  was 0.0417 days for all ETI-204 groups. CL (17.7 to 19.6 mL/day/kg) and  $V_{ss}$  (64.3 to 71.2 mL/kg) did not vary with dose. Repeated doses of ETI-204 at 100 mg/kg were well tolerated; therefore, the maximum tolerated dose (MTD) was not reached in this study.

**Methods**

Doses: Vehicle, 10, 30, or 100 mg/kg/dose  
Frequency of dosing: Three doses administered on Days 1, 4, and 7  
Route of administration: Slow bolus IV injection over approximately 45 seconds  
Dose volume: 1 mL/kg/dose  
Formulation/Vehicle: 0.9% sodium chloride for injection, USP  
Species/Strain: Sprague-Dawley rats (CrI:CD®(SD))  
Number/Sex/Group: 5 male rats/group  
Age: Approximately 8 weeks  
Weight: 195-259 g at randomization  
Satellite groups: None  
Deviation from study protocol: No deviations were described in the report.

**Observations and Results****Mortality**

Animals were observed twice daily observation for morbidity, mortality, injury, and availability of food and water.

No mortality was reported during the study.

**Clinical Signs**

A detailed clinical examination was performed daily (2-6 hours post-dose on dosing days).

No treatment-related findings were reported.

**Body Weights**

Body weights were recorded and reported prior to initiation of dosing on each dosing day and prior to termination on Day 15.

No effect of treatment on mean body weights was reported.

**Feed Consumption**

Not recorded

**Ophthalmoscopy**

Not performed

**ECG**

Not performed

**Hematology / Clinical Chemistry**

Blood samples for clinical pathology were collected from the vena cava prior to necropsy from all animals following an overnight fast.

No treatment-related effects were reported.

**Urinalysis**

Not performed

## Gross Pathology

Animals were euthanized on Day 15 by CO<sub>2</sub> inhalation followed by exsanguination via the abdominal vena cava, and a macroscopic examination was conducted.

No effect of treatment on macroscopic findings was reported.

## Organ Weights

Not performed

## Histopathology

### Adequate Battery

The following organs were removed and preserved in neutral buffered formalin for possible future examination: brain, stomach (glandular), heart, kidneys, liver, lung with bronchi, spleen, and gross lesions.

### Peer Review

No

### Histological Findings

Tissues were not examined.

## Toxicokinetics

Blood samples (approximately 0.3 mL) for toxicokinetics were collected from all animals via the sublingual vein prior to dosing (Day 1) and at 1, 12, 24, 48, and 72 (prior to the Day 4 second dose) hours post-dose Day 1, at 1 hour post-dose on Day 7, and prior to necropsy on Day 15. The animals were not fasted prior to blood collection, with the exception of the intervals that coincided with fasting for clinical pathology collections. Serum was separated and stored frozen at -50 to -90°C until analyzed. All analytical work was conducted by [REDACTED]<sup>(b) (4)</sup>, using an analytical method developed by [REDACTED]<sup>(b) (4)</sup>.

The report states that all animals in the control group (Group 1) had serum concentrations below the limit of quantitation (BLQ) at all time points (*Reviewer's comment: Ideally, these values should have been below the limit of detection*). All animals in treated groups were exposed to ETI-204 following IV administration. Following the first IV administration, T<sub>max</sub> occurred at the first blood collection time point after dosing (1 hr) for all three groups. Toxicokinetic parameters are shown in the Sponsor's tables below:

Mean ( $\pm$ SD) Toxicokinetic Parameters Following Three Intravenous Administrations of 10, 30, and 100 mg/kg/dose ETI-204 to Sprague-Dawley Rats			
Group	Dose (mg/kg/dose)	AUC <sub>0-3</sub> ( $\mu$ g*day/mL)	AUC <sub>inf</sub> ( $\mu$ g*day/mL)
2	10	316 (32.1)	603 (190)
3	30	870 (118)	1670 (339)
4	100	2640 (86.1)	5130 (443)

Mean ( $\pm$ SD) Toxicokinetic Parameters Following Three Intravenous Administrations of 10, 30, and 100 mg/kg/dose ETI-204 to Sprague-Dawley Rats (continued)				
Group	Dose (mg/kg/dose)	AUC <sub>inf</sub> /Dose ((day* $\mu$ g/mL)/mg/kg)	AUC <sub>0-14</sub> ( $\mu$ g*day/mL)	AUC <sub>0-14</sub> /Dose ((day* $\mu$ g/mL)/mg/kg)
2	10	60.3 (19.0)	1620 (337)	162 (33.7)
3	30	55.5 (11.3)	4890 (454)	163 (15.1)
4	100	51.3 (4.43)	16200 (1720)	162 (17.2)

Mean ( $\pm$ SD) Toxicokinetic Parameters Following Three Intravenous Administrations of 10, 30, and 100 mg/kg/dose ETI-204 to Sprague-Dawley Rats (continued)				
Group	Dose (mg/kg/dose)	T <sub>max</sub> (day)	C <sub>max</sub> ( $\mu$ g/mL)	C <sub>max</sub> /Dose (( $\mu$ g/mL)/mg/kg)
2	10	0.0417 (0.00)	228 (57.4)	22.8 (5.74)
3	30	0.0417 (0.00)	738 (98.8)	24.6 (3.29)
4	100	0.0417 (0.00)	2000 (121)	20.0 (1.21)

Mean ( $\pm$ SD) Toxicokinetic Parameters Following Three Intravenous Administrations of 10, 30, and 100 mg/kg/dose ETI-204 to Sprague-Dawley Rats (continued)					
Group	Dose (mg/kg/dose)	T <sub>1/2</sub> (day)	CL (mL/day/kg)	V <sub>z</sub> (mL/kg)	V <sub>ss</sub> (mL/kg)
2	10	2.79 (1.19)	17.7 (4.66)	65.8 (10.4)	64.3 (8.20)
3	30	2.62 (0.816)	18.6 (3.51)	66.8 (7.09)	69.1 (1.06)
4	100	2.26 (0.182)	19.6 (1.67)	63.6 (2.42)	71.2 (2.45)

Systemic exposure to ETI-204, as estimated by AUC<sub>inf</sub>, AUC<sub>0-3</sub>, AUC<sub>0-14</sub> and C<sub>max</sub>, increased in approximate proportion to dose between 10 and 100 mg/kg/dose. Mean T<sub>1/2</sub> was similar among dose levels, ranging from 2.26 to 2.79 days. CL (17.7 to 19.6 mL/day/kg) and V<sub>ss</sub> (64.3 to 71.2 mL/kg) did not vary with dose.

**Dosing Solution Analysis**

Not performed

**3. Study title: A 14-day definitive IV toxicity study of ETI-204 in Sprague-Dawley rats**

Study no.: TOX001 definitive  
(b) (4) no. 1984-006)

Study report location: Electronic submission of SDN 1, section 4.2.3.2

Conducting laboratory and location: (b) (4)

Date of study initiation: 7/24/2012

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: ETI-204, Lot Number 250241 (Lonza), 99.1% monomer  
MAb 103b (ETI-204), Lot Number: PBR-0024-001 (Baxter), 97% monomer

**Key Study Findings**

This definitive toxicology study included 8/sex/group plus satellite toxicokinetics animals. The purpose was to evaluate the potential subchronic toxicity and toxicokinetics (TK) of the test article, ETI-204, when administered to Sprague-Dawley rats on Days 1, 4, and 7. A secondary objective was to assess the comparability of ETI-204 manufactured from two different contract manufacturing organizations (CMOs) in terms of safety and TK profile. Material from Lonza will be used in all pivotal non-clinical studies and the clinical safety studies; whereas the Baxter material has been used in most non-clinical studies before Lonza material was available. Three treatment groups of eight male and eight female CD<sub>1</sub> [CrI:CD@ (SD)] rats/group were administered ETI-204 manufactured by Lonza at respective dose levels of 3, 10, and 30 mg/kg/dose. One treatment group of eight animals/sex was administered ETI-204 manufactured by Baxter at 30 mg/kg/dose. One additional group of eight animals/sex served as the control and received the vehicle, 0.9% Sodium Chloride for Injection, USP. The vehicle or ETI-204 was administered to all groups via intravenous (IV) injection, at a dose volume of 1 mL/kg/dose. Additionally, five groups of three, six, six, six, and six animals/sex/group (vehicle, Lonza 3, 10, and 30 mg/kg/, and Baxter 30 mg/kg, respectively) served as TK animals and received the vehicle (3 animals/sex) or ETI-204 (6 animals/sex/group) in the same manner as the main study groups.

All animals survived to terminal euthanasia. No test article-related findings were observed in clinical observations, body weights, food consumption, clinical pathology, macroscopic, microscopic, and organ weight parameters at any of the ETI-204 Lonza or Baxter doses evaluated. Therefore, the no-observable-adverse-effect-level (NOAEL) was 30 mg/kg/dose for both Lonza and Baxter ETI-204, the highest dosage tested

(AUC<sub>0-∞</sub> = 2130-2240 µg\*day/mL). The Baxter and Lonza manufactured ETI-204 were considered to have comparable TK and safety profiles. However, a large degree of variability in C<sub>max</sub> was observed with the Lonza material, resulting in a lower C<sub>max</sub> than that seen for the Baxter material.

## Methods

Doses: 0 (vehicle), 3, 10, and 30 mg/kg/dose (Lonza material), and 30 mg/kg/dose (Baxter material)  
 Frequency of dosing: Doses were administered on Days 1, 4, and 7.  
 Route of administration: IV injection (slow bolus) via the lateral tail vein  
 Dose volume: 1mL/kg/dose  
 Formulation/Vehicle: 0.9% Sodium Chloride for Injection, USP  
 Species/Strain: CD[CrI:CD®(SD)] rats  
 Number/Sex/Group: 8  
 Age: Approximately 7 weeks  
 Weight: Males weighed 248 to 287 g, and females weighed 173 to 210 g, at randomization  
 Satellite groups: Five groups of 3, 6, 6, 6, and 6 animals/sex/group (vehicle, Lonza 3, 10, and 30 mg/kg, and Baxter 30 mg/kg, respectively) served as toxicokinetics animals.  
 Deviation from study protocol: None that were considered to affect the quality or integrity of the study

## Observations and Results

### Mortality

All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily.

All animals survived to study termination.

### Clinical Signs

Observations for clinical signs and drug hypersensitivity were conducted daily. Detailed clinical examination of each main study animal was performed daily (at 2-6 hours post-dose on dosing days).

No test article-related findings were reported. Findings of blue and/or red discolored skin (tail) were observed in a few ETI-204 treated male and female animals. These findings were transient and were considered to be local irritation, inflammation, and/or hemorrhage. These findings were attributed to the injection procedure and not considered test article-related.

### Body Weights

Body weights were measured and recorded on Days 1, 4, 7, and 14. No test article-related findings were reported.

**Feed Consumption**

Food consumption for the main study animals was measured and recorded weekly. No test article-related findings were reported.

**Ophthalmoscopy**

Not performed

**ECG**

Not performed

**Clinical Pathology**

Blood and urine samples for clinical pathology evaluations were collected from main study animals prior to terminal necropsy following a 4 hour fast. Blood was collected from the vena cava after CO<sub>2</sub> inhalation. Urine was collected in metabolism cages at least 14 hours prior to necropsy.

All values for hematology, coagulation, clinical chemistry, and urinalysis parameters were considered to be within normal limits. No test article-related findings were reported.

**Gross Pathology**

At study termination (Day 15, according to the protocol) main study animals were euthanized. The protocol states, "Euthanasia will be by carbon dioxide inhalation followed by an (b) (4) SOP (NEC-12) approved method to ensure death, e.g. exsanguination." However, the final study report does not specify either the termination day or the method. Necropsy examinations were performed on main study animals. No test article-related macroscopic findings were reported. A renal cyst was found in one animal (see below under "Histopathology"), but it was not considered to be treatment-related.

**Organ Weights**

See the Sponsor's table below under "Histopathology" for the organs weighed. No test article-related findings were reported.

**Histopathology****Adequate Battery**

Tissues were microscopically examined for animals in the vehicle control, 30 mg/kg/dose (Lonza), and 30 mg/kg/dose (Baxter) groups. Tissues examined are shown in the Sponsor's table below:

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- Adrenal (2)*	- Larynx
- Aorta	- Liver [3 sections collected; 2 examined]*
- Bone with marrow [femur]	- Lung with bronchi [collected whole; 2 sections examined]*
- Bone with marrow [sternum]	- Lymph nodes: mandibular [2 collected; 1 examined] and mesenteric
- Bone marrow smear [2 collected]*	- Mammary gland [process females only]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*	- Pancreas
- Epididymis (2)*	- Pituitary*
- Eye including optic nerve (2)	- Prostate* and seminal vesicle (2)*
- GALT [gut associated lymphoid tissue]	- Salivary gland, mandibular/sublingual [2 collected; 1 examined]*†
- Gastrointestinal tract:	- Salivary gland, parotid [2 collected; 1 examined]
esophagus	- Sciatic nerve
stomach [glandular and nonglandular]	- Skeletal muscle, biceps femoris
duodenum	- Skin
jejunum	- Spinal cord [cervical, thoracic, and lumbar]
ileum	- Spleen*
cecum	- Thymus*
colon	- Thyroid/parathyroid (2)*
rectum	- Tongue
- Genads:	- Trachea
ovary (2)* with oviduct (2)	- Ureter (2)
testis (2)*	- Urinary bladder
- Gross lesions	- Uterus [both horns]/Cervix*
- Heart*	- Vagina
- Injection site, last	
- Kidney (2)*	
- Lacrimal gland, exorbital (2)	

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\*Bone marrow smears were collected at necropsy and held.

†The combined weight of the right mandibular/sublingual salivary gland was obtained.

(2) Paired organ

\*Organ weighed

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## Peer Review

No

## Histological Findings

No test article-related findings were reported.

Minimal subacute/chronic inflammation was present at the injection site in all groups, including control, at similar incidence. This was considered to be secondary to the injection procedure and not related to the test article.

For animal number 2503 [3 mg/kg/dose (Lonza)], a cyst in the kidney was noted on macroscopic observation. The kidney lesion of that animal was examined microscopically, revealing findings of renal tubular adenoma (amphophilic-vacuolar type), which was considered to be a spontaneous finding and not test article-related.

## Special Evaluation

Blood samples for evaluation of immunogenicity were collected from main study animals from the sublingual vein prior to initiation of dosing on Day 1 and on Day 14 prior to necropsy. Serum was separated and frozen at -50 to -90°C until analysis for determination of anti-therapeutic antibody (ATA).

Results of the analysis of these samples are not reported. It is unclear if the analysis was performed.

## Toxicokinetics

Blood samples for toxicokinetics were collected at 0.25 hours post-dose on Days 1, 4, 7 from satellite TK control animals. Samples were taken from satellite treated animals prior to dosing on Days 1, 4, 7, on Day 1 at 0.25, 4, 12, 24, and 48 hours post-dose, on Day 7 at 1 hour post-dose, and untimed samples on Days 10 and 15 as untimed samples. Animals were not fasted prior to collection. After blood collection, the

TK animals were euthanized and the carcasses were discarded. Blood was allowed to clot for at least 30 minutes. Samples centrifuged and serum collected and stored frozen at -50 to -90°C until analysis. Analysis was performed using an immunology method developed by (b) (4) and validated under (b) (4) study no. 1984-009

The toxicokinetic parameters for ETI-204 were described as similar in male and female rats; values for those parameters are combined rather than expressed separately by gender. Systemic exposure to ETI-204, as estimated by AUC<sub>0-∞</sub>, AUC<sub>0-3</sub>, AUC<sub>0-14</sub> and C<sub>max</sub>, increased approximately proportionally to dose between 3 and 30 mg/kg/dose. Mean t<sub>1/2</sub> was similar among dose levels, ranging from 2.46 to 3.57 days. Mean T<sub>max</sub> ranged from 0.0104 to 0.0885 days. CL (13.4 to 14.1 mL/day/kg) and V<sub>z</sub> (46.8 to 71.7 mL/kg) did not vary with dose. At a dose of 30 mg/kg/dose, all of the toxicokinetic parameters calculated for ETI-204 were similar between the materials provided by Lonza and Baxter, with the exception of C<sub>max</sub> (0.25 hours post-dose), which appeared to be higher for the material supplied by Baxter compared to Lonza. Because of the high variability in the Lonza mean C<sub>max</sub> (%CV = 62), the 30 mg/kg mean ETI-204 serum concentrations for the 4 hour post-dose Lonza and Baxter samples were compared. At a dose of 30 mg/kg, the mean ETI-204 concentrations for the 4 hours post-dose samples were similar between the Lonza and Baxter (672 and 793 µg/mL, respectively; see table below).

Dose Level (mg/kg/dose)	Timepoint (hr)	Mean ETI-204 Concentration (µg/mL)	%CV
30 (Lonza)	0.25	620	62
30 (Baxter)	0.25	1068	16
30 (Lonza)	4	672	17
30 (Baxter)	4	793	27

Toxicokinetic parameters for this study are presented in the Sponsor’s tables below:

Test article source	Dose (mg/kg/dose)	Sex	AUC <sub>0-∞</sub> (day·µg/mL)	AUC <sub>0-3</sub> /Dose ((day·µg/mL)/mg/kg)	AUC <sub>0-14</sub> (day·µg/mL)	
Lonza	3	Male	216	72.1	91.2	
		Female	215	71.6	104	
		Combined	216	71.8	97.4	
	10	Male	809	80.9	379	
		Female	648	64.8	317	
		Combined	729	72.9	348	
30	Male	Male	2170	72.4	1000	
		Female	2080	69.3	979	
		Combined	2130	70.9	991	
	Baxter	30	Male	2390	79.6	1130
			Female	2100	70.0	1320
			Combined	2240	74.8	1230

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Test article source	Dose (mg/kg/dose)	Sex	AUC <sub>0-12</sub> /Dose ((day*µg/mL)/mg/kg)	AUC <sub>0-18</sub> (day*µg/mL)	AUC <sub>0-18</sub> /Dose ((day*µg/mL)/mg/kg)
Lonza	3	Male	30.4	538	179
		Female	34.5	557	186
		Combined	32.5	547	182
	10	Male	37.9	2090	209
		Female	31.7	1680	168
		Combined	34.8	1890	189
30	Male	33.4	5560	185	
	Female	32.6	5340	178	
	Combined	33.0	5430	182	
Baxter	30	Male	37.8	6080	202
		Female	44.2	6940	231
		Combined	41.0	6500	217

Test article source	Dose (mg/kg/dose)	Sex	C <sub>max</sub> (µg/mL)	C <sub>max</sub> /Dose ((µg/mL)/mg/kg)	T <sub>max</sub> (day)
Lonza	3	Male	51.3	17.1	0.0104
		Female	73.3	24.3	0.0104
		Combined	62.4	20.8	0.0104
	10	Male	281	28.1	0.0104
		Female	269	26.9	0.0104
		Combined	275	27.3	0.0104
30	Male	667	22.2	0.0104	
	Female	697	23.2	0.167	
	Combined	682	22.7	0.0885	
Baxter	30	Male	1000	33.3	0.0104
		Female	1140	37.9	0.0104
		Combined	1070	35.6	0.0104

Test article source	Dose (mg/kg/dose)	Sex	CL (mL/day/kg)	t <sub>1/2</sub> (day)	V <sub>d</sub> (mL/kg)
Lonza	3	Male	13.9	3.82	76.5
		Female	14.0	3.32	66.8
		Combined	13.9	3.37	71.7
	10	Male	12.4	3.36	63.4
		Female	15.4	3.17	70.8
		Combined	13.9	3.36	67.0
30	Male	13.8	3.47	69.1	
	Female	14.4	3.36	70.0	
	Combined	14.1	3.42	69.3	
Baxter	30	Male	12.6	3.23	58.4
		Female	14.3	1.70	33.1
		Combined	13.4	2.46	46.8

### Dosing Solution Analysis

Samples were taken for homogeneity analysis from the 30 mg/kg/dose Baxter material on Day 1. The report states that homogeneity for the Lonza material in the placebo was previously demonstrated under Study No. 1984-004, however that would have been specific to the dosing solutions used in that study.

*Reviewer’s comment: Lack of homogeneity of the dosing solutions may be related to the variability seen in Cmax values.*

Samples for concentration analyses were taken from all dosing formulations on Days 1, 4, and 7. Stability analysis was not conducted because samples were prepared fresh on each day of dosing, and the report notes that the results of concentration analysis demonstrate the stability of the dosing solutions over that short time.

The report states that homogeneity analysis was conducted and results were within acceptance criteria (%CV within ≤ 20% and at least 2 of 3 reportable results within 30% of the expected concentration) and that dose formulation analysis indicated animals were dosed with appropriate concentrations of test article. No ETI-204 was measured in the vehicle samples.

**Study no. AP115** ( (b) (4) **study no. 1180-07059**), was a safety pharmacology study in cynomolgus monkeys that included general toxicology assessments. It was originally submitted to IND 12285 Serial no. 022 (P/T reviewer – Stephen Hundley, PhD) and was re-submitted in serial no. 091 amended to include anti-drug antibody (ADA) information. See the review above under Safety Pharmacology.

The NOAEL was the high dose, 30 mg/kg, resulting in an AUC of approximately 150,000 µg·hr/mL.

## 7 Genetic Toxicology

Not performed

## 8 Carcinogenicity

Not performed

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

Not performed

### 9.2 Embryonic Fetal Development

Two studies, a pilot study and a definitive study, were performed in rabbits. It should be noted that tissue cross-reactivity (TCR) studies have not been performed in rabbit tissues to assess the suitability of that species for toxicity testing of ETI-204.

#### 1. Study title: ETI-204: An intravenous range-finding developmental toxicity study in rabbits with toxicokinetic evaluation

Study no.:	EFT001 pilot ( (b) (4) study no. 1984-002)
Study report location:	Electronic submission, section 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/22/2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	MAB 103B (equivalent to ETI-204), Lot Number PBR-0024-001, 97.4% monomer

### Key Study Findings

This non-GLP study was conducted to determine doses and a dosing scheme for the definitive developmental toxicology study in rabbits. Three groups of time-mated females were administered doses of 32 mg/kg either on GD 6, 10, 13, and 17, on GD 6 and 10, or on GD 13 and 17 (Groups 1, 2, and 3, respectively). Doses were administered by slow IV bolus injection via the marginal ear vein. In-life assessments included clinical observations, body weights, food consumption, and toxicokinetics.

Animals were euthanized at Caesarean section on GD 29. Assessments included gravid uterine weights, viable and nonviable fetuses, early and late resorptions, implantations, corpora lutea, fetal weights, and external malformations and variations.

One Group 3 rabbit delivered early on GD 29. A mean body weight loss and decreased food consumption were determined for Group 3, but were attributable to this one animal. The findings in this animal were not considered to be test article-related.

With the exception of fetuses from the animal that delivered early, fetal body weights were similar across groups. The fetuses from that Group 3 litter were delivered dead and had low body weights. The relationship to treatment for these fetuses is unclear. There were no fetal malformations or variations in any group, and no test article-related maternal macroscopic pathology findings in any group.

All animals showed a similar exposure to ETI-204 within the 96 hours after their first dose. After IV bolus, ETI-204 was said to have distributed throughout serum and into limited extracellular fluid; exhibited low total body clearance; and exhibited long half-life estimates (ETI-204  $t_{1/2}$  ranged from 2.64-3.95 days). Exposure was stated to be 4 times that in healthy human subjects following a single therapeutic dose (8 mg/kg at that time). The dosing regimen utilized in Group 1 was said to provide sustained supra-maximal exposure throughout the period of organogenesis and was chosen for use in the definitive developmental toxicity study.

## Methods

Doses:	The dose for all groups was 32 mg/kg/dose.
Frequency of dosing:	The dosing schedule varied by group as follows: Group 1 – GD 6, 10, 13, and 17 Group 2 – GD 6, 10 Group 3 – GD 13, 17
Dose volume:	1 mL/kg/dose
Route of administration:	IV slow bolus injection over 45 seconds via the marginal ear vein
Formulation/Vehicle:	0.9% sodium chloride for Injection, USP
Species/Strain:	New Zealand White Hra:(NZW)SPF
Number/Sex/Group:	6 time-mated females per group
Satellite groups:	None
Study design:	In this pilot dose range-finding study, fetal evaluations were limited to body weights and external evaluation.
Deviation from study protocol:	None that were considered to affect the quality or integrity of the study

## **Observations and Results**

### **Mortality**

Cageside observations were made twice daily. There were no test article-related deaths. One Group 3 animal was euthanized on GD 13 prior to dosing due to complications from the blood collection procedure and was replaced with another animal.

### **Clinical Signs**

Detailed clinical observations were made daily from GD 6 through 29 (at approximately 60 to 90 minutes post-dose on dosing days).

No test article-related findings were reported. Clinical findings included red material in the cage pan for animal 218 (Group 3) that delivered early the morning of GD 29, and swelling in the cervical region of two animals (animal numbers 213 and 218) in Group 3 that correlated with necropsy findings of red, clotted material (i.e. hematoma) in the ventral neck region and was attributed to the blood collection procedure.

### **Body Weight**

Body weights were recorded on GD 0, 6, 10, 13, 17, 19, 21, 25, and 29. The report states that mean body weights and body weight changes were not affected by treatment. A mean body weight loss of 135 g was seen in Group 3 during GD 25-29, but was attributed to a single animal (no. 218) that lost 680g and delivered early on GD 29. This was not considered to be test article related.

### **Feed Consumption**

Feed consumption was measured daily and summarized over the same intervals as body weight measurements. The report states that mean food consumption was not affected by treatment. Mean food consumption was decreased in Group 3 during GD 25-29. This was also attributed to animal no. 218 that was noted to have several days of low food consumption during that interval.

### **Toxicokinetics**

Blood was collected for determination of serum concentrations of the test article as shown in the Sponsor's table below:

**Sample Collection Schedule**

Time-point (Post First Dose)	Gestation Day					
	Group 1		Group 2		Group 3	
	Sub A	Sub B	Sub A	Sub B	Sub A	Sub B
Prior to First Dose	6	-	6	-	13	-
End-of-Injection	-	6	-	6	-	13
4 h	6	-	6	-	13	-
8 h	-	6	-	6	-	13
16 h	6	-	6	-	13	-
24 h	-	7	-	7	-	14
48 h	8	-	8	-	15	-
72 h	-	9	-	9	-	16
96 h <sup>a</sup>	10	-	10	-	17	-
7 d <sup>a</sup>	-	13	-	13	-	20
11 d <sup>a</sup>	17	-	-	-	-	-
14 d	-	20	-	-	-	-
GD 25 <sup>b</sup>	25	25	25	25	25	25

<sup>a</sup>: Prior to the second dose for each cohort

<sup>b</sup>: Prior to scheduled sacrifice

Samples were allowed to clot for at least 30 minutes prior to centrifugation and removal of serum. Serum was frozen at -50 to -90°C until analysis. Samples were analyzed for ETI-204 by (b) (4), using a “validated analytical method” (details not specified). The lower limit of quantification (LLOQ) for ETI-204 was 50.0 ng/mL in serum.

Samples were also collected for immunogenicity evaluation prior to the first dose (GD 6 or 13) and prior to necropsy (GD 29). Serum was separated and frozen at -50 to -90°C and shipped to (b) (4) for possible future analysis.

All animals were exposed to ETI-204 following IV administration, and exposures in animals dosed on GD 6 were similar at 96 hours post-dose. After the first IV administration, mean serum ETI-204 concentrations decreased prior to the second dose. Following subsequent IV administration of ETI-204 (inter-dose interval of 3-4 days), no sudden drop of serum concentration at any time point for any animal was reported and was considered to indirectly suggest the absence of neutralizing anti-ETI-204 antibodies that may affect the toxicokinetic profile. Based on this, immunogenicity analysis was not conducted. The individual serum concentrations from non-pregnant rabbits, Animal 201, 212, 213, and 217 were listed for completeness, but were excluded from mean concentration calculations.

Following IV administration of 32 mg/kg ETI-204 to time-mated rabbits on GD 6, mean serum ETI-204 concentrations decreased gradually in all groups and was said to be consistent with the toxicokinetic profile following IV bolus dosing. Data were analyzed using non-compartmental analysis (NCA). Following the first IV administration of ETI-204, T<sub>max</sub> occurred immediately after dosing (0.25 hr) for all three groups. C<sub>max</sub> was 1020, 1100, and 1180 µg/mL, and the T<sub>1/2</sub> was 3.27, 3.95, and 2.64 days, for Groups 1, 2 and 3, respectively. The AUC<sub>0-96h</sub> was 1610, 1530, and 1420 µg\*day/mL,

with clearance of 11.8, 11.6, and 14.4 mL/day/kg, for Group 1, 2, and 3, respectively.  $V_z$  ranged from 54.9 to 66.4 mL/kg, and  $V_{ss}$  ranged from 52.9 to 59.8 mL/kg. The report states that the volume of distribution suggests that ETI-204 distributes throughout serum and into limited extracellular fluid.

The  $AUC_{0-last}$  for Group 1, in which rabbits received 4 IV bolus doses of ETI-204 on GD6, 10, 13, and 17, was 10100  $\mu\text{g}\cdot\text{day}/\text{mL}$ . The  $AUC_{0-last}$  for Group 2, in which rabbits received 2 IV bolus doses of ETI-204 on GD6 and 10, was 3990  $\mu\text{g}\cdot\text{day}/\text{mL}$ . The  $AUC_{0-last}$  for Group 3, in which rabbits received 2 IV bolus doses of ETI-204 on GD13 and 17, was 3530  $\mu\text{g}\cdot\text{day}/\text{mL}$ . The  $C_{min}$  after the second (GD10), third (GD13), and fourth (GD17) dose in Group 1 were at 461, 455 and 533  $\mu\text{g}/\text{mL}$ , on GD13, GD17 (prior to dose) and GD20, respectively, with no sudden drop of serum ETI-204 concentration in any individual animal observed. The report stated that this indicated that the dosing regimen in Group 1 provided a sustained systemic exposure of ETI-204 throughout the period of organogenesis.  $C_2(\text{min})$  for Group 2 and 3 were 454 and 357  $\mu\text{g}/\text{mL}$ , respectively, again with no sign of sudden drop of serum concentration at any time point for any animal. Differences in  $T_{1/2}$  and exposure were observed between Group 1 and 2 vs. Group 3, which was thought to reflect physiological differences between early and late gestational stages.

**Table 4: Toxicokinetic Parameters Following Intravenous Administrations of 32 mg/kg ETI-204 to Time-Mated Rabbits**

Group	$T_{max}$ (day)	$C_{max}$ ( $\mu\text{g}/\text{mL}$ )	$AUC_{0-96h}$ ( $\mu\text{g}\cdot\text{day}/\text{mL}$ )	$AUC_{inf}$ ( $\mu\text{g}\cdot\text{day}/\text{mL}$ )	$T_{1/2}$ (day)	CL (mL/day/kg)	$V_z$ (mL/kg)	$V_{ss}$ (mL/kg)	$AUC_{0-last}$ ( $\mu\text{g}\cdot\text{day}/\text{mL}$ )
1	0.0104	1020	1610	2710	3.27	11.8	55.8	52.9	10100
2	0.0104	1100	1530	2750	3.95	11.6	66.4	59.8	3990
3	0.0104	1180	1420	2220	2.64	14.4	54.9	54.0	3530

Exploratory, post-hoc toxicokinetic analysis using a two-compartment model for IV bolus data is presented in the table below. The report indicates that the difference for exposure, in terms of AUC, was less than 5% for all groups (with the exception of Group 2  $AUC_{0-last}$ ), between non-compartmental analysis and the two-compartment model.

**Table 5: Toxicokinetic Parameters Following Intravenous Administrations of 32 mg/kg ETI-204 to Time-Mated Rabbits Using Two-Compartment PK Model 07**

Group	$V_1^1$ (mL)	$K_{10}^2$ (1/day)	$K_{12}^3$ (1/day)	$K_{21}^4$ (1/day)	$AUC_{inf}$ ( $\mu\text{g}\cdot\text{day}/\text{mL}$ )	CL (mL/day/kg)	$V_{ss}$ (mL/kg)	$AUC_{0-last}$ ( $\mu\text{g}\cdot\text{day}/\text{mL}$ )
1	30.1	0.378	1.75	2.10	2810	11.4	55.2	10300
2	28.2	0.437	1.42	1.37	2600	12.3	57.7	5020
3	25.1	0.572	2.71	19.4	2230	14.3	60.0	3980

<sup>1</sup> Volume for compartment 1

<sup>2</sup> Elimination rate

<sup>3</sup> Transfer rate from compartment 1 to compartment 2

<sup>4</sup> Transfer rate from compartment 2 to compartment 1

*Reviewer's comment: The observed data appeared to be in agreement with simulations based on this 2-compartment model.*

The report states that the Group 1 dosing regimen provides approximately 10 times higher ETI-204 exposure, in term of  $AUC_{0-last}$ , in pregnant rabbits than in nulliparous rabbits receiving single dose of ETI-204 in the therapeutic range (Study no. AR010), and that the Group 1 dosing regimen provided 4-fold higher exposure, in terms of  $C_{max}$  and AUC, relative to that in healthy human subjects receiving 8 mg/kg (Study no. AH102).

### **Dosing Solution Analysis**

Information was not provided.

### **Necropsy**

Dams were euthanized by injection of euthanasia solution followed by exsanguination, and immediately subjected to cesarean section on GD29. No test article-related maternal gross necropsy findings were reported.

### **Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)**

No test article-related effects on uterine and ovarian parameters were reported.

The pregnancy index was 83.3, 83.3, and 66.7% in Groups 1, 2, and 3, respectively. There were one, one, and two animals not pregnant in Groups 1, 2, and 3, respectively. The pregnancy index in Group 3 was lower than Groups 1, 2, and the historical control range (83.3 to 100%). However, since the first dose for this group was on GD 13, after implantation should have occurred, the lower pregnancy index was not considered to be test article-related. Furthermore, timing of shipment and mating was considered to have been a stressor that may have contributed to the lower pregnancy index, and may have contributed to higher preimplantation loss in Groups 2 and 3 relative to Group 1, although the timing of dosing in Group 2 could have had an effect on pre-implantation loss. The numbers of corpora lutea, implantation sites, viable and nonviable fetuses, litter size, and total resorptions per animal were similar between groups (*Reviewer's comment: The report cites 0 nonviable fetuses per dam in the three pregnant animals in Group 3, but also states that all fetuses in the early delivery litter were born dead.*).

One animal (animal no. 218) in Group 3 delivered early on the morning of GD 29. This animal was losing weight and eating significantly less from GD 25-29. Since no early deliveries were seen in Group 1 animals (dosed at all 4 intervals), the early delivery in this one animal was not considered to be test article-related.

### **Offspring (Malformations, Variations, etc.)**

Mean fetal body weights were similar across groups and unaffected by treatment, with the exception of fetuses from the animal that delivered early. The fetuses from that Group 3 litter were delivered dead and had low body weights.

No fetal external malformations or variations were reported.

## 2. Study title: A definitive IV dose study for effects of ETI-204 on embryo-fetal development in rabbits

Study no.: EFT001 Phase B (definitive) ( (b) (4) study no. 1984-005)

Study report location: Electronic submission, section 4.2.3.5.2

Conducting laboratory and location: (b) (4)

Date of study initiation: March 26, 2012

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: ETI-204 Drug Substance ( (b) (4) cell line), Lot Number 250241, 99.1% monomer

### Key Study Findings

This study was conducted for Elusys Therapeutics, Inc., to determine the developmental toxicity, including the teratogenic potential, of the test article, ETI-204, in rabbits. Two treatment groups of 30 time-mated female New Zealand White Hra:(NZW)SPF rabbits/group were administered ETI-204 at respective dose levels of 16 or 32 mg/kg/dose. One additional group of 22 time-mated females served as the control and received the vehicle, sterile 0.9% Sodium Chloride for Injection, USP. The vehicle or ETI-204 was administered to all groups via intravenous (IV) injection on Gestation Day (GD) 6, 10, 13, and 17, at a dose volume of 1 mL/kg/dose.

All animals that received the full dosing regimen survived to terminal euthanasia on GD 29, with the exception of one animal at 32 mg/kg/dose that delivered early on GD 28. This animal was losing weight and eating significantly less (0-5 g per day) from GD 13-27, however early deliveries have been observed in recent historical control data for this laboratory; therefore the early delivery limited to this one animal was not considered test article-related (*Reviewer's comment: This animal appears to be similar to one dam in the pilot study that was dosed on GD 13 and 17 and delivered early following a period of decreased food consumption and weight loss*). Additionally, one animal (animal number 140) at 16 mg/kg/dose died immediately following dosing on GD 6 and was replaced. No definitive cause of death could be determined from macroscopic findings, however, no other animals at this dose or at 32 mg/kg/dose died, therefore this death was not considered to be test article-related.

Results from immunogenicity analysis confirmed two animals to be positive for antibodies to ETI-204.

Systemic exposure to ETI-204, as estimated by  $AUC_{0-\infty}$ ,  $AUC_{0-4}$  and  $C_{max}$ , increased in approximate proportion to dose between 16 and 32 mg/kg/dose. Mean  $t_{1/2}$  was similar between dose levels, ranging from 3.60 to 4.08 days. Mean  $T_{max}$  was 0.00207 days. CL (9.74 to 10.3 mL/day/kg) and  $V_z$  (50.3 to 54.4 mL/kg) did not vary with dose.

There were no ETI-204-related effects on mortality, maternal body weight, body weight gain, food consumption, clinical pathology, macroscopic findings, microscopic brain findings, and uterine implantation data. Likewise, no ETI-204-related effect was evident on fetal body weight and external, visceral, and skeletal evaluations. Therefore, the No-Observed-Effect Level (NOEL) of ETI-204 for maternal and developmental

toxicity was 32 mg/kg/dose, the highest dose tested. The report states that systemic exposure associated with this dose in the preliminary study was approximately four times higher than that of healthy human subjects administered an IV dose of 8 mg/kg. Since the clinical dose is more likely to be 16 mg/kg, this may represent a 2-fold safety margin.

## Methods

- Doses: 0 (vehicle), 16, and 32 mg/kg/dose  
(The report states that systemic exposure associated with the high dose in the preliminary study was approximately four times higher than that of healthy human subjects administered an IV dose of 8 mg/kg.)
- Frequency of dosing: Based on prior data that the half-life of ETI-204 in the rabbit is approximately 4 days, it was determined that doses would be administered at approximately 4-day intervals, i.e. on GD 6, 10, 13 and 17.
- Dose volume: 1 mL/kg/dose
- Route of administration: Slow IV bolus injection over approximately 45 seconds via the marginal ear vein
- Formulation/Vehicle: Sterile 0.9% sodium chloride for injection, USP
- Species/Strain: New Zealand White Hra:(NZW)SPF rabbits
- Number/Sex/Group: 22 time-mated females for the control group, and 30 time-mated females for each treated group. Animals were 5-7 months of age at receipt.
- Satellite groups: None
- Study design: Doses were administered during the period of organogenesis. On GD 29, each surviving female was euthanized by injection of sodium pentobarbital, followed by exsanguination. The uterus was excised, the gravid uterine weight was recorded, the location of viable and nonviable fetuses, early and late resorptions for each uterine horn, and the total number of implantations were recorded. The number of corpora lutea on each ovary was also recorded.

All fetuses were evaluated for external, visceral, and skeletal anomalies. The left brain hemispheres of five pregnant/animals/group were collected, fixed in 10% formalin, and shipped at ambient temperature to (b) (4) for subsequent examination.

Deviation from study protocol: Serum samples for immunogenicity testing were analyzed beyond known long term stability; the impact of this was stated to be undetermined.

Rabbit anti ETI-204 antiserum initially was stored at -70°C, but stability had not been established at that temperature. This was not considered to have any impact on the integrity or quality of study.

None of the other deviations were considered to have any effect on the quality or integrity of the study.

## Observations and Results

### Mortality

All animals were observed twice daily for morbidity, mortality, injury, and availability of food and water.

One animal (animal number 140) at 16 mg/kg/dose died immediately following dosing on GD 6 and was replaced. No definitive cause of death could be determined on necropsy. Since no other animals treated with ETI-204 died, this death was not considered to be test article-related. Data for this animal were not reported.

One animal (animal number 181) at 32 mg/kg/dose delivered early on GD 28 and was subsequently euthanized. All other animals survived to terminal euthanasia on GD 29.

### Clinical Signs

Cageside observations were made twice daily. Detailed clinical observations were made daily from GD6 through 29 (at 60-90 minutes post-dose on dosing days).

No test article-related clinical findings were observed in any group. On Day 28, red discharge from the vulva and red material in the pan was observed for animal number 181 at 32 mg/kg/dose; those signs were consistent with early delivery. Three high dose animals (animal numbers 178, 181, and 182) exhibited inappetence and were offered supplemental food for up to 13 days during the study period. The inappetence observed in these three animals was not considered to be test article-related, but consistent with reduced food consumption said to be seen in rabbits in late pregnancy.

### Body Weight

Body weights were recorded on GD 0, 6, 10, 13, 17, 20, 25, and 29. Individual body weight change was calculated for the following GD intervals: 0-6, 6-10, 10-13, 13-17, 17-20, 20-25, 25-29, 6-17, 17-29, and 0-29. Adjusted body weight (GD 29 body weight minus gravid uterine weight) and adjusted body weight change (GD 0 to 29) were also calculated.

No test article-related changes in mean body weights and body weight change were reported. Animal number 181 at 32 mg/kg/day was losing weight during the later

stages of gestation (GD 17-25); this was considered to be consistent with the observed inappetence and early delivery in this animal and not test article-related.

### Feed Consumption

Food consumption was recorded daily and reported on corresponding body weight reporting days. Daily food consumption was not analyzed statistically.

There were no test article-related changes in mean food consumption. Inappetence was observed in animal 181 at 32 mg/kg/day and was consistent with the lower weight gain and early delivery in this animal. This was considered to be incidental and not test article-related.

### Toxicokinetics

Blood samples (approximately 1.0 mL) were collected from all treated animals (unfasted) via the jugular vein on GD 6 pre-dose, immediately post-dose (within 1 minute), at 1, 24, 48, 72, and 96 (pre-dose GD 10) hours, on GD 10 at 1 hour post-dose, on GD 13 and 17 pre-dose, on GD 20 and 25, and prior to termination on GD 29. Blood samples were collected at all of the above time points for the control animals, however, only the 1 hour post-dose samples were analyzed. Serum was separated and stored frozen at -50 to -90°C until analysis. All analytical work was conducted by (b) (4), using analytical methods developed and validated by that laboratory. Toxicokinetic analysis was performed using a non-compartmental methods (WinNolin®, Pharsight Corporation, Version 6.1).

Serum concentrations of ETI-204 increased as dose increased on GD 6. Systemic exposure to ETI-204, as estimated by  $AUC_{0-\infty}$ ,  $AUC_{0-4}$ ,  $AUC_{0-15}$ , and  $C_{max}$ , increased approximately proportional to dose between 16 and 32 mg/kg. Mean half-life ranged from 3.60 to 4.08 days and was similar between dose levels. Mean  $T_{max}$  was 0.00207 days. CL (9.74 to 10.3 mL/day/kg) and  $V_z$  (50.3 to 54.4 mL/kg) did not vary with dose. Parameters are shown in the Sponsor's tables below:

Mean ( $\pm$ SD) Toxicokinetic Parameters for ETI-204 in Pregnant Rabbits given an Intravenous Dose on Gestation Day 6				
Dose (mg/kg/dose)	$AUC_{0-\infty}$ (day $\cdot$ $\mu$ g/mL)	$AUC_{0-4}$ (day $\cdot$ $\mu$ g/mL)	$AUC_{0-15}$ (day $\cdot$ $\mu$ g/mL)	$C_{max}$ ( $\mu$ g/mL)
16	1710 $\pm$ 389	896 $\pm$ 125	4990 $\pm$ 1010	588 $\pm$ 86.4
32	3220 $\pm$ 638	1800 $\pm$ 170	10000 $\pm$ 1090	1180 $\pm$ 171

Mean ( $\pm$ SD) Toxicokinetic Parameters for ETI-204 in Pregnant Rabbits given an Intravenous Dose on Gestation Day 6				
Dose (mg/kg/dose)	$t_{1/2}$ (day)	$T_{max}$ (day)	CL (mL/day/kg)	$V_z$ (mL/kg)
16	4.08 $\pm$ 1.50	0.00207 $\pm$ 0.00748	9.74 $\pm$ 1.94	54.4 $\pm$ 13.8
32	3.60 $\pm$ 1.46	0.00207 $\pm$ 0.00748	10.3 $\pm$ 1.89	50.3 $\pm$ 11.3

## Clinical pathology

Clinical pathology evaluations were conducted on all animals (unfasted) pretest and at termination. Blood samples (4.8-5.8 mL) were collected via the jugular vein for evaluation of hematology, coagulation and clinical chemistry parameters. Urine samples were collected using steel pans placed under the cages for at least 16 hours.

No test article-related changes were observed. Differences noted from control that were statistically significant included increased leukocytes and lymphocytes in high dose females at termination and increased glucose in low dose females at termination; these values appeared to be within normal limits and were consistent with biologic variation.

## Immunogenicity

Blood samples (1.0 mL) were collected prior to dosing on GD 6 and prior to terminal necropsy from all animals (unfasted) via the jugular vein for determination of the anti-therapeutic antibody levels. Samples were processed to serum and frozen at -50 to -90°C until shipment to (b) (4), for serum antibody analysis using analytical methods said to be developed and validated at that laboratory.

Of the 164 samples that were screened, 51 samples were screened as positive. Of those 51 samples, two were confirmed to have antibodies to ETI-204. Twenty-three percent of samples were hemolyzed, but the report states that hemolysis had no effect on the results. The titers in one control animal (#114) terminal sample and in one 16 mg/kg animal (#127) terminal sample were both reported in a summary table as 5.

## Dosing Solution Analysis

Samples from the middle of the flask of dosing solutions were sampled during the first and second weeks for analysis of concentration only.

The report states that homogeneity was established under (b) (4) Study Number 1984-004 (Elusys Study AR014) for the concentration range of 12 to 120 mg/mL at the batch sizes used in this study under refrigerated (2 to 8° C) conditions; therefore no homogeneity analysis was performed.

*(Reviewer's comment: These data are not applicable to the current dosing solutions.)*

The report states that no stability analysis was performed because the Sponsor has provided documentation that the ETI-204 formulation is stable at the concentrations used on this study (study reference not provided).

Samples were stored refrigerated at 2 to 8°C until analysis. All analytical work was conducted by (b) (4) using an analytical method said to be developed and validated by that laboratory.

The report states that dose formulation analysis indicated animals were dosed with appropriate concentrations of the test article. No ETI-204 was measured in the vehicle samples. The appended analytical report indicates that dosing solution concentrations were near the expected concentrations.

## **Necropsy**

On GD 29, animals were euthanized by injection of sodium pentobarbital followed by exsanguination. Animals underwent caesarean section and ????. The left brain hemispheres of 5 pregnant animals/group were collected, fixed and sent for pathological examination.

No test article-related macroscopic findings were reported for female rabbits at terminal necropsy. Mild to moderate tan discoloration of liver was present in two females at 32 mg/kg/dose and was microscopically correlated to minimal to mild diffuse vacuolation of hepatocytes. This finding was considered to be secondary to pregnancy and unrelated to the test article.

Lung, liver, kidney, brain, and heart were weighed at terminal necropsy. No test article-related differences in the organ weights were reported.

No test article-related microscopic findings were reported in female rabbits at terminal necropsy. A variable degree of centrilobular, periportal, or diffuse vacuolation of hepatocytes was present in the liver in all groups, including the control group. There was no evidence of dose response reported, and the finding was considered to be secondary to pregnancy.

Microscopic evaluation of multiple sections of the left brain hemispheres revealed no microscopic change at any dose level. Brains of animals at both the low (16 mg/kg/dose) and high (32 mg/kg/dose) doses were considered to be microscopically normal, as were the brains of the control group (0 mg/kg/dose).

## **Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)**

No test article-related effects on uterine or ovarian parameters were reported. The pregnancy index was 86.4, 96.7, and 100% in the control, 16, and 32 mg/kg/dose groups, respectively. Three control animals and one 16 mg/kg/dose animal were not pregnant. One animal (animal number 181) in the 32 mg/kg/dose group delivered early on GD 28 after losing weight and eating significantly less than controls from GD 17-25. The report states that early deliveries have been observed in recent historical control data for this laboratory and this instance was not considered to be test article-related. However, this finding is similar to one 32 mg/kg female in the dose range-finding study; while the frequency may be within historical control values, it is unusual that it should be seen only in a high dose animal in both the dose range-finding and definitive embryo-fetal toxicity studies.

## **Offspring (Malformations, Variations, etc.)**

No test article-related effects on fetal sex ratio or on mean fetal body weights were reported. Mean fetal body weights were similar across groups. Three viable and two dead fetuses were evaluated from animal 181 at 32 mg/kg/dose that delivered early the morning of GD 28. All of these fetuses had low body weights (18.2-23.5 g). However, the mean fetal body weight for the high dose group was still not statistically different from control, so low fetal body weights in this litter were considered to be an isolated finding.

No test article-related effects on fetal external malformations or variations were reported. No fetal external malformations or variations were observed in the control or 32 mg/kg/dose group. An open eye (malformation) was observed in one fetus in the 16

mg/kg/dose group. This finding was not seen in the higher dose group, was within historical control experience, and was considered to be unrelated to test article.

No test article-related effects on fetal visceral malformations or variations were reported. There were several fetal visceral malformations (gall bladder absent, aortic arch and interventricular septum discontinuous, larger than normal heart, and transposition of the great vessels) observed in the treated groups, however, they were observed at a low incidence (one fetus) or were within the historical control range for the conducting laboratory, and were considered to be spontaneous and unrelated to treatment. The incidence of fetal visceral variations in the treated groups was low and either comparable to controls or within the historical control range for the conducting laboratory.

No test article-related effects on fetal skeletal malformations or variations were reported. The fetal skeletal malformations and variations observed in the treated groups were low in incidence (1-2 fetuses), not dose-dependent, or were similar to controls or historical control data for the conducting laboratory.

### 9.3 Prenatal and Postnatal Development

Not performed

## 10 Special Toxicology Studies

From Dr. Alexandra Worobec's review of the original IND 12285 submission:

1. Study no. AH001 ( (b)(4) study IM1082): Cross-reactivity study of Anthim™ with normal human tissues ( (b)(4) 12/13/2004) and

2. Study no. ARR003 ( (b)(4) study IM1083): Cross-reactivity study of Anthim™ with normal rat tissues ( (b)(4) 12/13/2004)

**Tissue Cross Reactivity:** The ATR has been observed to be expressed in a number of different tissues including the CNS, heart, lung, and lymphocytes. Tissue crossreactivity studies were conducted by the sponsor using human and 344 Fischer rat tissues. For the human tissue crossreactivity study ( (b)(4) Study No. IM1082, (b)(4) 12/13/2004), Anthim was applied to cryosections of normal human tissues (3 sources/tissue) at 2 concentrations: 10 µg/mL and 1 µg/mL (IND # 12285, Vol. 1.21, page 21-073). A (+) (anthrax protective antigen) and (-) control Ab (Ab H419E9: human PTHrP or hypercalcemia of malignancy peptide, N-terminal fragment, amino acid residues 1-34) was used and showed appropriate staining patterns (IND # 12285, Vol. 1.21, page 21-014). Anthim stained cytoplasmic filaments in a majority of tissues evaluated at the higher concentration of Ab (endothelium, smooth muscle (vascular or intrinsic), myofibroblasts, blood cells, macrophages, glia in the CNS, pia mater, perineurium, and epithelium (numerous subtypes). In a smaller subset of tissues, cytoplasmic granules reacted positively with Anthim. The only membranous staining noted (in the entire study) was for the visceral epithelial cell (VEC) of the kidney, at the higher concentration of Ab. For the rat tissue crossreactivity study ( (b)(4) Study No. IM1083, (b)(4) 12/13/2004), Anthim was applied

to normal rat (Harlan Fischer F344) tissues (2 sources/tissue—not always the same 2 animals) at 2 concentrations: 10 µg/mL and 1 µg/mL (IND # 12285, Vol. 1.21, page 21-073). A (+) (anthrax protective antigen) and (-) control Ab (Ab H419E9) was used and showed appropriate staining patterns. Grading of staining patterns was on a 0-4+ scale (0=none, 1+ = weak, 2+ = moderate, 3+ = strong, 4+ = intense (IND # 12285, Vol. 1.21, page 21-019). In the rat immunohistochemistry study, Anthim generally stained vascular smooth muscle in the adrenal, breast, cerebrum, cerebellum, eye, colon, esophagus, small intestine, stomach, heart, kidney, liver, lung, pancreas, peripheral nerve, prostate, salivary gland, skin, spinal cord, striated skeletal muscle, testis, thyroid, ureter, urinary bladder and cervix. In all these tissues staining was of the cytoplasmic filaments which were felt to represent a cytoskeletal or structural component of the cell, but no definitive identification of the component was made by the sponsor. The Ab also stained epithelial cells in the esophagus, stomach, kidney, pancreas, salivary gland, skin, and thymus—which was weak-strong staining of cytoplasmic granules (again, identity of these cellular structures is unknown) (IND # 12285, Vol. 1.21, page 21-073). In general, the findings in the rat immunohistochemistry study were not dissimilar from the findings noted in the human immunohistochemistry study, however a far fewer tissues were found to have cytoplasmic staining than in the human, at a lower intensity, and membrane staining of the visceral epithelial cell of the glomerulus was not noted in the rat study, as it was in the human. Findings of these studies are described in detail in Appendices 8.3 and 8.4 but are summarized in the following table (IND # 12285, Vol. 1.21, pages 21-010-21-068, 21-071-21-094):

Tissue	Human Tissue Crossreactivity (n=3 specimens)		344 Fischer Rat Tissue Crossreactivity (n=2 specimens)	
	(+) Crossreactivity at the 10 µg/mL conc. of PA mAb	(+) Crossreactivity at the 1 µg/mL conc. of PA mAb	(+) Crossreactivity at the 10 µg/mL conc. of PA mAb	(+) Crossreactivity at the 1 µg/mL conc. of PA mAb
*Adrenal: smooth muscle, vascular	3-4+ (rare-occas), n=3	(-)	1-2+ (occas), n=2	(-)
*Adrenal: endothelium	2-3+ (frequent), n=1	1+ (rare), n=1	(-)	(-)
*Adrenal: epithelium	1+ (frequent), n=1	(-)	(-)	(-)
# Blood cells: Neutrophils	1-2+ (rare), n=3	(-)	Not examined.	Not examined.
# Blood cells: Lymphocytes	2-3+ (freq), n=3	1+ (freq), n=1 1+ (rare), n=2	Not examined.	Not examined.
# Blood cells: Monocytes	2-3+ (freq), n=3	1+ (freq), n=1 1+ (rare), n=2	Not examined.	Not examined.
# Blood cells: Platelets	3-4+ (freq), n=3	1-2+ (freq), n=3	Not examined.	Not examined.

*Bone Marrow: smooth muscle	1-2+ (rare), n=1	1+ (rare), n=1	(-)	(-)
# Bone Marrow: hematopoietic cells	2-3+ (occas), n=1	(-)	(-)	(-)
*Brain (Cerebrum): endothelium	1+ (rare-occas), n=3	(-)	(-)	(-)
*Brain (Cerebrum): smooth muscle	2-3+ (rare- occas), n=3	(-)	1-3+ (occas), n=2	1+ (occas), n=2
*Brain (Cerebellum): Glia	1-2+ (rare-freq), n=2	(-)	(-)	(-)
*Brain (Cerebellum): Smooth muscle	2-3+ (rare), n=1	(-)	1-3+ (occas), n=2	1+ (occas), n=1
*Breast: endothelium	1-3+ (occas- freq), n=3	1+ (occas), n=1	(-)	(-)
* Breast: epithelium, glands and ducts	<b>2-4+ (freq), n=3</b>	1-2+ (occas), n=2	(-)	(-)
Breast: nipple skin and external duct	<b>3-4+ (freq), n=1</b>	2-3+ (occas), n=1	(-)	(-)
* Breast: smooth muscle, vascular and intrinsic	3-4+ (freq), n=1 1-2+ (occas), n=1	1-2+ (freq), n=1	1-2+ (occas), n=2	(-)
*Eye: smooth muscle, vascular and intrinsic	Not examined.	Not examined.	1-2+ (occas- freq), n=2	1-2+ (freq), n=1
*GI Tract: Colon: endothelium	1-2+ (occas), n=3	(-)	(-)	(-)
*GI Tract: Colon: smooth muscle, vascular, intrinsic	<b>2-4+ (freq), n=3</b>	1+ (occas), n=1	1-3+ (freq), n=2	(-)
*GI Tract: Colon: epithelium	1-3+ (freq), n=3	(-)	(-)	(-)
*GI Tract: Colon: GALT	2-3+ (freq), n=1	(-)	(-)	(-)
*Esophagus: endothelium	1-2+ (occas), n=3	(-)	(-)	(-)
*Esophagus: epithelium, submucosal glands	<b>3-4+ (freq), n=3</b>	1-2+ (rare- occas), n=3	(-)	(-)
*Esophagus: epithelium, surface, basal layers	1-2+ (occas), n=3	(-)	1-3+ (occas), n=1	(-)
*Esophagus: smooth muscle,	2-4+ (occas), n=3	1-2+ (rare- occas), n=1	2-3+ (occas), n=1	(-)

vascular and intrinsic				
<b>*Esophagus:</b> mononuclear cells, submucosal lymphoid nodules	2-3+ (occas), n=1 1-2+ (occas), n=1 1+ (rare), n=1	(-)	(-)	(-)
<b>*GI Tract: Small intestine:</b> endothelium	1-2+ (freq), n=3	1-2+ (occas), n=3	(-)	(-)
<b>*GI Tract: Small intestine:</b> epithelium	<b>3-4+ (freq), n=2</b>	1-2+ (freq), n=2	(-)	(-)
<b>*GI Tract: Small intestine:</b> myofibroblasts, lamina propria	<b>2-4 + (freq), n=3</b>	1-3+ (freq), n=3	(-)	(-)
<b>*GI Tract: Small intestine:</b> smooth muscle, vascular and intrinsic	<b>3-4+ (freq), n=3</b>	1-3+ (freq), n=3	1-2+ (occas), n=2	(-)
<b>*GI Tract: Small intestine:</b> GALT	2-3+ (freq), n=1 1-2+ (occas), n=1	1+ (occas-freq), n=2	(-)	(-)
<b>*GI Tract: Stomach:</b> endothelium	<b>2-3 + (freq), n=3</b>	1-2+ (occas), n=3	(-)	(-)
<b>*GI Tract: Stomach:</b> epithelium	1-3+ (freq), n=3	1+ (occas), n=3	1-3+ (freq), n=2	(-)
<b>*GI Tract: Stomach:</b> smooth muscle, vascular and intrinsic	<b>3-4+ (freq), n=3</b>	2-3+ (freq), n=3	1-2+ (freq), n=2	(-)
<b>*GI Tract: Stomach:</b> GALT	2-3+ (freq), n=1	1+ (occas), n=1	(-)	(-)
<b>*Heart:</b> smooth, muscle, vascular	<b>3-4+ (freq), n=3</b>	(-)	1+ (rare), n=1	(-)
<b>*Heart:</b> endothelium	1-2+ (occas), n=2	(-)	(-)	(-)
<b>*Kidney:</b> endothelium, glomerulus, and interstitial	2-3+ (freq), n=3	1+ (rare), n=2	(-)	(-)
<b>*Kidney:</b> mesangium,	2-3+ (freq), n=1	(-)	(-)	(-)

glomerulus				
<b>*Kidney:</b> smooth muscle, vascular	<b>3-4+ (freq), n=3</b>	(-)	1-2+ (occas), n=2	(-)
<b>Kidney:</b> epithelium, visceral, glomerulus ( <b>membrane staining</b> )	2-3+ (freq), n=3	(-)	(-)	(-)
<b>*Kidney:</b> epithelium, parietal, glomerulus	2-3+ (freq), n=3	(-)	(-)	(-)
<b>*Kidney:</b> epithelium, tubules (collecting ducts/Henle's loop)	<b>3-4+ (occas), n=2</b> 1-2+ (occas), n=1	1-3+ (rare), n=2	1+ (occas), n=1	(-)
<b>*Liver:</b> endothelium	2-3+ (freq), n=1 1-2+ (occas), n=2	1-2+ (occas), n=2	1-2+ (rare-occas), n=2	(-)
<b>*Liver:</b> epithelium, biliary	<b>3-4+ (freq), n=3</b>	1-2+ (freq), n=1	(-)	(-)
<b>*Liver:</b> smooth muscle, vascular	<b>3-4+ (occas), n=3</b>	1-2+ (rare), n=1	2-3+ (freq), n=1 1+ (occas), n=1	2-3+ (occas), n=1
<b>*Liver:</b> hepatocytes	2-3+ (freq), n=1 1-2+ (freq), n=1 1+ (rare-occas), n=1	1+ (occas), n=1	(-)	(-)
<b>*Liver:</b> Kupffer cells	<b>2-4+ (occas-freq), n=3</b>	2-3+ (freq), n=1	1-2+ (rare-occas), n=1	(-)
<b>* Lung:</b> endothelium	1-2+ (occas-freq), n=3	1+ (occas), n=1	(-)	(-)
<b>* Lung:</b> alveolar MØs	<b>2-4+ (freq), n=3</b>	1-3+ (occas-freq), n=2	(-)	(-)
<b>* Lung:</b> smooth muscle, vascular	2-4+ (occas-freq), n=3	(-)	2-3+ (freq), n=1	1-2+ (occas), n=1
<b>* Lung:</b> epithelium, alveolar	1-2+ (occas), n=2	1-2+ (occas), n=2	(-)	(-)
<b>*Lymph node:</b> high endothelial venules	<b>3-4+ (freq), n=2</b> 2-3+ (occas), n=1	1-2+ (occas), n=3	(-), 1 missing specimen.	(-), 1 missing specimen.
<b>*Lymph node:</b> endothelium, blood vessels	2-3+ (freq), n=1 1-2+ (occas), n=1	1-2+ (occas), n=2	(-), 1 missing specimen.	(-), 1 missing specimen.
<b>*Lymph node:</b> smooth muscle, vascular	<b>3-4+ (freq), n=2</b>	1-3+ (occas), n=2	(-), 1 missing specimen.	(-), 1 missing specimen.

* <b>Lymph node:</b> lymphocytes	2-3+ (freq), n=2	1-2+ (freq), n=1	(-), 1 missing specimen.	(-), 1 missing specimen.
* <b>Lymph node:</b> MØs, medullary	1+ (freq), n=1	(-)	(-), 1 missing specimen.	(-), 1 missing specimen.
* <b>Ovary:</b> endothelium	1-2+ (freq), n=2	1+ (occas), n=2	(-)	(-)
* <b>Ovary:</b> epithelium, inclusion cysts	2-3+ (freq), n=1	(-)	(-)	(-)
* <b>Ovary:</b> smooth muscle, vascular	2-3+ (freq), n=2	(-)	(-)	(-)
* <b>Ovary:</b> granulosa- theca cells	2-3+ (freq), n=1	1+ (occas), n=1	(-)	(-)
* <b>Fallopian tube:</b> endothelium	1-2+ (occas), n=3	1+ (rare-occas), n=2	(-)	(-)
* <b>Fallopian tube:</b> smooth muscle, vascular and intrinsic	<b>3-4+ (freq), n=3</b>	(-)	(-)	(-)
* <b>Fallopian tube:</b> epithelium	<b>2-4+ (freq), n=3</b>	1-2+ (occas- freq), n=2	(-)	(-)
* <b>Pancreas:</b> endothelium	1-3+ (freq), n=3	1+ (occas), n=3	(-)	(-)
* <b>Pancreas:</b> smooth muscle, vascular	<b>3-4+ (freq), n=3</b>	1-3+ (occas), n=3	1+ (occas), n=2	(-)
* <b>Pancreas:</b> epithelium, ducts	<b>2-4+ (freq), n=3</b>	1-3+ (occas), n=3	(-)	(-)
* <b>Pancreas:</b> epithelium, acinar	1-3+ (freq), n=3	1+ (occas-freq), n=2	1+ (freq), n=1	(-)
* <b>Parathyroid:</b> chief cells	1-2+ (occas), n=2	(-)	2 missing specimens	2 missing specimens
* <b>Parathyroid:</b> endothelium	2-3+ (occas), n=1	(-)	2 missing specimens	2 missing specimens
* <b>Peripheral nerve:</b> endothelium	1-2+ (occas), n=3	(-)	(-)	(-)
* <b>Peripheral nerve:</b> smooth muscle, vascular	2-3+ (freq), n=2	1-2+ (rare), n=2	2-3+ (freq), n=1 1+ (rare), n=1	(-)
* <b>Peripheral nerve:</b> perineurium	1-2+ (occas), n=2	(-)	(-)	(-)
# <b>Pituitary:</b> epithelium, Rathke's pouch	<b>3-4+ (occas- freq), n=3</b>	1-3+ (occas), n=3	(-)	(-)
* <b>Pituitary:</b> epithelium, anterior pituitary	1-2+ (rare- occas), n=3	(-)	(-)	(-)
* <b>Placenta:</b>	1+ (rare), n=1	(-)	(-)	(-)

endothelium				
<b>*Placenta:</b> trophoblasts, chorionic plate	2-3+ (freq), n=2	1+ (rare-occas), n=2	(-)	(-)
<b>*Placenta:</b> trophoblasts, chorionic villi	1-3+ (rare- occas), n=2	(-)	(-)	(-)
<b>*Prostate:</b> epithelium, acinar	1-4+ (occas), n=3	2-3+ (rare- occas), n=1	(-)	(-)
<b>*Prostate:</b> smooth muscle, vascular	<b>2-4+ (freq), n=2</b> 1-2+ (occas), n=1	1+ (rare-occas), n=2	1+ (occas), n=1	1+ (occas), n=1
<b>*Salivary gland:</b> endothelium	1-2+ (freq), n=3	1+ (freq), n=3	1+ (occas), n=1	(-)
<b>#Salivary gland:</b> epithelium, acinar, duct	2-3+ (freq), n=3	1-2+ (freq), n=3	1-2+ (occas), n=2	1-2+ (occas), n=2
<b>*Salivary gland:</b> myoepithelium	<b>3-4+ (freq), n=3</b>	2-3+ (occas- freq), n=3	(-)	(-)
<b>*Salivary gland:</b> smooth muscle, vascular and intrinsic	<b>3-4+ (freq), n=3</b>	2-3+ (occas- freq), n=3	<b>2-3+ (freq), n=2</b>	1+ (occas), n=2
<b>*Skin:</b> endothelium	1-3+ (freq), n=3	1-2+ (occas- freq), n=3	(-)	(-)
<b>*Skin:</b> epithelium, surface, sebaceous/apocrine glands and follicles	<b>3-4+ (freq), n=3</b>	1-3+ (freq), n=3	<b>2-3+ (freq), n=1</b> 1-2+ (occas), n=1	1-2+ (occas), n=2
<b>*Skin:</b> smooth muscle, vascular and intrinsic	<b>3-4+ (freq), n=3</b>	1-3+ (occas- freq), n=3	1-2+ (occas), n=1	(-)
<b>*Spinal cord:</b> endothelium	<b>2-3+ (freq), n=3</b>	<b>1-2+ (freq), n=3</b>	(-)	(-)
<b>*Spinal cord:</b> pia mater	1-2+ (freq), n=3	1+ (occas), n=3	(-)	(-)
<b>*Spinal cord:</b> perineurium, spinal nerves	1+ (occas), n=3	1+ (occas), n=3	(-)	(-)
<b>*Spinal cord:</b> neuropil, grey matter >> white matter	2-3+ (freq), n=3	1+ (freq), n=3	(-)	(-)
<b>*Spinal cord:</b> smooth muscle,	<b>3-4+ (freq), n=3</b>	<b>2-3+ (freq), n=3</b>	1+ (occas), n=1	(-)

vascular				
<b>*Spleen:</b> endothelium	1-2+ (freq), n=3	1+ (occas), n=3	(-)	(-)
<b>*Spleen:</b> lymphocytes, follicles, and PALS	1+ (freq), n=3	1+ (occas), n=3	(-)	(-)
<b>*Spleen:</b> RES, red pulp	<b>3-4+ (freq), n=3</b>	1-3+ (freq), n=3	(-)	(-)
<b>*Spleen:</b> sm muscle, vascular, intrinsic	<b>3-4+ (freq), n=3</b>	1-2+ (occas), n=3	(-)	(-)
<b>*Striated muscle:</b> endothelium	1-2+ (occas), n=3	(-)	(-)	(-)
<b>*Striated muscle:</b> smooth muscle, vascular	2-3+ (freq), n=3	1-2+ (occas), n=3	2-3+ (freq), n=2	1+ (rare-occas), n=2
<b>*Striated muscle:</b> tissue MØs	<b>3-4+ (occas), n=1</b>	(-)	(-)	(-)
<b>*Testis:</b> endothelium	1-2+ (occas), n=3	1+ (occas), n=3	(-)	(-)
<b>*Testis:</b> lining cells, seminiferous tubules	1-2+ (rare- occas), n=3	(-)	(-)	(-)
<b>*Testis:</b> epithelium, rete testis	1-2+ (freq), n=1	1+ (freq), n=1	(-)	(-)
<b>*Testis:</b> Leydig cells	1-2+ (freq), n=3	1+ (occas), n=3	(-)	(-)
<b>*Testis:</b> smooth muscle, vascular and intrinsic, incl. peritubular	<b>3-4+ (freq), n=3</b>	<b>2-3+ (freq), n=3</b>	1-2+ (occas), n=1	(-)
<b>*Thymus:</b> endothelium	1-2+ (occas), n=3	1+ (occas), n=3	(-)	(-)
<b>*Thymus:</b> squamous epithelium, Hassall's corpuscles	<b>3-4+ (freq), n=1</b>	1-2+ (freq), n=1	2-3+ (freq), n=2	(-)
<b>#Thymus:</b> lymphocytes, medulla >> cortex	2-3+ (freq), n=2 1-2+ (occas), n=1	1-2+ (rare- occas), n=3	(-)	(-)
<b>*Thymus:</b> smooth muscle, vascular	<b>3-4+ (freq), n=3</b>	1-2+ (freq), n=3	(-)	(-)
<b>*Thyroid:</b> endothelium	1-2+ (freq), n=3	1-2+ (occas), n=3	(-)	(-)
<b>#Thyroid:</b> follicular	<b>3-4+ (freq), n=3</b>	<b>2-3+ (freq), n=3</b>	(-)	(-)

epithelium				
<b>#Thyroid:</b> C-cells	1-2+ (freq), n=3	1+ (freq), n=3	(-)	(-)
<b>*Thyroid:</b> smooth muscle, vascular	<b>3-4+ (freq), n=3</b>	<b>2-3+ (freq), n=3</b>	1+ (rare), n=1	(-)
<b>*Tonsil:</b> endothelium	1-2+ (freq), n=3	1-2+ (occas), n=2	Not examined.	Not examined.
<b>#Tonsil:</b> lymphocytes	1-2+ (occas), n=3	1+ (occas), n=2	Not examined.	Not examined.
<b>*Tonsil:</b> lymphoepithelium	2-3+ (freq), n=3	1+ (occas-freq), n=3	Not examined.	Not examined.
<b>*Tonsil:</b> smooth muscle, vascular and intrinsic	<b>3-4+ (freq), n=3</b>	1-3+ (freq), n=3	Not examined.	Not examined.
<b>*Ureter:</b> endothelium	<b>2-3+ (freq), n=3</b>	1-2+ (occas), n=3	(-)	(-)
<b>*Ureter:</b> epithelium	<b>2-4+ (freq), n=3</b>	1-2+ (occas), n=3	(-)	(-)
<b>*Ureter:</b> smooth muscle, vascular and intrinsic	<b>3-4+ (freq), n=3</b>	<b>2-3+ (freq), n=3</b>	<b>1-3+ (freq), n=2</b>	(-)
<b>*Urinary bladder:</b> epithelium	<b>3-4+ (occas-freq), n=2,</b> 1-2+ (rare), n=1	2-3+ (rare), n=1 1+ (occas), n=1	(-)	(-)
<b>*Urinary bladder:</b> smooth muscle, vascular and intrinsic	2-3+ (freq), n=1 1-3+ (occas), n=2	(-)	1-2+ (occas-freq), n=3 (3 specimens examined here).	1+ (occas), n=1
<b>*Urinary bladder:</b> endothelium	2-3+ (freq), n=1 1+ (rare-occas), n=2	(-)	(-)	(-)
<b>*Uterus (Endometrium):</b> endothelium	2-3+ (freq), n=3	1-2+ (occas), n=3	(-)	(-)
<b>*Uterus (Endometrium):</b> epithelium	<b>3-4+ (freq), n=1</b> 2 missing specimens	1-2+ (occas), n=1 2 missing specimens	(-)	(-)
<b>*Uterus (Endometrium):</b> smooth muscle, vascular and intrinsic	2-3+ (freq), n=3	1+ (occas-freq), n=3	(-)	(-)
<b>*Uterus (Cervix):</b> endothelium	1-3+ (rare-occas), n=3	(-)	1-2+ (rare), n=1	(-)
<b>Uterus (Cervix):</b> epithelium, internal	<b>3-4+ (freq), n=1</b>	1-2+ (occas), n=1	(-)	(-)

OS				
<b>*Uterus (Cervix):</b> epithelium, external OS	1-2+ (occas), n=1	1+ (rare), n=1	(-)	(-)
<b>*Uterus (Cervix):</b> smooth muscle, intrinsic	1+ (occas), n=1	(-)	(-)	(-)

**\*Indicates cytoplasmic filaments were (+) under immunohistochemical staining.**  
**# Indicates that cytoplasmic granules were (+) under immunohistochemical staining.**

The following three reports were originally submitted to IND 12285, Serial no. 006 (b) (4). There does not appear to be any written review of these nonclinical studies, and they are reviewed here by the current reviewer:

**3. (b) (4) study no. IM1215: Cross reactivity study of AnthIM™ with normal human, rat and cynomolgus monkey tissues.**

In this GLP-compliant study, test article was applied to tissues at concentrations of 1 or 10 µg/mL. In tissues from all three species, human, Fischer 344 rats, and cynomolgus monkey, the test article was said to stain various cell types in most tissues examined. The tissue cross-reactivity profiles were said to be “very similar” for all three species in terms of histologic nature, subcellular and histologic patterns, intensity, frequency, and affinity of staining. The report states that in all cases, staining was cytoplasmic and was specifically localized to cytoplasmic granules and/or filaments, often associated with cell borders. While it was unknown with what tissue structures, the test article was reacting, the report states that the pattern of staining was suggestive of intracellular cytoskeletal elements. No membrane staining was reported. Positive and negative controls reacted as expected. The study and peer review pathologists stated that the data demonstrated that both the rat and cynomolgus monkey were appropriate and relevant models for toxicity testing of ETI-204. They also state that, although the potential clinical and toxicological significance of the binding was not determined, it is unlikely that cytoplasmic structures would be accessible *in vivo*.

*Reviewer’s comment: In reference to the concentrations used in tissue cross-reactivity (TCR) studies, an earlier pre-EUA submission stated, “These concentrations are much lower than those seen with human doses of 4 to 16 mg/kg.” It is unclear whether or not use of higher concentrations would have been feasible in TCR studies, but this does bring into question the relevance of these studies to actual clinical exposures.*

**4. (b) (4) study no. IM1219: Processing and detection of test article in support of (b) (4) study no 03553 entitled “Repeat-dose intravenous and intramuscular toxicity of antibody (b) (4) in male Fischer 344 rats”**

In this GLP-compliant follow-up study, immunohistochemical staining was performed to detect antibody (b) (4) in tissues from animals in the general toxicology study submitted to the original IND submission. The reactions in positive and negative control materials and tissues were said to indicate that the assay was specific for detection of the test article (b) (4) (AnthIM™) in paraffin tissue sections from the rat toxicology study. Lungs, kidneys, heart, liver, mandibular salivary glands, eyes, skin, spleen, colon, cerebellum, skeletal muscle (biceps femoris), and sciatic nerves were examined. No staining was observed in tissues from vehicle-treated animals. Staining was observed in most tissues from all three (b) (4)-treated rats that were examined. The most frequent observation was staining of intravascular protein (interpreted as (b) (4)-containing serum) in all organs. In general, staining was limited to the intravascular spaces or limited local staining of the interstitial fluid compartment, with no apparent binding to any cellular or structural tissue element. There was one exception: Within the kidney of one treated animal, there was staining of material within Bowman’s space indicating movement of degraded test article across the glomerulus. Membrane or intracellular staining was not observed in any organ or tissue.

Although not submitted to this BLA, a report of a Genebank search for potential Anthim cross-reactive epitopes was provided in serial no. 006, in response to a request from Dr. Worobec:

(b) (4)

The following study was submitted to IND 12285, Serial no. 016 (meeting briefing package). The nonclinical studies was not previously reviewed, but is reviewed here by the current reviewer:

One of the topics for discussion at this meeting was a manufacturing change in which a new production cell line (b) (4) was introduced to replace the low-producing research cell line (b) (4) previously used.

**5. (b) (4) study no. IM1368: Limited human tissue cross-reactivity study with (b) (4) and (b) (4)**

This GLP-compliant study was conducted in order to evaluate the potential cross-reactivity of (b) (4) and (b) (4) antibodies and to demonstrate that the staining for both antibodies would be the same as that previously seen with (b) (4) and submitted to the Agency. Results for positive and negative controls were as expected. Both antibodies stained endothelial cells (localized to cytoplasmic granules), structural and contractile cells (limited to cytoplasmic granules and filaments, depending on cell type), epithelial cells (localized to cytoplasmic granules), neural elements, and blood cells and lymphocytes (localized to cytoplasmic granules). The TCR profiles for both (b) (4) and (b) (4) were said to be “very similar” in terms of histologic nature, subcellular and histologic patterns, intensity, and frequency of staining, as well as the concentrations of antibody at which staining was observed. The profiles were said to be consistent with that seen in previous TCR studies of (b) (4). Staining was reported to be limited to the cytoplasm, and no membrane staining was reported in any tissue.

Neurotoxicity assessment

**6. Report no 358-0006: Combined review of neuropathology data to determine the potential brain toxicity of ETI-204: review of multiple studies in an inhalational *Bacillus anthracis* exposure therapeutic model and a single study using intravenous dosing (without *B. anthracis* exposure)**

This report was prepared by (b) (4) and was based on the following studies:

SPONSOR AP201/ (b) (4) 834-G924202  
 SPONSOR AP204/ (b) (4) 1121-G924204  
 SPONSOR AP203/ (b) (4) 1219-100005989  
 SPONSOR AR033/ (b) (4) 1185-100003006  
 SPONSOR AR0315/ (b) (4) 1142-G924203  
 SPONSOR AR028/ (b) (4) 2395-100008193  
 SPONSOR EFT001, PHASE B/ (b) (4) 1984-005

Evaluation for neuropathological changes was performed on tissue from 3 studies in infected rabbits, one non-infected study in rabbits, and 3 studies in infected monkeys. These were single dose studies in which the highest test article doses ranged from 8-32 mg/kg. A comprehensive examination of brain and meninges, including the pia and arachnoid layers of overlying meninges, ventricular system, cerebral cortex/neocortex, basal nuclei/striatum, thalamus/hypothalamus, midbrain, pons/pontine nuclei, limbic system/hippocampus, cerebellum, and medulla oblongata was performed. For each brain, 15 (monkeys) or 8 (rabbits) coronal sections were embedded and cut at approximately 5 microns. H&E stained sections were examined by two separate reviewing pathologists (at least one was blinded to treatment groups), with their combined consensus being reported.

Studies AP201, AP203, and AP204 were GLP-compliant trigger-to treat studies of IV ETI-204 treatment of cynomolgus monkeys in a model of inhalation anthrax.

Brains from all animals in AP201 and AP204 were evaluated. For AP203, brains of all survivors were evaluated, but non-survivors with treatment exposure of less than 12 hours were excluded, and only five animals were randomly selected from each group for neuropathological assessment.

Study AR033 was a GLP-compliant trigger-to treat study of IV ETI-204 treatment of rabbits in a model of inhalation anthrax. Three survivors and three non-survivors from each group were chosen for evaluation, but since there were no survivors in the control group, only three nonsurvivors were evaluated from that group. All animals chosen were required to be positive for bacteremia prior to treatment.

Study AR315 was a non-GLP post-exposure prophylaxis study of IM ETI-204 administration in the rabbit model of inhalation anthrax. Brains from all animals were evaluated.

Study AR028 was a non-GLP study of combined delayed treatment with levofloxacin and ETI-204. Four animals from the saline control group, 4 survivors from each of the levofloxacin and levofloxacin + ETI-204 groups, and 8 non-survivors from each of the levofloxacin and levofloxacin + ETI-204 groups were evaluated. Evaluated animals chosen based on bacteremia, duration of treatment exposure, and, for control animals, evidence of bacterial infection in the brain.

Study EFT001 was a GLP-compliant embryo-fetal toxicity study in non-infected rabbits. Animals were administered 4 doses at approximately 4-day intervals, i.e. on GD 6, 10, 13 and of 16 or 32 mg/kg. The report for that study states that left brain hemispheres from 5 does per treatment group were evaluated.

The numbers of animals evaluated at the respective doses are shown in the Applicant's table below:

**Total Animals Examined at <sup>(b) (4)</sup> (by Species) in Various Treatment Groups**

<b>Primates</b>				
Treatment	Total Animals	Survivors	Non-Survivors	Total
Saline	37	5	32	37 (saline)
4 mg/kg ETI-204	30	15	15	78 (test article) 41 survivors 37 non-surv.
8 mg/kg ETI-204	21	12	9	
16 mg/kg ETI-204	16	8	8	
32 mg/kg ETI-204	11	6	5	
<b>Rabbits</b>				
Saline	17	0	17	29 (no test article) 4 survivors (all levofloxacin) 25 non-surv.
Levofloxacin only	12	4	8	
1 mg/kg ETI-204	6	3	3	84 (test article) 51 survivors 33 non-surv.
4 mg/kg ETI-204	30	19	11	
8 mg/kg ETI-204	6	3	3	
16 mg/kg ETI-204	30	22	8	
16 mg/kg ETI-204 + Levofloxacin	12	4	8	

The following is excerpted from the pathologist's report:

“In primates and rabbits exposed to inhalational anthrax *that did not survive* (found dead or moribund sacrificed animals), administration of ETI-204 at doses at and above 4 mg/kg is associated with an increased incidence (frequency) of histological findings, consistent with a severe acute inflammatory response and characterized by extravascular bacteria (meninges/brain), inflammation (meninges/brain), hemorrhage (meninges/brain), and occasionally necrosis (brain), as compared to the untreated, challenged control animals. The effect is most pronounced in monkeys (where the effect is consistently statistically significant; Fisher's Exact Test;  $p < 0.05$ ) but is also present in rabbits. Similar changes were not noted at the 1 mg/kg dose level in the single rabbit study (AR033) included in this report.

The changes in the non-survivors, including those treated with only saline, with ETI-204, or with levofloxacin, were consistent with morphologic lesions/hemorrhagic meningoencephalitis previously reported in monkeys and rabbits with inhalation anthrax (Fritz, 1995; Henning, 2012; Twenhafel, 2007; Vasconcelos, 2003; Zaucha, 1998).

Biologically significant reactions (hemorrhage, inflammation, necrosis) in non-survivors were clearly associated with the presence of extravascular bacteria in all dose groups, including saline controls. The occurrence of an acute inflammatory response in the ETI-204 treated non-survivors did not exhibit a dose response relationship (i.e.,

changes were not more pronounced at higher doses), and administration of the ETI-204 was not associated with any biologically significant morphologic reactions in surviving animals exposed to inhaled *B. anthracis* or in rabbits not exposed to anthrax spores given up to 32 mg/kg intravenous ETI-204 (EFT001).”

The report discusses findings in published studies of inhalation anthrax in animal models. The state that Zaucha, et al. (1998) describe hemorrhage as the only finding in the brain/meninges in rabbits (in 4 of 22 animals; the number of brain sections examined was not provided). It was also stated that there was less leukocytic infiltration in the brain and meningeal lesions in rabbits relative to that described in reported cases of inhalational anthrax in humans. The report also cites Vasconcelos et al. (2003) as describing CNS findings in 14 monkeys with inhalational anthrax. Those findings were characterized by extravascular bacteria, necrotizing vasculitis, meningeal hemorrhage, parenchymal hemorrhage, meningitis, and leukomalacia. The report includes the following table, comparing findings in the latter study to those in studies AP201 and AP204:

**Comparison of Published Morphologic Changes in the Brain of monkeys with inhalational anthrax (Vasconcelos, 2003) to morphological changes in Study AP201/834- G924202 and Study AP204/1121-G924204**

Diagnosis	Percentage Published Study (Vasconcelos, 2003)	Study AP201 Controls (%)	Study AP204 Controls (%)	Study AP201, 4.0 mg/kg ETI-204 (%)	Study AP204, 4.0 mg/kg ETI-204 (%)	Study AP201, 8.0 mg/kg ETI-204 (%)	Study AP201, 16.0 mg/kg ETI-204 (%)
Bacteria, extravascular (Meninges, Bacteria) <sup>2</sup>	71%	50%	19%	21%	63%	27%	44%
Necrotizing vasculitis (Meninges, Vasculitis)	14%	7%	13%	14%	56%	20%	44%
Hemorrhage, meningeal (Meninges, Hemorrhage)	57%	7%	19%	14%	69%	27%	38%
Hemorrhage, parenchymal (Hemorrhage, brain, any area)	29%	7%	13%	14%	56%	20%	38%
Meningitis, suppurative (Meninges, Inflammation)	21%	7%	13%	14%	63%	20%	44%
Leukomalacia (Molecular Layer, Vacuoles, brain, any area)	14%	0%	0%	0%	6%	7%	6%
(Brain, Perivascular Inflammation) <sup>1</sup>	--	21%	13%	14%	56%	33%	44%
(Brain, Vasculitis) <sup>1</sup>	--	0%	13%	14%	50%	20%	44%
Overall mortality	100%	86% 7/14 FD 5/14 MS 2/14 SS	94% 9/16 FD 6/16 MS 1/16 SS	21% 2/14 FD 1/14 MS 11/14 SS	75% 7/16 FD 5/16 MS 4/16 SS	27% 1/15 FD 3/15 MS 11/15 SS	50% 3/16 FD 5/16 MS 8/16 SS

<sup>1</sup>Diagnosis not used in the published study

<sup>2</sup>For study AP201 only, this diagnosis includes animals with intravascular and extravascular bacteria. For study AP204, this includes only extravascular bacteria.

The percentages reflect males and female data combined.

FD=found dead; MS=moribund sacrifice; SS=scheduled sacrifice (survivor)

In general, survivors either had no lesions or incidental changes not considered to be related to infection or treatment. Some of non-survivors had no neurological findings and were believed to have died before the development of a bacteremia. Some non-survivors had only intravascular bacteria and slight to mild hemorrhage; these were not considered to have morphologic lesions in the brain of biological significance. Remaining non-survivors had biologically significant findings including extravascular bacteria and prominent inflammation, hemorrhage, and/or necrosis, consistent with anthrax infection. In the one included study in rabbits not exposed to anthrax (EFT001), there were no morphologic changes in the brain at either the 16 mg/kg or the 32 mg/kg dose.

The report cites similarity of these findings to findings with raxibacumab and with AVP-21D9. It notes that significant higher grade biological reaction was primarily associated with the presence of extravascular bacteria and that similar findings were not found in survivors or in un-infected animals.

*Reviewer's comment: These findings appear to be suggestive of the inability of antibody to cross the blood-brain barrier prior to CNS involvement.*

## 11 Integrated Summary and Safety Evaluation

### Pharmacodynamics studies:

GLP-compliant studies in NZW rabbits and cynomolgus macaques were performed to evaluate the efficacy of ETI-204 in animal models of inhalation anthrax alone or in combination with levofloxacin. After inhalation exposure to 200 LD<sub>50</sub> equivalents of B. anthracis spores, animals were monitored for a trigger to treat, significant increase in body temperature, presence of anthrax PA in blood using an electrochemiluminescence assay, or after a defined elapsed time after exposure. Treatment with saline or ETI-204 was initiated, using doses ranging from 1-16 mg/kg in rabbits, and 4-32 mg/kg in cynomolgus monkeys. Survival varied and was not always dose-dependent. Survival in control animals in some studies confounded results, but most studies showed significant increase in survival in treated animals over controls. Pathology and neuropathology of animals that did not survive was consistent with anthrax in the respective species. The neuropathologist noted that pathologic changes in the brain indicating a response by the host animal (principally inflammation, including vasculitis and hemorrhage) were, in general, more pronounced in the test article treated animals as compared to the saline treated controls in studies in cynomolgus monkeys. Other findings were considered to be incidental and did not appear to be related to ETI-204 treatment. One study in cynomolgus monkeys compared Baxter and Lonza product and found similar results in the two groups.

It should be noted that, in efficacy studies in animal models, the control groups did not always exhibit 100% mortality. Some infected animals died with no lesions, which may not be consistent with referenced articles describing LD<sub>50</sub> anthrax exposures and typical anthrax lesions in test species. Not all studies demonstrated a dose-related trend in survival or a significant difference in survival between control and ETI-204-treated animals.

Safety pharmacology studies:

Two safety pharmacology studies were conducted to evaluate cardiovascular function in cynomolgus monkeys. In the first study, compound-related elevation in blood pressure at 2 and 4 hours after IV (5 mg/kg) and IM (5 or 10 mg/kg) administration was seen, as well as apparent increase in QT interval on ECG. Anti-drug antibodies were noted.

In the second study, following doses of 10 or 30 mg/kg IV, the changes in blood pressure were not seen; blood pressures were in the normal range for this species. No QT prolongation was reported.

In Study no. AP204, a trigger-to-treat study in cynomolgus monkeys challenged with *B. anthracis* spores and administered ETI-204 at 4 and 16 mg/kg via a single IV bolus dose, neurobehavioral examinations were conducted by a veterinarian on all monkeys pre-test and on all surviving monkeys at Days 28 and 56 post-challenge. No treatment-related effects were reported on behavior, head posture, coordination, oculomotor nerve function, range of motion, extensor-flexor function, deep pain, response to noxious stimuli, Babinski reflex, superficial sensation, anal reflex, and assessment of muscle atrophy.

General toxicology studies:

The second safety pharmacology study in cynomolgus monkeys included limited general toxicology evaluation of test article generated in the (b) (4) cell line, but did not include pathological evaluation. The NOAEL was 30 mg/kg, which resulted in an AUC of approximately 150,000 µg·hr/mL.

General toxicology studies were performed in rats. The initial study no. ARR002 ((b) (4) study no. 03553) was performed in 10-13 week old male Fischer 344 rats at repeated IV and IM doses up to 2.91 mg/rat (approximately 10.6 mg/kg) of the (b) (4) antibody. Doses were administered on Days 1, 4, and 7, with sacrifice on Day 10/11. No test article-related findings were reported for mortality, clinical signs, hematology, clinical chemistry, organ weights, or gross or microscopic pathology. The NOAEL was determined to be the high dose, 10.6 mg/kg.

Pilot and definitive GLP-compliant toxicology studies were performed in Sprague-Dawley rats. In the pilot study, doses of vehicle (saline), 10, 30, or 100 mg/kg were administered by slow IV bolus injection on Days 1, 4, and 7 to five male rats per group. No effects were noted on survival, clinical observations, body weights, clinical pathology or gross pathology. The study concluded that the maximum tolerated dose had not yet been reached. In the definitive study, 8 rats/sex/group were administered vehicle (saline), 3, 10, or 30 mg/kg by IV injection on Days 1, 4, and 7. The test article was the new material manufactured by Lonza, and this study included an additional high dose (30 mg/kg) group utilizing material manufactured by Baxter in order to compare material made by the two manufacturers. Additional satellite groups were used for pharmacokinetics. No test article-related findings were reported in clinical observations, body weights, food consumption, clinical pathology, organ weights, or macroscopic or microscopic pathology. While no differences between material made by the two manufacturers were reported in the toxicologic or toxicokinetic profiles, the report did note increased variability in Cmax with the Lonza material, resulting in a lower Cmax

than that seen for the Baxter material. The NOAEL was the high dose, 30 mg/kg, for both the Lonza and Baxter material ( $AUC_{0-\infty} = 2130-2240 \mu\text{g}\cdot\text{day}/\text{mL}$ ).

#### Developmental and reproductive toxicology:

In a developmental toxicity study in rabbits, two treatment groups of 30 time-mated female New Zealand White Hra:(NZW)SPF rabbits/group were administered ETI-204 at doses of 16 or 32 mg/kg/dose, respectively. A vehicle control group of 22 time-mated females were administered sterile 0.9% Sodium Chloride for Injection, USP. The vehicle or ETI-204 was administered to all groups via intravenous (IV) injection on Gestation Day (GD) 6, 10, 13, and 17, at a dose volume of 1 mL/kg/dose. All but one high dose animal that received the full dosing regimen survived to terminal euthanasia on GD 29. That animal exhibited inappetance and body weight loss and delivered early on GD 28, similar to one animal in the pilot study that received 32 mg/kg on GD 13 and 17. Since this was an isolated occurrence, it was not considered to be test article-related. The No-Observed-Effect Level (NOEL) of ETI-204 for maternal and developmental toxicity was determined to be 32 mg/kg/dose, the highest dose tested, with an associated  $AUC_{0-\infty}$  of  $3220 \pm 638 \mu\text{g}\cdot\text{day}/\text{mL}$ . The report states that systemic exposure associated with this dose in the pilot study was approximately four times higher than that of healthy human subjects administered an IV dose of 8 mg/kg. Since the clinical dose is more likely to be 16 mg/kg, this may represent a 2-fold safety margin. Results from immunogenicity analysis confirmed two animals to be positive for antibodies to ETI-204.

#### Special toxicology:

Tissue cross-reactivity (TCR) studies were performed in human, rat, and cynomolgus monkey tissues. The test article was said to stain various cell types in most tissues examined in a similar manner in all three species. Staining was reported to be cytoplasmic and was specifically localized to cytoplasmic granules and/or filaments, often associated with cell borders, suggestive of intracellular cytoskeletal elements, and therefore not relevant. One additional TCR study was performed in a limited set of human tissues to compare antibody derived from (b) (4) and (b) (4) cell lines after a manufacturing change from the former to the latter. Staining was reported to be similar for both test articles, and consistent with previous findings for the (b) (4) antibody. In these studies, ETI-204 concentrations of 1-10  $\mu\text{g}/\text{mL}$  were used, although it is unclear if these concentrations were high enough to be relevant to clinical exposures.

An assessment for neuropathological changes was performed on tissue from studies in infected monkeys and infected and non-infected rabbits. In primates and rabbits exposed to inhalational anthrax that did not survive (found dead or moribund sacrificed animals), administration of ETI-204 at doses of 4 mg/kg and higher was associated with an increased incidence of histological findings consistent with an acute inflammatory reaction. The changes in the non-survivors, including those treated with only saline, with ETI-204, or with levofloxacin, were stated to be consistent with morphologic lesions/hemorrhagic meningoencephalitis previously reported in monkeys and rabbits with inhalation anthrax. Biologically significant reactions (hemorrhage, inflammation, necrosis) in non-survivors were associated with the presence of

extravascular bacteria in all dose groups, including saline controls. The occurrence of an acute inflammatory response in the ETI-204 treated non-survivors did not exhibit a dose response relationship (i.e., changes were not more pronounced at higher doses). The administration of the ETI-204 was not associated with any biologically significant morphologic reactions in surviving animals exposed to inhaled *B. anthracis*. Similarly, no significant neuropathological lesions were reported in rabbits not exposed to anthrax spores and given up to 32 mg/kg intravenous ETI-204 (EFT001) in a reproductive toxicology study.

The margins of safety for ETI-204, based on cumulative exposures, are summarized in the Applicant's table below:

### Margins of Safety Based on Exposures at Animal NOAEL

Study No.	Nonclinical Study	Route of Admin.	NOAEL (mg/kg/dose)	No. of Doses	AUC <sup>a</sup> (µg·day/mL)	Margin of Safety <sup>b</sup>
TOX001 Pilot	15-Day Repeat-Dose Toxicity in Rats <sup>c</sup>	IV bolus	100 <sup>d</sup>	3	M – 16,200	M – 3X
TOX001	14-Day Repeat-Dose Toxicity in Rats <sup>c</sup>	IV bolus	30	3	M – 5560/6060 <sup>e</sup> F – 5340/6940 <sup>e</sup>	M – 1X F – 1X
AP115	17-Day Monkeys <sup>f</sup>	IV infusion	30 <sup>e</sup>	2	M – 6667 F – 6083	M – 1X F – 1X
EFT001 Pilot	Embryofetal Toxicity Rabbits	IV bolus	32 <sup>e</sup>	2, 4	F – 10100 <sup>h</sup>	F – 2X
EFT001	Embryofetal Toxicity Rabbits	IV bolus	32	4	F – 10000	F – 2X
AH104	Human PK	IV infusion	-	1	5170 <sup>i</sup>	-

AUC – area under the concentration curve; F – female; M – male; NOAEL – no-observed-adverse-effect level

<sup>a</sup>: Cumulative exposures were utilized, including AUC<sub>0-14</sub> via nonparametric superpositioning for TOX001 Pilot and TOX001, AUC<sub>last</sub> for AP115, AUC<sub>last</sub> for EFT001 Pilot, and AUC<sub>0-15</sub> via nonparametric superpositioning for EFT001.

<sup>b</sup>: Margin of Safety = AUC in animal at NOAEL dose ÷ AUC in humans at 16 mg/kg clinical dose.

<sup>c</sup>: Rats received ETI-204 via slow IV bolus over 45 ± 5 seconds on Days 1, 4, and 7.

<sup>d</sup>: A NOAEL/NOEL was not defined in the study report, which was conducted to identify the MTD. MTD was not established.

<sup>e</sup>: ETI-204 concentration and AUC data for the 30 mg/kg dose group is reported as Lonza value/Baxter value.

<sup>f</sup>: Vehicle or ETI-204 via slow IV bolus over 60 ± 2 minutes with 8 days between doses.

<sup>g</sup>: A NOAEL was not set for this study due to a lack of standard toxicological endpoints (primarily histopathology), but ETI-204 was well-tolerated at the highest tested dose.

<sup>h</sup>: Mean AUC value from all 3 dosing schedules combined.

<sup>i</sup>: Overall mean AUC<sub>0-∞</sub> value from Study No. AH104, 16 mg/kg IV dose.

It is notable, however, that a higher clinical dose may ultimately be recommended. While there may be some data to support clinical doses higher than 16 mg/kg, the MTD has not been reached for this drug substance in toxicology studies. The Applicant's table above did make species extrapolations based on AUC, which is appropriate if their antibody distributes outside of the vascular compartment.

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/s/  
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AMY C NOSTRANDT  
12/11/2015

WENDELYN J SCHMIDT  
12/16/2015

I concur with Dr. Nostrandt's interpretation of the data and assessment of the completeness of the NDA submission. The NDA can be approved from the pharm/tox perspective.

Comments on BLA 125509 obiltoxaximab

From A. Jacobs, AD

Date 12/11/15

1. I concur that there are no pharmtox approval issues.
2. I have conveyed other comments to the reviewer and she will address them as appropriate.

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/s/  
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ABIGAIL C JACOBS  
12/15/2015

**MEMO TO FILE: IND 12,285 (078)**

**DATE:** July 30, 2012  
**TO:** File, IND 12,285 (078)  
**FROM:** Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist, DAIP  
**THROUGH:** Wendelyn J. Schmidt, Ph.D.  
Pharmacology Supervisor, DAIP  
**RE:** IND 12,285, ETI-204 (Anthim®), SDN #78, submitted 2/24/2012

**Comment for Sponsor: Yes**  
See comment at the end of this review.

The Sponsor has provided two protocols for pharmacokinetics studies. Both were scheduled to begin a few days before the protocols were submitted. Both will be conducted by (b) (4) in compliance with GLPs.

**1. Protocol no. 1984-004: A dose escalation study to evaluate tolerability and pharmacokinetic profile of intramuscularly administered ETI-204 in rabbits**

New Zealand White Hra:(NZW)SPF albino rabbits (15/sex) will be administered the control or test article as a single dose on Day 1 as shown in the table below.

Group	Dose Level mg/kg	Number of Dose Sites	Number of Animals	
			M	F
1	0	1	3	3
2	3	1	3	3
3	10	1	3	3
4	30	1	3	3
5	30	2 (15 mg/kg/dose site)	3	3

The test article and vehicle will be administered by intramuscular bolus injection into the lumbar musculature. The vehicle will be 40 mM histidine, 200 mM sorbitol, 0.01% polysorbate 80 (Tween 80).

Evaluations will included a complete physical examination pretest, ophthalmological examinations pretest and prior to the scheduled sacrifice, twice daily cageside observations for morbidity, mortality, injury, and availability of food and water, and weekly detailed clinical examinations. Any animals in poor health will be identified for further monitoring and possible euthanasia. Body weights will be measured and recorded within 3 days of arrival, at least once prior to randomization, and weekly during the study. Food consumption will be measured and recorded daily and reported weekly during the study.

Blood collection for serum test article concentrations will be performed on Day 1 prior to dosing and at 8, 16, 24, 36, 48, and 72 hours after dosing, and on Days 5, 9, 13, 21, and 29 at an unspecified time. The test animals will not be fasted before blood collection. The blood samples will be collected at all time points in the control group for consistency among groups; however, only the sample at 24 hours post dose (approximately  $T_{max}$ ) will be analyzed (all other control samples will be discarded following collection). Approximately 1 mL of whole blood will be collected from the jugular, or other suitable vein into serum separator tubes. Samples will be allowed to clot for at least 30 minutes at room temperature prior to centrifugation. Serum will be separated and frozen at  $-50^{\circ}$  to  $-90^{\circ}\text{C}$ . Whenever possible, a terminal blood sample for evaluation of plasma test article concentrations will be collected from any animal euthanized *in extremis* to help determine exposure associated with morbidity. The serum will be analyzed for ETI-204 by a Sponsor-contracted laboratory, (b) (4) using analytical methods developed and validated by that laboratory. A noncompartmental module will be used to determine  $C_{max}$ ,  $T_{max}$ ,  $AUC_{0-t}$  (where t equals to the last collected time point) or  $AUC_{last}$  and  $AUC_{0-t}/\text{Dose}$  or  $AUC_{last}/\text{Dose}$  for the test article from mean concentration-time data in the test species. Based on the data, appropriate parameters will be estimated and may include, but not be limited to,  $AUC_{inf}$ ,  $T_{1/2}$ ,  $C_{max}/\text{Dose}$ , and  $AUC_{inf}/\text{Dose}$ .

Additional blood samples (1 mL) will be collected from the jugular, or other suitable vein from all surviving animals (unfasted) prior to dose on Day 1 and prior to termination for determination of the antibody level in the blood. The blood will be collected into plastic tubes, allowed to clot for at least 30 minutes, and processed to serum at room temperature. Following centrifugation, the serum will be frozen at approximately  $-70^{\circ}\text{C}$ . The serum will be analyzed for antibodies to ETI-204 by a Sponsor-contracted laboratory, (b) (4) using an analytical method developed and validated by that laboratory.

Any moribund animals will be euthanized, and necropsy examination and histopathology of a full tissue set will be performed.

## 2. Protocol no.1984-003: A dose escalation study to evaluate tolerability and pharmacokinetic profile of intramuscularly administered ETI-204 in naïve cynomolgus monkeys

A similar study will be performed in cynomolgus monkeys (*Macaca fascicularis*, 12/sex), approximately 2 to 5 years old and 2 to 5 kg in weight.

Group	Dose Level mg/kg	Number of Dose Sites	Number of Animals	
			M	F
1	3	1	3	3
2	10	1	3	3
3	30	1	3	3
4	30	2 (15 mg/kg/dose site)	3	3

The test article will be administered by intramuscular bolus injection into the lumbar musculature on Day 1. The vehicle will be 40 mM histidine, 200 mM sorbitol, 0.01% polysorbate 80 (Tween 80). No control group is included.

Evaluation of Groups 1 and 2 animals will be terminated on Day 29, while that for Groups 3 and 4 will be terminated on Day 57. Evaluations will include a complete physical examination pretest, ophthalmological examinations pretest and prior to the scheduled sacrifice, twice daily cageside observations for morbidity, mortality, injury, and availability of food and water, and weekly detailed clinical examinations. Any animals in poor health will be identified for further monitoring and possible euthanasia. Body weights will be measured and recorded within 3 days of arrival, at least once prior to randomization, and weekly during the study. A qualitative assessment of food consumption will be conducted as a part of the twice daily cageside observations.

Blood collection for serum test article concentrations will be performed on Day 1 prior to dosing and at 6, 12, 24, 48, and 72 hours after dosing, and on Days 5, 9, 13, 21, and 29 at an unspecified time from all animals. Additional collections from animals in Groups 3 and 4 will be collected on Days 41 and 57. The test animals will not be fasted before blood collection. Approximately 1 mL of whole blood will be collected from the femoral artery or vein into serum separator tubes. Samples will be allowed to clot for at least 30 minutes at room temperature prior to centrifugation. Serum will be separated and frozen at  $-50^{\circ}$  to  $-90^{\circ}\text{C}$ . Whenever possible, a terminal blood sample for evaluation of plasma test article concentrations will be collected from any animal euthanized *in extremis* to help determine exposure associated with morbidity. The serum will be analyzed for ETI-204 by a Sponsor-contracted laboratory, (b) (4), using analytical methods developed and validated by that laboratory. A noncompartmental module will be used to determine  $C_{\max}$ ,  $T_{\max}$ ,  $AUC_{0-t}$  (where t equals to the last collected time point) or  $AUC_{\text{last}}$  and  $AUC_{0-t}/\text{Dose}$  or  $AUC_{\text{last}}/\text{Dose}$  for the test article from mean concentration-time data in the test species. Based on the data, appropriate parameters will be estimated and may include, but not be limited to,  $AUC_{\text{inf}}$ ,  $T_{1/2}$ ,  $C_{\max}/\text{Dose}$ , and  $AUC_{\text{inf}}/\text{Dose}$ .

Additional blood samples (1 mL) will be collected from the femoral artery or vein from all surviving animals (unfasted) prior to dose on Day 1 and on Day 29 for determination of the antibody level in the blood. The blood will be collected into plastic tubes, allowed to clot for at least 30 minutes, and processed to serum at room temperature. Following centrifugation, the serum will be frozen at approximately  $-70^{\circ}\text{C}$ . The serum will be analyzed for antibodies to ETI-204 by a Sponsor-contracted laboratory, (b) (4) using an analytical method developed and validated by that laboratory.

Any moribund animals will be euthanized, and subjected to a routine necropsy examination.

The Sponsor also has the following question:

“Reference is made to a meeting between Elusys and the Agency on May 10, 2011 and the Agency’s minutes of that meeting dated June 8, 2011. In response to question 6, the Agency’s minutes noted that “The previously conducted nonclinical pharmacology/toxicology studies and the proposed embryo-fetal developmental toxicity study in rabbits (Ref. 15.1.5.3) appear to be sufficient to support the NDA/BLA submission.” ETI-204 has no endogenous target in humans, and in a tissue cross-reactivity study with ETI-204 using rat tissues (Study IM1219) ETI-204 staining was limited to intravascular spaces with no apparent binding to intravascular blood cells, endothelial cells, perithelial cells, or any other cellular or acellular tissue element. With

this information in mind, can the Agency confirm that a nonclinical biodistribution study is not required for licensure of ETI-204?”

This was discussed with the Medical Officer and the Clinical Pharmacology Team Leader. Since large molecules, such as monoclonal antibodies, are generally restricted to the vascular compartment, there does not appear to be any reason to conduct a biodistribution study.

The following comment was forwarded to the Sponsor:

“A biodistribution study for this IV administered monoclonal antibody does not appear to be warranted.”

It was not taken into consideration at that time that the test article might be administered IM. It is unclear how an IM-administered monoclonal antibody would distribute into the vascular compartment from an IM injection site, if we assume that a molecule that size is unable to get out of the vascular compartment. If these studies demonstrate a pharmacokinetic profile for this product, it is possible that the issue of a biodistribution study may have to be reconsidered.

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/s/  
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AMY C NOSTRANDT  
08/22/2012

WENDELYN J SCHMIDT  
08/22/2012

**MEMO TO FILE: IND 12,285 (069)**

**DATE:** August 7, 2012

**TO:** File, IND 12,285 (069)

**FROM:** Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist, DAIP

**THROUGH:** Wendelyn J. Schmidt, Ph.D.  
Pharmacology Supervisor, DAIP

**RE:** IND 12,285, ETI-204 (Anthem®), SDN #69, submitted 11/29/2011

**Comment to Sponsor: None**

The Sponsor has provided two pharmacokinetics study reports.

1. <sup>(b) (4)</sup> **Study no. 1180-04527: A single-dose intravenous and intramuscular pharmacokinetics study of ETI-204 (Anthem™) in cynomolgus monkeys**

This report presents pharmacokinetic data in a study that also evaluated blood pressure and ECG effects of ETI-204 in cynomolgus monkeys. Groups of 4 male and 4 female cynomolgus monkeys (2-3 kg) received either 5 mg/kg IV or IM (0.4 mL/kg) or 10 mg/kg IM (0.8 mg/kg) of ETI-204 (12.5 mg/mL). IV bolus dosing was by slow push over 3-5 minutes, while IM injections were split to deliver half of the dose to the muscle of each thigh. Blood samples were collected from the femoral or cephalic vein pre-dose, at 1, 2, 4, 8, 24, and 32 hours and at 3, 4, 5, 6, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 29, 31, 33, 36, 38, 40, and 43 days post-injection. Serum was separated and frozen at -70°C until analysis by PAA3 ELISA. Serum from all monkeys on Days 0, 13, 26, and 43 were also analyzed for anti-ETI-204 antibody.

Anti-product antibodies were detected in samples from 2 monkeys on Day 13 and an additional 6 monkeys on Day 26, for a total of 8 out of 12 monkeys, or 75%. The reports states that data up to Day 13 would be the most meaningful to understand the disposition of the drug before development of an immune response, so pharmacokinetic analysis and data presented in the report only include serum concentrations through Day 13. Summary pharmacokinetic parameters are presented in the Sponsor's table below.

### Summary of Pharmacokinetic Parameters of ETI-204 in Cynomolgus Monkeys

Males			
Route of Administration	IV	IM	IM
Dose, mg/kg	5	5	10
C <sub>max</sub> , µg/mL	N/A	40.34 ± 4.73	94.91 ± 13.83
T <sub>max</sub> <sup>a</sup> , d	N/A	2.5 (1-6)	0.8 (0.3-1.3)
AUC(0-inf), µg·d/mL	752.47 ± 105.99	614.37 ± 63.54	1284.96 ± 64.18
Clearance, L/d/kg	0.0068 ± 0.0011	N/A	N/A
V <sub>ss</sub> , L/kg	0.0896 ± 0.0507	N/A	N/A
T <sub>1/2</sub> <sup>b</sup> , d	7.9 ± 3.3	6.8 ± 2.9	8.4 ± 2.2
F, %	N/A	81.65	85.38

Females			
Route of Administration	IV	IM	IM
Dose, mg/kg	5	5	10
C <sub>max</sub> , µg/mL	N/A	40.29 ± 7.77	75.64 ± 18.46
T <sub>max</sub> <sup>a</sup> , d	N/A	1.3 (0.08-1.3)	1.3 (1.3-4.0)
AUC(0-inf), µg·d/mL	717.81 ± 153.67	481.79 ± 86.51	1399.46 ± 99.64
Clearance, L/d/kg	0.0072 ± 0.0013	N/A	N/A
V <sub>ss</sub> , L/kg	0.0922 ± 0.0098	N/A	N/A
T <sub>1/2</sub> <sup>b</sup> , d	9.7 ± 1.6	6.0 ± 2.0	12.0 ± 1.9
F, %	N/A	67.12	97.48

<sup>a</sup> Expressed as median and range

<sup>b</sup> Expressed as harmonic mean and pseudo SD based on jackknife variance

N/A - Not Applicable

Clearance was low, and volume of distribution was small at steady state. The terminal half-life was 7.9-9.7 days. Mean bioavailability of IM-administered product was 82-84%. There was no significant difference in pharmacokinetics between males and females, except for AUC<sub>0-inf</sub> after the 5 mg/kg IM dose.

## 2. Perry Scientific Study 06-0082: A pharmacokinetic study of (b) (4) and (b) (4) in rabbits following intravenous and intramuscular administration

The report describes (b) (4) and (b) (4) as monoclonal antibodies to anthrax protective antigen, but it is unclear how they differ from each other or how they are different or similar to ETI-204. The pharmacokinetics of (b) (4) was investigated following an IV dose of 20 mg and IM doses of 10 or 20 mg in NZW rabbits as dilute solution (8.64 mg/mL in buffer). An IM dose of a more concentrated solution (84.1 mg/mL in buffer) was administered at a dose of 16.82 mg. An additional group of rabbits received an IM injection of (b) (4) (10 mg/mL in PBS) at a dose of 20 mg. Three female rabbits were dosed in each group. Blood samples were collected from the ear artery of each animal pre-dose, at 0.5, 1, 2, and 4 hours and at 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days post-injection. Plasma was separated and frozen at -80°C until analysis by PAA3 ELISA.

Pharmacokinetic parameters are presented in the Sponsor's table below.

Summary of Pharmacokinetic Parameters of (b) (4) and (b) (4) in Rabbits

	H46			H25	
	Group 1	Group 2	Group 4	Group 3	Group 5
Route of Administration	IV	IM	IM	IM	IM
Dose, mg	20	10	20	16.82	20
Dosing Solution, mg/mL	8.64	8.64	8.64	84.1 (High Conc)	10.25
C <sub>max</sub> , µg/mL	N/A	31.86 ± 4.24	60.22 ± 7.15	47.95 ± 6.41	65.81 ± 7.68
T <sub>max</sub> <sup>a</sup> , d	N/A	2.0 (1-2)	1.33 (1-2)	2.00 (2-2)	1.67 (1-2)
AUC, µg.d/mL	557.98 ± 36.42	265.60 ± 38.42	606.61 ± 150.78	307.88 ± 90.56	458.91 ± 73.34
Clearance, L/d	0.036 ± 0.002	N/A	N/A	N/A	N/A
V <sub>dss</sub> , L	0.199 ± 0.031	N/A	N/A	N/A	N/A
T <sub>1/2</sub> <sup>b</sup> , d	3.1 ± 2.5	1.7 ± 1.1	5.7 ± 0.8	1.4 ± 0.7	1.5 ± 0.8
F, %	N/A	95.20	108.72	65.61	N/A

<sup>a</sup> Expressed as median and range

<sup>b</sup> Expressed as harmonic mean and pseudo SD based on jackknife variance

N/A - Not Applicable

Clearance was low, and volume of distribution at steady state was small. Elimination half-life was 3.1 days after IV administration. IM bioavailability was 95% or greater. Pharmacokinetic parameters (AUC, half-life, bioavailability) for (b) (4) following dosing with the higher concentration solution were lower than with the low concentration dosing solution. The mean C<sub>max</sub> values following a 20 mg dose of (b) (4) or (b) (4) were similar, but the half-life and AUC values for (b) (4) were considerably greater.

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/s/  
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AMY C NOSTRANDT  
08/22/2012

WENDELYN J SCHMIDT  
08/22/2012

**MEMO TO FILE: IND 12,285 (071)**

**DATE:** August 17, 2012

**TO:** File, IND 12,285 (071)

**FROM:** Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist, DAIP

**THROUGH:** Wendelyn J. Schmidt, Ph.D.  
Pharmacology Supervisor, DAIP

**RE:** IND 12,285, ETI-204 (Anthem®), SDN #71, submitted 12/21/2011

**Comment for Sponsor: None**

The Sponsor has provided an efficacy study report.

**1. Study no. AP201 ( (b) (4) study no. 834-G924202): Evaluating the efficacy of ETI-204 when administered therapeutically in the cynomolgus macaque inhalational anthrax model**

The efficacy of ETI-204, an anti-toxin antibody described as binding and neutralizing the cell binding component of anthrax protective antigen (PA), was evaluated in cynomolgus monkeys (2.6-5.1 years of age, 2.5-5.3 kg) in this GLP-compliant study (*Reviewer's comment: The report describes these animals as juveniles; it is unclear whether or not the Sponsor intends for this study to support pediatric use. No information was provided to compare cynomolgus monkeys of this age to human children with respect to post-natal development.*). The study was conducted at (b) (4). Forty-five animals were obtained for the study. The monkeys surgically implanted with TA-D70 telemetry transmitters. One animal died prior to the implantation surgery, possibly due to a reaction to the anesthetic. Remaining animals were randomized by weight into 2 groups of 15 and one of 14. Each group was approximately 50% male and 50% female. The animals were further randomized to one of 3 aerosol challenge days (cohorts). Study Day 0 was the day of challenge for each cohort.

Monkeys were challenged via the inhalation route with *B. anthracis* (Ames strain) on Study Day 0. Anesthetized monkeys were placed into a plethysmography chamber in a Class III biosafety cabinet system. They then were aerosol challenged with a targeted 200 LD<sub>50</sub> dose (1.24 x 10<sup>7</sup> spores) of *B. anthracis* (Ames strain) spores, as determined by Vasconcelos et al. (Vasconcelos 2003). The spore concentration and Spray Factor characterization for the spore lot used on this study (Spore Lot B35) were used to calculate the estimated aerosol concentration. Spores were aerosolized by a Collison nebulizer and delivered via a head-only inhalation exposure chamber. Estimated aerosol concentration and individual respiratory parameters were used to determine the length of time of aerosol exposure. Aerosol concentrations of *B. anthracis* were quantified by determination of colony forming units (cfu) collected by an in-line all-glass

impinger. Particle size distributions were obtained, and mass median aerodynamic diameters and geometric means and standard deviations were calculated.

Monkeys were observed approximately every six hours ( $\pm 1$  hour) beginning approximately 24 hours post-median challenge time and ending 8 days post median challenge time for clinical signs including anorexia, lethargy, respiratory distress, moribundity, activity (recumbent, weak, or unresponsive), seizures, and other abnormal clinical signs. Appetite was monitored twice daily during this observation period. From Study Day 9 until Study Day 30, clinical observations were recorded twice daily. On Day 30, clinical observations were recorded prior to euthanasia.

Body temperature and activity were monitored and stored electronically beginning prior to challenge until the end of the in-life animal phase (30 days post-challenge) via implanted TA-D70 DSI telemetry devices. Temperature was recorded in degrees C and converted to degrees F for analysis. Activity data were recorded in counts per minute. Baseline body weights were recorded prior to challenge and on Day 0. Day 0 body weights were used for treatment calculations. No post challenge body weights recorded; the report stated the lack of observation of prolonged inappetence as justification.

Blood samples were collected from a femoral artery or vein, saphenous vein or other vein according to the schedule below. Times are relative to the median challenge time.

**Table 2 Blood Collection and Assay Schedule<sup>a</sup>**

Approximate Time Point	Blood Tube type/ Approximate Blood volume	Bacteremia (Culture)	CBC/ CRP	Serum PA levels (via ECL and PA-ELISA)	Serum for ETI-204 dose confirmation	* Retention Serum for Potential Future Use
Day -7	EDTA ~1.5ml SST ~2.0ml	X	X	X	X	X
^24hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^30hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^36hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^42hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^48hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
^54hr PC	EDTA ~0.5ml SST ~1.5ml	X		X		
PTT <sup>d</sup>	EDTA ~1.5 ml SST ~2.0ml SPS ~1.0 ml	X <sup>#</sup>	X	X		X
5 min PT	SST ~1.0ml					X
6hr PT	SST ~1.0ml					X
24hr PT	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>	X	X
96hr PT	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
7 days PC	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
14 days PC	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
21 days PC	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
30 days PC	EDTA ~1.5 ml SST ~2.0ml	X	X	X <sup>c</sup>		X
Terminal <sup>b, d</sup>	EDTA ~1.5 ml SST ~2.0ml	X	CRP only	X <sup>c</sup>		X

PC = Post-Challenge PTT = Prior to Treatment PT = Post-Treatment

<sup>a</sup> Post-challenge pre-treatment bleed time points were relative to a median challenge time for a challenge cohort. Post-treatment bleed times were calculated from the time each animal's IV treatment ended. Blood sample collection occurred within ±60 minutes of the calculated time, except for the 5 min PT, 6hr PT and 96hr PT samples which occurred within 2 min, 15 min and 3 hours of their calculated times, respectively. The Day -7, Day 7 PC, Day 14 PC, Day 21 PC and Day 30 PC bleeds were relative to the day of challenge.

<sup>b</sup> If collection was possible

<sup>c</sup> Samples tested via PA-ELISA only

<sup>d</sup> Terminal samples were not collected on animals euthanized at the end of study as day 30 PC samples were already scheduled

<sup>e</sup> If remaining serum was sufficient after aliquots for other analyses were made. Samples will be stored for potential future testing

<sup>f</sup> Post-Challenge, pre-treatment sampling stopped once decision to treat was made

<sup>g</sup> PTT Bacteremia enrichment performed on sample collected in SPS tube

Qualitative assessment of protective antigen (PA) was performed using an electrochemiluminescence (ECL) assay for determination of the time to initiate treatment. Quantitative assessment of PA was by ELISA. ETI-204 dose confirmation was performed by PAA3 ELISA.

The test article, ETI-204, used for treatment was Baxter lot no. 103B20-X109-TR06 (Elusys lot no. ET 472-084), protein concentration 117 mg/mL, purity 98% monomer (HPLC-SEC), potency 69 ng/mL (LNA). The control was 0.9% saline. Treatment groups were administered single IV bolus doses via the saphenous vein of saline, 4 or 8 mg/kg ETI-204 at a volume of 0.5 mL/kg. Initiation of treatment was based on positive serum PA-ECL assay result post-challenge. If there was no positive PA-ECL result by 54 hours post-challenge, animals were to be administered test article or control at that point. The staff assessing animals and administering test article were blinded to treatment.

Gross necropsy was performed on all early decedents and on survivors at termination on Day 30. Sections of target tissues, including brain/meninges, lungs, liver, spleen, kidney and

mediastinal lymph nodes, and gross lesions were examined by a board-certified veterinary pathologist. Additional microscopic evaluation of the brain was performed to characterize potential test article-related changes by a neuropathologist, who was blinded to treatment. Samples of spleen and mediastinal or bronchial lymph nodes were collected at necropsy for qualitative assessment of bacteria in these tissues.

#### Results:

One animal died prior to telemetry implantation, presumably as a result of the anesthetic. A second animal was removed from study due to abnormalities noted on pre-study evaluation. A total of 43 animals were challenged. The average aerosol exposure dose for all animals on study was  $199 \pm 60$  LD<sub>50</sub> *B. anthracis* spores. The average mass median aerodynamic diameter was 1.11-1.12  $\mu\text{m}$ .

There were 12/14 deaths in the saline control group, 3/14 in the 4 mg/kg group, and 4/15 in the 8 mg/kg group, resulting in 14%, 79%, and 73% survival, respectively. Survival rates in treated groups were statistically significantly greater than control. Statistical analysis revealed a significant relationship between survival and quantitative bacteremia prior to treatment in treated groups, i.e. greater magnitude of bacteremia was associated with lower survival. A similar relationship was observed between PA-ELISA values prior to treatment and survival in treated groups.

After challenge, the report states that the majority of animals presented with clinical signs consistent with anthrax, including lethargy, hunched posture, inappetence, stool abnormalities and respiratory abnormalities. Animals that died on study demonstrated a progression of signs beginning with lethargy and inappetence, progressing to respiratory abnormalities, and finally to moribundity. Surviving animals had hunched posture, lethargy and inappetence, but returned to normal between 8-10 days post-challenge.

The standard deviation of all pre-challenge, baseline-adjusted temperatures was calculated for each animal and twice this value was used to determine the threshold for an elevated temperature. Elevated body temperatures, according to these criteria were reached in 43-73% of animals in each of the groups.

Qualitative serum PA-ECL was used as the trigger for treatment. All but 5 animals were treated based on a positive PA-ECL test result. Three animals in the 4 mg/kg group and one in the 8 mg/kg group were treated as directed by the study director following failure of ECL plates to meet acceptance criteria (protocol deviation). One control animal was treated after 54 hours post-challenge in spite of a negative ECL test result. Elsewhere in the report, it is stated that there were no significant differences in the proportion of animals positive for PA-ECL values at any time point and that only 2 animals (1 saline control and 1 4 mg/kg) did not have positive ECL prior to treatment. The average time from challenge to treatment was similar between groups, ranging from 41.3 to 44.5 hours

Quantitative serum PA-ELISA testing was also performed. There were no significant dose group effects for time from challenge until a positive PA-ELISA result; geometric means for the three groups were reported to range from 41.49-44.36 hours (range 25-83 hours). There was also no significant difference reported for time from treatment until resolution for PA-ELISA. The percentages of monkeys positive by PA-ELISA prior to treatment ranged from 73-86%, with no significant differences between groups. However, the report indicates that the group geometric mean PA-ELISA values prior to treatment were lower for monkeys that survived to the end of the study compared to non-survivors in ETI-204 treated groups suggesting

that the test article was more likely to be effective in less affected animals. The report states that animals with PA levels below 100 ng/mL were more likely to survive if treated with ETI-204 than were saline controls. Day 7 data was suggestive of a more rapid decrease in PA-ELISA values in ETI-204-treated survivors than in the two saline control survivors.

100% of monkeys were positive for bacteremia by either qualitative or quantitative culture evaluation prior to treatment. Blood samples from 18 of the 19 early decedents were also positive for bacteremia; blood could not be obtained from one animal found dead. The report states that bacteremia was completely resolved in treated animals by 96 hours post-treatment. For the two surviving controls, bacteremia was resolved by Day 7 (qualitative) or 14 (quantitative) post-challenge. There were no significant group differences reported in the proportion of animals bacteremic prior to treatment or for the time from challenge until bacteremic. Of the 19 animals that died on study, all but one had a positive bacterial tissue culture.

Geometric means of test results indicated that positive results would first be seen for bacteremia, then abnormal PA-ECL, then abnormal PA-ELISA after challenge. The bacteremia (quantitative) and PA-ELISA values had overall significant positive correlations at the PTT and 24 hours post-treatment time points, indicating that elevated bacteremia levels correlated with elevated circulating PA levels. Additionally, there was a significant relationship between PA-ELISA levels at the PTT time point and survival (as discussed above) and quantitative bacteremia levels at the PTT time point and survival. For both of these parameters, greater values at the PTT time point were associated with lower probabilities of survival.

Hematology evaluation revealed that red blood cell parameters were decreased significantly relative to baseline in all 3 groups at most time points. For the ETI-204 treated groups, the maximum decreases were seen at Day 7 post-challenge and were 21-28% below baseline. For the control group, the maximum decrease was 44-48% on Day 14 post-challenge in the 2 surviving monkeys. Many of the decreased mean values in the control animals were significantly different from those in treated animals. Other significant changes seen at various time points post-challenge were decreases for MCV and MCH and increases for MCHC.

White blood cells (WBC) were significantly increased relative to baseline in all 3 groups from the prior to treatment time point through Day 14 post-challenge. The maximum increase in the control group was greater than those for the treated groups. Lymphocyte counts exhibited biphasic responses with maximum decreases at 24 hours post-treatment and maximum increases at Day 14 post-challenge. Mean neutrophil counts were increased for all groups, but those for the saline group increased slightly later, remained elevated longer, and were higher than those in treated groups. Maximum values were 24 hours post-treatment for the treated groups and Day 7 post-challenge for the saline control group. The report states that the WBC changes are consistent with those reported in the literature in non-human primate (NHP) models of inhalational anthrax. At least partial recovery was evident by Day 30 post challenge.

Biphasic responses were also seen in platelets. Initial decreases were followed by increases. The magnitude and duration of the decreases were greater for the saline control group than for treated groups. Maximal decreases at 24 hours post-treatment in treated groups were 19-20% lower than baseline, while maximum decrease in the saline group was approximately 30% on Day 7 post-challenge. In treated groups, platelet counts peaked at Day 14 post-challenge. Full or partial recovery was evident by the end of the study.

C-reactive protein (CRP) was significantly increased at most time points relative to baseline. The increases in the treatment groups at 24 hours post-treatment were significantly

greater than that in the saline control group. At least partial recovery was observed in all surviving animals at the end of the study.

Dose confirmation analysis (pharmacokinetics) revealed approximately dose-proportional concentrations at 24 hours post-treatment. Values were 38-83 µg/mL for the 4 mg/kg group and 87-168.95 for the 8 mg/kg group. However, there were anomalous (low or none detected) results in both treatment groups, and one control sample contained 133.22 µg/mL ETI-204. There appears to have been some mistakes in labeling or switching samples; the utility of these data are therefore limited.

At necropsy, gross lesions were described as consistent with previously described and published findings in cynomolgus monkeys with inhalational anthrax. Red discoloration in the brain of one female control monkey was described. It was considered to be consistent with gross findings of anthrax, but there were no corresponding microscopic findings. Microscopic findings in other organs of that animal were consistent with anthrax. All animals found dead or euthanized *in extremis* had gross and/or microscopic findings consistent with anthrax. Of the surviving animals euthanized on Day 30, five had gross findings that were neither attributed to anthrax or to test article administration, but appeared to be incidental or common findings in this species. None of the animals surviving until study termination had microscopic findings attributed to either anthrax or test article administration.

A neuropathology examination was also performed. The neuropathologist's assessment was reported to be similar to that of the study pathologist. While there were no notable findings in animals that survived to termination, there were findings of bacterial meningitis and meningeal vasculitis as well as bacterial encephalitis and vasculitis in the brain in animals that died or were euthanized prior to study termination. The findings tended to predominate in the meninges, with less pronounced findings in the brain, usually adjacent to meningeal changes. It is notable, that, in the early decedents, test article treated animals were more likely to have a more severe inflammatory response to the bacteria than controls. The report speculates that test article-treated animals' immune systems were more able to mount an inflammatory response, but the exact cause of this difference is unknown. Another possible conclusion would be that the test article was not protective if the disease had progressed to include encephalitis and/or meningitis.

It should be noted that there were a large number of protocol deviations, many attributed to inadequate training of personnel. Included in these were unexpected findings in pharmacokinetic monitoring that indicated that samples were mis-labeled or mixed up. Findings in this study may not be entirely reliable.

In summary, forty-three monkeys were challenged with an average dose of 199 ( $\pm$  60) LD<sub>50</sub> equivalents of *Bacillus anthracis* (Ames strain) spores via aerosol exposure. Initiation of ETI-204 or saline (control) treatment for each monkey was based on a positive result in a qualitative protective antigen (PA) assay (PA-ECL). Monkeys treated intravenously with ETI-204 at doses of 4 and 8 mg/kg demonstrated survival rates of 79% and 73%, respectively, which were statistically significantly greater than the 14% percent of control animals receiving saline (intravenously) that survived until scheduled termination. However, no clear dose-response relationship was demonstrated. One-hundred percent of the monkeys had a positive bacteremia result prior to treatment. WBC counts, neutrophil counts, N/L ratios, and CRP values were increased and red cell parameters and platelet values were decreased following challenge. In general, these changes were more pronounced in saline-treated monkeys as compared with those that received ETI-204. For ETI-204-treated monkeys that survived until scheduled sacrifice,

recovery or a trend towards recovery was evident by Day 30 post challenge. Monkeys that were found dead or euthanized in a moribund condition prior to Day 30 all had gross and/or microscopic findings consistent with anthrax, whereas monkeys that survived to Day 30 demonstrated no gross and/or microscopic findings indicative of anthrax infection at termination. The report concluded that ETI-204 was protective against mortality due to inhalation anthrax when given as a single IV dose of 4 or 8 mg/kg in this cynomolgus monkey model.

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/s/  
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AMY C NOSTRANDT  
08/22/2012

WENDELYN J SCHMIDT  
08/22/2012

**MEMO TO FILE: IND 12,285 (097 and 098)**

**DATE:** August 17, 2012

**TO:** File, IND 12,285 (097 and 098)

**FROM:** Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist, DAIP

**THROUGH:** Wendelyn J. Schmidt, Ph.D.  
Pharmacology Supervisor, DAIP

**RE:** IND 12,285, ETI-204 (Anthem®), SDN #97, submitted 8/7/12, and SDN #98, submitted 8/13/12

**Comment for Sponsor: Yes**

See comment at the end of this review.

In SDN #97 (sequence no. 96) the Sponsor has provided two protocols, along with associated protocol amendments:

1. **TOX-001: A 14-Day Definitive Intravenous Toxicity Study of ETI-204 in Sprague-Dawley Rats ( (b)(4) Study No. 1984-006)**
2. **AR037: Evaluating the Post-Exposure Effect of Intramuscularly Administered ETI-204 in Inhalational Anthrax Challenged Rabbits ( (b)(4) Protocol No. FY12-097)**

The former is a repeated dose toxicology study performed to compare the proposed drug substance produced from two different manufacturers. According to the protocol, the in-life portion of the study was completed on the same day that this document was submitted to the Agency. The second study is an efficacy study in a rabbit model of inhalational anthrax.

In SDN #98 (sequence no. 97) the Sponsor also has provided two protocols, along with associated protocol amendments:

1. **AP307: Study to Evaluate the Post -Exposure Efficacy of ETI -204 via Intramuscular (IM) Administration in the Cynomolgus Macaque Inhalation Anthrax Model ( (b)(4) study No. 2597 -100011517)**
2. **AR034: Re-challenge of Rabbits Treated Previously for Inhalational Anthrax with Intravenous ETI-204 to Assess Protective Immunity ( (b)(4) study No. 2637-100012211)**

Both are efficacy studies designed to explore the prevention of mortality from inhalational anthrax by ETI-204. Dates of study initiation are not indicated, but the protocols were signed over a month before submission, indicating that they may well be underway at this time.

The Sponsor has no questions regarding any of these protocols, and does not request Agency feedback. At least one of studies appears to be in progress or completed. It is unclear why they have been submitted. This is not the first time the Sponsor has submitted protocols for studies that have already begun. Since they have no questions, and it is too late to revise any protocol for which the study is already underway, there is no need to review these protocols in any detail. In future, any submission of protocols from this Sponsor without a specific question or request for feedback will not be reviewed.

**Comment to be conveyed to the Sponsor:**

It is unclear why protocols of nonclinical studies that are completed or in progress are being submitted for review. It is sufficient to submit these protocols as appendices to the final study reports.

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AMY C NOSTRANDT  
08/22/2012

WENDELYN J SCHMIDT  
08/22/2012

**MEMO TO FILE: IND 12,285 (085)**

**DATE:** August 3, 2012

**TO:** File, IND 12,285 (085)

**FROM:** Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist, DAIP

**THROUGH:** Wendelyn J. Schmidt, Ph.D.  
Pharmacology Supervisor, DAIP

**RE:** IND 12,285, ETI-204 (Anthem®), SDN #85, submitted 5/7/2012

**Comment for Sponsor: Yes**

See response to Question 2 below.

The Sponsor has provided a revised plan for development for the treatment of inhalational anthrax as well as prophylaxis and post-exposure prophylaxis. The Sponsor intends to develop the product for (b) (4) IV use. From a pharmacology/toxicology perspective, the following is noteworthy:

- Models to be used in efficacy studies will be NZW rabbits and cynomolgus monkeys. The Sponsor states that both of these species meet the criteria for an animal model and have clinical courses of anthrax similar to humans.
- Tissue cross-reactivity studies demonstrated cytoplasmic binding sites only. The Sponsor states that these are not relevant to *in vivo* administration; this is consistent with the ICH S6 “Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals” (May 2012).
- Cardiovascular safety pharmacology studies were performed in monkeys.
- Completed toxicology studies include repeat dose studies of IV administered ETI-204 in rats and monkeys and IM administered ETI-204 in rats. The highest doses were considered to be NOELs, approximately 10 mg/kg (3 doses, 3 days apart) in rats and 10 mg/kg (2 doses, 8 days apart) in monkeys. Neuropathology in these studies compared the presence of bacteria and/or lesions in surviving and early decedents and in treated vs. controls.
- Pharmacokinetics studies have been conducted in rabbits and monkeys. AUC was similar for IV and IM administered drug in one study; the similarity or dissimilarity in Cmax was not mentioned. Half-lives were 1.4-5.7 days in rabbits and 6-12 days in monkeys. (*Reviewer’s comment: These seem a bit short relative to the duration of*

*antibiotic treatment required for anthrax; perhaps repeated dosing may need to be considered.)*

- Some anti-drug antibody has been seen in individual animals, but no anaphylactic reactions are described.
- Efficacy studies have been conducted and are planned to assess treatment, prophylaxis, and post-exposure prophylaxis.
- A segment II teratology study in rabbits is planned or ongoing; no other developmental or reproductive toxicology studies are planned.
- A repeat dose general toxicology study in Fischer rats is planned to assess the comparability of test article manufactured by two contract manufacturers (Lonza and Baxter)
- Studies of interaction with (b) (4) antibiotic are planned or ongoing (added benefit). Other planned studies include a study of development of protective immunity (rechallenge), evaluation of added benefit study over antibiotics alone, (b) (4)

[Redacted]

The Sponsor also has the following questions:

Question 1.

As outlined in the development plan, Elusys proposes that generating data to support (b) (4) administration of ETI-204 and to support the dosing, safety and efficacy of ETI-204 across a range of potential clinical scenarios is an important and appropriate target for development and subsequent label of this anthrax (b) (4). Does the Agency agree?

Does the Agency have additional advice or feedback for the sponsor regarding the data required (beyond that outlined in the development plan) to support both indications and both modes of administration?

Rationale:

As outlined in the development plan, Elusys is considering the potential for two indications for ETI-204:

1. ETI-204 is indicated for the treatment of patients with (b) (4) inhalational anthrax due to *B. anthracis*. (b) (4)

[Redacted]

2.

(b) (4)

Elusys intends to provide a single dosage recommendation for both indications

(b) (4)

Elusys will develop a pediatric dosing recommendation for ETI-204 based on PK modeling of exposure in older subjects and animals together with knowledge of both pediatric dosing with other monoclonal antibodies as well as the pathophysiology of severe infection in children.

*Pharmacology/Toxicology internal comment only:*

(b) (4)

#### Sponsor Question 2.

As outlined in the development plan and discussed with FDA in May 2011, Elusys is conducting a segment II teratology study examining embryo-fetal development in rabbits in support of an indication for the treatment of inhalational anthrax. Elusys proposes that additional reproductive toxicity studies (e.g., fertility and pre/postnatal development) are not required to support use of ETI-204 administration in the post postexposure /presumed exposed population or to support pediatric use of the product. Does the Agency agree?

#### Rationale:

ETI-204 is an IgG<sub>1κ</sub> isotype immunoglobulin that binds specifically to protective antigen, thereby neutralizing the cell-binding component of anthrax toxin. ETI-204 has no endogenous target. ETI-204 is intended to be given as a single administration to subjects with symptomatic inhalational anthrax or to subjects known or presumed exposed to inhalational anthrax.

**Pharmacology/Toxicology response:** At this time, the Sponsor's proposal appears to be acceptable. However, should results of ongoing studies indicate the potential for unexpected toxicity, it may be necessary to conduct those studies.

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/s/  
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AMY C NOSTRANDT  
08/22/2012

WENDELYN J SCHMIDT  
08/22/2012

**MEMO TO FILE: IND 12,285**

**DATE:** June 15, 2012

**TO:** File, IND 12,285

**FROM:** Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist, DAIP

**THROUGH:** Wendelyn J. Schmidt, Ph.D.  
Pharmacology/Toxicology Supervisor, DAIP

**RE:** IND 12,285, supporting document no. 82, submitted 4/9/12, for Anthim® (ETI-204)

ETI-204 is a humanized IgG that binds to *B. anthracis* protective antigen (PA) that is being developed (b) (4) for post-exposure treatment in humans. The sponsor has provided a protocol for a pharmacokinetics and efficacy study of ETI-204 administered IM to rabbits exposed to *B. anthracis* spores by inhalation.

The following information was provided:

**Study title:** Pharmacokinetics of Intramuscularly Administered ETI-204 in Inhalational Anthrax Challenged Rabbits at Various Post-Exposure Time-Points

Study no.: AR035

Conducting laboratory and location:

(b) (4)

GLP compliance: Yes

Drug, lot #, and % purity: ETI-204 (Lonza Biologics), lot no. 250241

## Methods

Doses: Group 1: 0 (vehicle) at 18 hours post-challenge,  
Group 2: 16 mg/kg at 18 hours post-challenge,  
Group 3: 16 mg/kg at 24 hours post-challenge,  
Group 4: 16 mg/kg at 30 hours post-challenge

Frequency of dosing: Single dose

Route of administration: IM

Dose volume: 0.25 mL/kg

Formulation/Vehicle: ETI-204 as a 64 mg/mL solution in vehicle containing 40 mM histidine, 200 mM sorbitol, and 0.01% polysorbate 80

Species/Strain: New Zealand white (NZW) rabbits, certified *Pasteurella*-free

Number/Sex/Group: 10 males per group

Age: 6-7 months

Weight: 2.5-3.75 kg

Satellite groups: None

Unique study design: Prior to challenge, nose-only plethysmography will be performed to assess inhalation volume for each animal. Challenge exposure will be on Study Day 0. *Bacillus anthracis* spores, Ames strain, will be aerosolized using a Collison MRE 3-jet nebulizer and delivered in a nose-only dynamic aerosol chamber housed in a Class III biological safety cabinet. Exposure time will be calculated to achieve a target dose of  $200 \pm 50 \text{ LD}_{50}$  (LD<sub>50</sub> has been previously reported to be (b) (4) spores). The bacterial aerosol will be sampled downstream from the rabbits' nares using an all-glass impinger. Samples will be cultured, and actual challenge doses will be calculated using the viable/culturable spore concentration in the exposure atmosphere as sampled by the impingers and the inhaled volume of each animal as determined by plethysmography.

The Sponsor's table of study design and sampling schedule is shown below:

**Table 2. Timeline of Experimental Endpoints**

	D-7	SD0	18H	19H	24H	25H	30H	31H	36H	42H	48H	54H	66H	72H	78H	90H	96H	102H	D7	D14	D21	D28
Temp	X	X	Hourly observations and temperature collections will occur from 18 to 72 hr post-challenge, clinical observations (obs) and temps every 6 hr will occur from 78 hr to Day 7, twice daily obs and temps will be performed from Day -7 to Day 0, and from Day 8 to Day 27, and once daily on Day 28 prior to the study termination.																			
BW	X	Every other day from Day 0 until Study Termination.																				
Challenge		X																				
ETI-204 Dose			X <sup>a</sup>		X <sup>b</sup>		X <sup>c</sup>															
QT <sup>+</sup> -BAC <sup>1</sup>	X <sup>a,b,c</sup>	X <sup>a,b,c</sup>	X <sup>a,b,c</sup>		X <sup>a,b,c</sup>		X <sup>a,b,c</sup>	X <sup>a,b,c</sup>	X <sup>a</sup>	X <sup>b</sup>	X <sup>c</sup>	X <sup>a</sup>	X <sup>b</sup>	X <sup>c</sup>	X <sup>a</sup>	X <sup>b</sup>	X <sup>c</sup>	X <sup>a,b,c</sup>				
ETI-204 <sup>2,3</sup>	X <sup>a,b,c</sup>			X <sup>a</sup>	X <sup>d</sup>	X <sup>b</sup>	X <sup>d</sup>	X <sup>c</sup>	X <sup>b</sup>	X <sup>d,c</sup>	X <sup>b</sup>	X <sup>c</sup>	X <sup>d</sup>	X <sup>b</sup>	X <sup>c</sup>	X <sup>d</sup>	X <sup>b</sup>	X <sup>c</sup>	X <sup>d,b,c</sup>	X <sup>d,b,c</sup>	X <sup>d,b,c</sup>	X <sup>d,b,c</sup>
ATA <sup>2</sup>	X																					X
Anti-PA IgG <sup>2</sup>	X																					X
Tissue Fixation		Tissues will be collected from animals found dead, euthanized due to moribund status or at completion of study.																				
Tissue Burden		Bacterial load determination to be performed on tissues collected from animals found dead, euthanized due to moribund status, or at completion of study.																				

<sup>1</sup>Quantitative bacteremia, prior to treatment as applicable.

<sup>2</sup>Blood to be collected in EDTA tube.

<sup>3</sup>Blood to be collected in SST tube.

<sup>3</sup> Blood collection for ETI-204 for Group 1 at D-7 and 19H only, (collections for bacteriology will continue to be collected)

<sup>a</sup> Groups 1 and 2

<sup>b</sup> Group 3

<sup>c</sup> Group 4

<sup>d</sup>Group 2 only

## Observation and Times:

**Clinical observations / Mortality and moribundity:** Twice daily, with the following exceptions: hourly observations will be made from 18 hours post-challenge to 72 hours post-challenge, observations will be made every 6 hours from 78 hours post-challenge through Day 7, and observations will be made in the morning only on Day 28 prior to euthanasia.

Body temperature assessment will be made at the same time as clinical observations, beginning hourly at 18 hours post-challenge.

**Body weights:** Study Day 0 and every other day thereafter

**Blood collection:** Blood will be drawn from the central ear artery or marginal ear vein for the following assessments in order of priority:

**Quantitative bacteremia:** Animals will be bled for evaluation of bacteremia prior to challenge, at 18, 24, 30, and 36 hours post-challenge, at 24, 48, and 72 hours post-treatment, at 7, 14, and 21 days post-challenge, and at terminal necropsy. Each sample will consist of approximately 0.5 mL of whole blood, collected in a K<sub>2</sub>-EDTA serum separator tube.

**Serum ETI-204 concentration:** See toxicokinetics section below.

**Anti-PA IgG ELISA:** Evaluation of serum will be performed prior to challenge and at terminal necropsy. The protocol states that methodology will be added by protocol amendment.

**Immunogenicity (ATA):** Serum separated from 1 mL whole blood samples will be tested for anti-therapeutic antibodies prior to challenge and at terminal necropsy. This evaluation will be performed at (b) (4) along with pharmacokinetic evaluations.

**Hematology / Clinical chemistry / Urinalysis:** Not to be performed after initiation of the study. CBC and serum chemistry will be evaluated pre-test to assure the health of the animals starting the study.

Gross pathology: On Day 28, rabbits will be euthanized by IV overdose of euthanasia solution. Animals found dead or euthanized *in extremis* will be necropsied. Selected tissues will be collected for bacteriology and for fixation. Tissues to be sampled for bacterial burden will be lung, liver, spleen, brain, heart, kidney, and mediastinal lymph node. Samples of those tissues along with thymus, gross lesions, and the injection site will be retained in fixative.

Histopathology: Not planned for this study, but may be added if needed

Pharmacokinetics: Serum (at least 100 µL from a 1.0 mL whole blood sample) is to be collected for measurement of ETI-204 concentrations at Day -7, at 1, 12, 24, 48, and 72 hours post treatment, at 7, 14, and 21 days post-challenge, and at terminal necropsy in treated groups. The control group will be sampled on Day -7 and at 1 hour post-treatment only. Samples will be frozen at -70 to -90°C and shipped to (b) (4) for analysis. The protocol indicates that the laboratory will develop and validate an assay for the test article. Data analysis and calculation of pharmacokinetic parameters will be performed by (b) (4).

**Reviewer comments:**

This study is intended to be a preliminary study to assess the pharmacokinetics and optimal dosing times for ETI-204 administered IM to rabbits challenged with inhalational anthrax. Inclusion of only one gender is acceptable for this preliminary study. However, pivotal studies should include male and female animals. Pathological evaluations may need to be more comprehensive for a pivotal study, as well.

There is no need to forward comments to the Sponsor at this time.

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AMY C NOSTRANDT  
06/25/2012

WENDELYN J SCHMIDT  
06/25/2012

**MEMO TO FILE: IND 12,285 (081)**

**DATE:** 6/9/12

**TO:** File, IND 12,285 (081)

**FROM:** Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist, DAIP

**THROUGH:** Wendelyn Schmidt, Ph.D.  
Pharmacology/Toxicology Supervisor, DAIP

**RE:** IND 12,285, Anthim® (ETI-204) from Elusys Therapeutics, Inc.

The Sponsor has provided the attached toxicology study synopsis for comment. It is intended to compare drug substance produced by the old (Baxter) and current (Lonza) manufacturers.

In the absence of a complete protocol, the study design appears to be reasonable.

Study Synopsis

<b>Title of Study: A General Toxicity Study in Fischer Rats with Repeated Intravenous Administration of ETI-204 (Protocol Number TOX-001)</b>					
Investigator: (b) (4)					
Study center: (b) (4)					
Publications: None					
Study Period: 14 days in life					
Objective: The objective of this study is to assess the comparability of ETI-204 manufactured at two CMOs in terms of safety and TK.					
Methodology: This is a general toxicity study with repeated IV doses of ETI-204 in Fischer rats. The study compares the TK profile at 1, 3, and 10 mg/kg of ETI-204 from Lonza, and at 10mg/kg of ETI-204 from Baxter. Hematology, clinical chemistry, necropsy, and histopathology data will be generated and compared to those of the control group					
Study Design:					
Group	Number of Animals (Male/Female)	Number of Animals On Study (Male/Female)	Number of Animals for TK (Male/Female)	Dose Level (mg/kg)/Frequency	ETI-001 Source
1	22 (11/11)	16 (8/8)	6 (3/3)	0 (saline control) daily/SD1, 4, and 7	
2	22 (11/11)	16 (8/8)	6 (3/3)	1 mg/kg daily/SD1, 4 & 7	Lonza
3	22 (11/11)	16 (8/8)	6 (3/3)	3 mg/kg daily/SD1, 4 & 7	Lonza
4	22 (11/11)	16 (8/8)	6 (3/3)	10 mg/kg daily/SD1, 4 & 7	Lonza
5	22 (11/11)	16 (8/8)	6 (3/3)	10 mg/kg daily/SD1, 4 & 7	Baxter
For on-study rats, the blood samples will be taken prior to dosing and at the end of study for hematology and clinical chemistry parameters. The rats will be observed specifically for difficulty breathing, stridor, cyanosis, rash, erythema, and increased scratching behavior, in addition to general clinical observation. Animals will be euthanized on Study Day (SD) 14; necropsy and histopathology data will be collected.					
For rats in TK group, the bloods samples will be collected prior to dosing, 0.25 hr, 4h, 12h, 24h and 48h after first dose, SD 4 (prior to 2 <sup>nd</sup> dose), SD7 (prior to 3 <sup>rd</sup> dose), SD7 (1 h after 3 <sup>rd</sup> dose), SD10, and SD14. Anti-therapeutic antibody will be measured from blood samples collected prior to study and at the end of the study. Animals will be euthanized following the final blood collection.					
Number of Animals: 110 Fischer344 rats are randomized by weight into 5 groups of 22 animals (with each group containing ~50% male, ~50% female).					
Test Products, Dose, Mode of Administration, Batch Nos: ETI-204 supplied as Lot No: 250241 (Lonza) is administered at 1, 3, and 10 mg/kg IV bolus on Study Day (SD) 1, 4, 7 to Group 2, 3, and 4, respectively. ETI-204 supplied as Lot No: PBR-0024-001 (Baxter) is administered at 10 mg/kg via IV bolus on SD1, 4, 7 in Group 5.					
Reference Therapy, Dose, Mode of Administration, Batch Nos: Saline is administered via IV bolus at the same volume as 10 mg/kg ETI-204 from Lonza on SD1, 4, and 7.					
Duration of Treatments: Three doses of IV bolus in rats on SD 1, 4, and 7 followed by 7-day observation period.					
Criteria for Evaluation:					
<ul style="list-style-type: none"> <li>Safety and tolerability of ETI-204 in rats</li> <li>TK at different dose levels</li> <li>TK comparison from two different lots of ETI-204 at 10mg/kg</li> <li>Study drug induced hypersensitivity, such as for difficulty breathing, stridor, cyanosis, rash, erythema, and increased scratching behavior, will be observed closely and compared that of the control group</li> </ul>					
control group					
Statistical Methods:					
Descriptive statistical analysis on weight, food consumption, and clinical observation					
Non-compartment analysis for TK analysis					
Date of the Protocol:					

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AMY C NOSTRANDT  
06/13/2012

WENDELYN J SCHMIDT  
06/14/2012

**MEMO TO FILE: IND 12,285**

**DATE:** 2/15/2012  
**TO:** File, IND 12,285

**FROM:** Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist, DAIP

**THROUGH:** Wendelyn J. Schmidt, Ph.D.  
Pharmacology/Toxicology Supervisor, DAIP

**RE:** IND 12,285, SD#73, submitted 1/13/12, protocol for a segment II embryo-fetal developmental toxicology study in rabbits

ETI-204 is described as a high affinity, humanized, de-immunized monoclonal antibody targeting *Bacillus anthracis* protective antigen. It is being developed for IV treatment of patients with inhalation anthrax. It appears that the Sponsor intends for the treatment to consist of a single administration, but the exact dose is unknown at this time.

The Sponsor has conducted a pilot study in rabbits (EFT001 Phase A) to determine the optimal dosing regimen, due to the long half-life of the test article and the potential for anti-therapeutic antibody (ATA) formation. Three groups of time-mated rabbits were dosed with an IV injection of 32 mg/kg of ETI-204 (The protocol states that a single IV administration of 16 mg/kg was the "maximum efficacious dose" in the rabbit). Group 1 was dosed on Gestation Day (GD) 6, 10, 13, and 17, Group 2 was dosed on GD 6 and 10, and Group 3 was dosed on GD 13 and 17. The in-life portion of the study is completed and the PK and ATA analyses are underway.

The Sponsor states that the submitted protocol was designed assuming that every fourth day dosing would be adequate to maintain sufficient ETI-204 exposure during the period of organogenesis. Group sizes for the definitive study were increased by 8 animals, based on the assumption of potential for low level ATA formation, so that those animals that did develop ATA could be excluded from data analysis, leaving an adequate number of evaluable animals and litters.

The Sponsor has provided the following protocol:

**Study title: A definitive intravenous dose study for effects of ETI-204 on embryo-fetal development in rabbits**

Study no.: 11-085226 (EFT001 Phase B)  
Conducting laboratory and location: [REDACTED] (b) (4)  
GLP compliance: Yes  
Drug, lot #, and % purity: ETI-204 (Anthim®), lot no., etc., to be provided

## Methods

Doses:	0, 16, 32 mg/kg/dose
Frequency of dosing:	Approximately once every 4 days, on GD 6, 10, 13, and 17
Dose volume:	1 mL/kg/dose
Route of administration:	IV injection as a slow bolus over approximately 45 seconds
Formulation/Vehicle:	Sterile 0.9% sodium chloride for injection, USP
Species/Strain:	New Zealand White rabbits (Hra:(NZW))SPF
Number/Sex/Group:	30 time-mated females per treated group, 22 for the control group; 5-8 months of age, 2.5-3.7 kg, randomized by body weight
Satellite groups:	None
Study design:	Dams will be treated during the period of organogenesis as described above. Sacrifice and Caesarean section will be performed on GD 29.

## Observations

### Mortality / Clinical Signs

All animals will be observed at least twice daily for morbidity, mortality, injury, and availability of food and water. Detailed clinical examinations will be made daily from GD 6 through GD 29, at 60-90 minutes post-dose on dosing days.

### Body Weight

Body weight will be recorded on GD 0, 6, 9, 13, 17, 20, 25, and 29.

### Feed Consumption

Food consumption will be recorded daily and reported on the corresponding body weight days.

### Toxicokinetics

Blood will be sampled from a jugular or other suitable vein. Two cohorts of 11/group (Group 1) or 15/group (Groups 2 and 3) will be bled at alternating time points. The test animals will not be fasted before blood collection. The blood samples will be collected at all time points in the control group for consistency among groups; however, only the sample at 24 hours post-dose (approximately T<sub>max</sub>) will be analyzed. Time points for blood collection will be: prior to dose on GD 6, immediately post-dose (within 1 minute), at 4, 8, 16, 24 (GD 7), 48 (GD 8), 72 (GD 9), and 96 hours (GD 10 prior to dose), on GD 13 (prior to dose) and GD 17 (prior to dose), on GD 20 and 25, and on GD 29 (prior to termination). Serum will be collected and frozen at -50 to -90°C. Serum will be analyzed for ETI-204 concentration and appropriate toxicokinetic parameters will be calculated.

## Other

Blood samples (1 mL per time point) will be collected from the jugular, or other suitable vein from non-fasted animals for determination of the antibody level in the blood (ATA). All animals prior to the first dose and all surviving animals prior to necropsy will be sampled. Serum will be collected and frozen at approximately -70°C until analysis.

## Dosing Solution Analysis

The test article will be provided as a sterile, pre-formulated stock solution that will be stored refrigerated (2 to 8 °C) when not in use. Fresh dosing formulations will be prepared weekly stored refrigerated (2 to 8 °C) when not in use. If the test article stock solution is not provided sterile or if sterility is compromised, each dosing formulation will be filtered through a 0.22 µm low protein-binding filter prior to dosing.

Test article formulations prepared for the study will be evaluated for concentration. Samples will be taken while the preparations are stirring and after filtration (as applicable). Six 1 mL samples will be taken from the middle of the preparation at Week 1 and at Week 2; two will be analyzed, and four will be held as backup.

Homogeneity and stability will not be performed.

*Reviewer's comment: Data to justify this omission was not provided in the protocol and should be provided to the IND.*

## Necropsy

Euthanasia will be by overdose with sodium pentobarbital solution via the ear vein/artery or other suitable vein followed by exsanguination by severing the femoral vessels.

A complete necropsy will be performed on all does under procedures approved by a veterinary pathologist. A uterine examination will be conducted on all does that deliver, die, or are euthanized within 24 hours of scheduled euthanasia. Fetuses from these does will be evaluated. Does that deliver, die, or are euthanized earlier than 24 hours before scheduled euthanasia also will be given a uterine examination. Fetuses from these does will be examined externally to the fullest possible extent, and fetuses with findings will be placed in 10% neutral buffered formalin for possible further examination.

## Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

On GD 29, each female will be euthanized, followed immediately by cesarean section. The location of viable and nonviable fetuses, and early and late resorptions for each uterine horn will be recorded. The number of corpora lutea on each ovary will be recorded. The uterus will be excised, and gravid uterine weight will be recorded. The fetuses will be removed. The placentae will be grossly examined. Each implant will be categorized according to the following criteria:

- Viable fetus - responds to touch
- Nonviable fetus - does not respond to touch, no signs of autolysis
- Late resorption - recognizable fetal form, but undergoing autolysis
- Early resorption - implantation site, tissue has no recognizable fetal characteristics.

Uteri from females that appear nongravid will be opened and placed in 10% ammonium sulfide solution for detection of implantation sites. The foci, if detected, will be considered early resorptions, and data from this female will be included in mean calculations. If no foci are seen, the female is considered to be nonpregnant.

**Offspring (Malformations, Variations, etc.)**

Each fetus will be individually examined for external malformations and variations. Following completion of the external examination of all fetuses in the litter, each fetus will be euthanized via intraperitoneal administration of sodium pentobarbital solution, weighed, and subjected to a fresh fetal soft tissue dissection. Visceral malformations and variations will be recorded. The sex of each fetus will be documented. After dissection and examination of internal organs is complete, each fetus will be prepared and stained with Alizarin Red S for subsequent skeletal examination for malformations and developmental variations.

**Sponsor's Question:**

Does the Agency have any comments to the adequacy of the design and/or dosing rationale for study EFT001 to support BLA submission and labeling?

Agency response: The design of the study appears to be adequate. The exposure to the test article may be sufficient as long as it exceeds clinical exposure by a sufficient multiple. It is unclear whether or not the study will test to maternal toxicity. The protocol states that homogeneity and stability analysis of dosing solutions will not be performed; justification for this omission should be provided with the study report.

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/s/  
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AMY C NOSTRANDT  
02/21/2012

WENDELYN J SCHMIDT  
02/22/2012

**MEMO TO FILE: IND 12,285**

**DATE:** 2/10/2012  
**TO:** File, IND 12,285

**FROM:** Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist, DAIP

**THROUGH:** Wendelyn J. Schmidt, Ph.D.  
Pharmacology/Toxicology Supervisor, DAIP

**RE:** IND 12,285, SD#68, submitted 11/21/11, protocol for a nonclinical efficacy study with blinding

The Sponsor has provided the following protocol:

**1. Study title: AP203: Evaluating the Efficacy of Intravenous ETI-204 when Administered Therapeutically in the Cynomolgus Macaque Inhalational Anthrax Model**

Study no.:	1219-100005989
Conducting laboratory and location:	 (b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	ETI-204, Baxter Lot No. 103820-XI09-TR06 (PBR-0024-001) (bulk drug substance, BDS)

## Methods

Doses:	Vehicle control, 8, and 32 mg/kg/day
Frequency of dosing:	Single dose, administered within 3 hours of determining a positive serum PA-ECL assay result. If no positive ECL result has been obtained by 54 hours post-challenge, treatment will be administered at that time point.
Route of administration:	IV bolus
Dose volume:	1 mL/kg
Formulation/Vehicle:	Sterile saline
Species/Strain:	Cynomolgus macaques Pre-screened for tuberculosis, Simian Immunodeficiency Virus SIV), Simian T-lymphotropic Virus-1 (STLV-1), Macacine herpesvirus 1 (Herpes B virus), and Simian Retrovirus (SRV1 and SRV2).
Number/Sex/Group:	8
Age:	Juvenile (< 5 years)
Weight:	2.0-5.0 kg
Satellite groups:	None
Unique study design:	Study Day 0 will be the day of aerosol challenge for each cohort. After acclimation to the BL-3 facility, animals will be anesthetized and placed in a plethysmography chamber and Class III cabinet system. They will be aerosol-challenged with a targeted 200xLD <sub>50</sub> dose of <i>B. anthracis</i> spores (Ames strain) using a head-only exposure chamber. Aerosol concentrations of <i>B. anthracis</i> will be determined by collection of effluent streams by an in-line impinger, with samples plated for determination of CFUs. Blood samples will be taken at 24 hours and then every 6 hours until serum PA is detected by ECL assay, triggering the initiation of treatment.

## Observations

### Mortality / Clinical Signs

Animals will be observed approximately every 6 hours beginning 24 hours after the median challenge time and ending 8 days following the median challenge time. Thereafter, observations will be made twice daily, at least 6 hours apart, and once on study day 29 prior to euthanasia.

### Feed Consumption

Appetite will be monitored twice daily during the observation period.

### Hematology / Clinical Chemistry / Bacteriology

Blood will be collected as in the Sponsor's table below:

**Table 2. Blood collection and assay schedule.**<sup>a</sup>

Approximate Time Point	Blood Tube type/ Approximate Blood volume	Bacteremia (Culture)	CBC/ CRP	Serum for PA-ECL	Serum PA levels (via ELISA)	Serum for ETI-204 PK	Serum for Anti-ETI-204 Antibodies
Day -6	EDTA ~1.5 mL SST ~3.0 mL	X	X	X	X	X	X
^24hr PC	EDTA ~0.5 mL SST ~1.5 mL	X		X	X		
^30hr PC	EDTA ~0.5 mL SST ~1.5 mL	X		X	X		
^36hr PC	EDTA ~0.5 mL SST ~1.5 mL	X		X	X		
^42hr PC	EDTA ~0.5 mL SST ~1.5 mL	X		X	X		
^48hr PC	EDTA ~0.5 mL SST ~1.5 mL	X		X	X		
^54hr PC	EDTA ~0.5 mL SST ~1.5 mL	X		X	X		
PTT <sup>#</sup>	EDTA ~0.5 mL SST ~1.5 mL SPS ~1.0 mL	X <sup>#</sup>		X	X		
15 min PT	SST ~1.0 mL				X	X	
2hr PT	SST ~1.0 mL					X	
6hr PT	SST ~2.0 mL					X	
24hr PT	EDTA ~0.5 mL SST ~1.5 mL	X			X	X	
48hr PT	EDTA ~0.5 mL SST ~1.5 mL	X			X	X	
96hr PT	EDTA ~0.5 mL SST ~1.5 mL	X			X	X	
5 days PT	SST ~1.5 mL					X	
7 days PT	EDTA ~0.5 mL SST ~1.5 mL	X			X	X	
16 days PC	EDTA ~1.5 mL SST ~1.5 mL	X	X		X	X	
23 days PC	EDTA ~1.5 mL SST ~4.0 mL	X	X		X	X	
28 days PC	EDTA ~1.5 mL SST ~4.0 mL	X	X		X	X	X
Terminal <sup>b, c</sup>	EDTA ~1.5 mL SST ~4.0 mL	X	CRP only		X	X	X

PC = Post-Challenge, PTT = Prior to Treatment, PT = Post-Treatment

<sup>a</sup> Post-challenge pre-treatment bleed time points are relative to a median challenge time for a challenge cohort. Post-treatment bleed times are calculated from the time each animal's IV treatment ends. Blood samples will occur within ±60 minutes of the calculated time, except for the 15 min PT, 2hr PT, 6hr PT and 96hr PT samples which will occur within 5 min, 10 min, 15 min and 3 hours of their calculated times respectively. The Day -6, Day 5 PT, Day 7 PT, Day 16 PC, Day 23 PC and Day 28 PC bloods will be relative to the day of treatment or challenge.

<sup>b</sup> If collection is possible.

<sup>c</sup> Terminal samples will not be collected on animals euthanized at the end of study as Day 28 PC samples are already scheduled.

<sup>^</sup> Post-Challenge, pre-treatment sampling stops once decision to treat has been made.

<sup>#</sup> PTT Bacteremia enrichment performed on sample collected in SPS tube (see section 7.7)

## Gross Pathology

Surviving animals will undergo terminal sacrifice on Day 28. Gross necropsy will be performed on all animals found dead or euthanized (including the terminal sacrifice). Samples of brain,

spleen, lung, liver, kidney, and mediastinal or bronchial lymph node will be collected for qualitative bacteriology.

### **Histopathology**

Tissues including, but not limited to, brain/meninges, lungs, liver, spleen, spinal cord, kidney, mediastinal and bronchial lymph nodes, and gross lesions will be collected, fixed, and examined microscopically.

### **Pharmacokinetics**

Pharmacokinetic parameters will be determined, including AUC, C<sub>max</sub>, T<sub>max</sub>, volume of distribution, clearance, and half-life.

The Sponsor presents the following proposal for blinding the study, along with the related question:

“The Study Director, Sponsor, microbiologists, pathologist, technicians performing the dosing, and all technicians assessing the animals are blinded to the contents in each dosing vial. Once animals have been randomized to a group, challenge day, and challenge order, the results will be sent to the Study Coordinator and Quality Assurance Auditor. QA will verify the randomization and then provide the Study Director with the randomization information regarding animal ID, challenge day, and challenge order but NOT the group assignments. Group assignments will remain blinded to the Study Director and Sponsor until sample analysis is complete and data audited. The paperwork that documents the treatment group and dosage information on each vial will be maintained by the Quality Assurance Unit until the collections/assays performed at the (b) (4) are complete; bacteremias (quantitative), tissue bacterial assessments, hematology, C-reactive protein (CRP), and PA-ELISA (quantitative). Completion of these collections/assays will be defined as the completion of audited final summary tables. Once the summary tables audit is complete, the group assignments will be released to the Study Director but the Study Director will not release the group assignment information to the study pathologist or neuropathologist. The pathologist and neuropathologist will remain blinded until their respective histopathology slides have been read and documentation has been provided to the Study Director indicating this is complete.

Does the Agency agree that employing the blinding process described above addresses the Agency’s comment and is sufficient to ensure integrity of blinding for the definitive efficacy studies?”

*Reviewer’s comment: From a pharmacology/toxicology standpoint, the proposal is reasonable. However, the request for blinding of the study came from another discipline. It would be best if definitive evaluation of the blinding proposal and the answer for the Sponsor came from the requesting discipline.*

*No Pharmacology/Toxicology comments are recommended for the Sponsor at this time.*

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/s/  
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AMY C NOSTRANDT  
02/17/2012

WENDELYN J SCHMIDT  
02/22/2012