U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

ANIMAL EFFICACY STUDIES

NDA/BLA #:	BLA 125509
Drug Name:	Anthim®(obiltoxaximab) 16 mg/kg IV
Indication(s):	Treatment and Prophylaxis of Inhalational Anthrax due to *Bacillus anthracis*
Applicant:	Elusys Therapeutics, Inc.
Date(s):	Submission date: 3/20/2015. PDUFA due date: 3/18/2016
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Keywords: Animal efficacy studies

Table of Contents

LIST OF TABLES

LIST OF FIGURES

1 EXECUTIVE SUMMARY

The applicant submitted this BLA to seek the approval of a monoclonal antibody ETI-204 (Obiltoxaximab) for the treatment of adult and pediatric patients with inhalational anthrax due to *Bacillus anthracis* in combination with appropriate antibacterial drugs and for prophylaxis of inhalational anthrax when alternative therapies are not available or are not appropriate. Due to ethical reasons, the Animal Rule was used for the development of this product. There were 22 efficacy studies in cynomolgus monkeys or New Zealand White rabbits used to evaluate the efficacy of ETI-204 alone (monotherapy) for treatment, post-exposure prophylaxis, and pre-exposure prophylaxis. A separate statistical review will assess the effect of ETI-204 when given with antibacterial therapy.

The studies were conducted with varying doses, different administration times before or after exposure, two administration routes (intravenous or intramuscular), and products manufactured at different manufacturing facilities. This led to a wide range of study results. These studies were randomized and some were blinded. Animals were challenged with *B. anthracis* spores. The primary endpoint was survival at the end of a study, usually 28 days after challenge. Most of these 22 studies demonstrate a statistically significant treatment effect. The number of studies demonstrating a significant treatment effect was consistent with the underpowered design in some studies. Bacteremia level or protective antigen (PA, a toxin from anthrax) level prior to treatment was found to be associated with survival and a high level of bacteremia or PA could be a reason for failure in some studies.

The proposed dose of 16 mg/kg IV was found to be effective in two monkey and two rabbit studies where treatment was started after the development of clinical signs/symptoms (i.e., treatment studies). Prophylaxis studies were conducted where treatment was initiated either prior to exposure (pre-exposure prophylaxis studies) or post-exposure. In post-exposure prophylaxis studies, doses given closer to the time of challenge gave higher survival rates, as did IV dosing compared to IM dosing, and higher doses compared to lower doses. A 16 mg/kg IM dose given to monkeys and rabbits by 24 hours was effective. In pre-exposure studies, a 16 mg/kg IM dose was effective when treatment was given 30 minutes to 3 days prior to challenge. We can extrapolate that the IV dose would also be effective in the prophylaxis setting. Additionally, in a re-challenge trial, 100% of the animals who were previously treated with ETI-204 16 mg/kg IV survived after a second challenge and 89% of the animals who were previously treated with ETI-204 16 mg/kg IV and levofloxacin survived after a second challenge.

Overall, these studies demonstrated that 16 mg/kg IV of ETI-204 was effective in the treatment, post-exposure prophylaxis and pre-exposure prophylaxis of inhalational anthrax. There is adequate evidence that the Lonza product, which is the to-be-marketed product, is effective in both the treatment and prophylaxis of anthrax using data from rabbits and monkeys.

2 INTRODUCTION

2.1 Overview

A medical product for treatment and prophylaxis of anthrax infection can be administered for treatment after development of clinical signs and/or symptoms, for pre-exposure prophylaxis (PrEP) before exposure to *Bacillus anthracis* (*B. anthracis*) spores, and for post-exposure prophylaxis (PEP) after exposure to *B. anthracis* spores, but before the development of clinical signs and/or symptoms.

ETI-204 (Obiltoxaximab) is a monoclonal antibody [redacted (b) (4)] that binds the protective antigen (PA) of *B. anthracis*. PA is the cell-binding component of anthrax and a key component of *B. anthracis* virulence and pathogenesis. The proposed indication is for the treatment of adult and pediatric patients with inhalational anthrax due to *B. anthracis* in combination with appropriate antibacterial drugs and for prophylaxis of inhalational anthrax when alternative therapies are not available or are not appropriate. Regarding prophylaxis, the applicant has submitted both pre- and post-exposure prophylaxis studies. ETI-204 is to be administered as a single intravenous (IV) 16 mg/kg infusion over 90 minutes.

The applicant only seeks the approval for IV administration for ETI-204. [redacted (b) (4)] The applicant considers that ETI-204 has two advantages: 1) it can be administered IV faster than raxibacumab (90 minutes versus 135 minutes); [redacted (b) (4)].

For the development of a product for the prophylaxis and treatment of anthrax, it is unethical or not feasible to conduct clinical trials in humans. Therefore, the "Animal Rule" regulation is used for the development of this product. In order to demonstrate efficacy of ETI-204, animal studies were conducted in cynomolgus monkeys or New Zealand White (NZW) rabbits. The development of ETI-204 dates back to 2003. In 2003, a pre-IND meeting was held to discuss chemistry and manufacturing controls, preclinical, and clinical development plans. In 2005 a new IND 12285 was created. During the IND development two issues of concern were raised with the applicant. The first was regarding the demonstration that ETI-204 would not diminish the efficacy of an antibacterial product given concomitantly with ETI-204. The applicant addressed this by conducting both rabbit and monkey studies in which ETI-204 given with an antibacterial was compared against antibacterial alone. This added-benefit of ETI-204 will be addressed in a separate statistical review by Dr. Ling Lan. Another issue of concern was regarding the change in manufacturing facilities during the development of the product. The majority of the animal efficacy studies were conducted using the product manufactured at the Baxter manufacturing site. Later studies were conducted using product from the Lonza manufacturing site. Due to the failure of ETI-204 to obtain the expected survival rates in the first

monkey treatment study (AP203) using the Lonza product, concern arose as to if it signified a problem with the product. The applicant explored possible reasons for the failed study and hypothesized that the failure was due to the increased severity of illness just prior to treatment as measured by both pre-treatment bacteremia and PA levels. The applicant also conducted an additional monkey treatment study (AP202) under a special protocol assessment. This review will assess this concern as well as the general efficacy of ETI-204 in the treatment and prophylaxis of anthrax.

This submission contains 26 studies which assess the efficacy of ETI-204. A total of 22 studies, most of which contained multiple doses of ETI-204, are included in this review. These 22 studies were all randomized trials with ETI-204 as monotherapy. There are 5 treatment studies in monkeys and 4 in rabbits using IV administration. There are 3 post-exposure prophylaxis studies in monkeys and 6 in rabbits using either the IV or IM administration. Additionally, there are 4 supportive studies in monkeys and rabbits assessing the pre-exposure prevention and re-challenge. The four studies not covered in this review include AM002 (a pre-exposure prophylaxis study in mice) and three combination studies that did not include an ETI-204 alone group. As stated above, Dr. Ling Lan's statistical review of this BLA will cover antibacterial combination efficacy studies.

All of the studies with a name beginning with "A" were conducted by the applicant. "AP" signified monkey studies and "AR" signified rabbit studies. Studies beginning with "NIAID" were conducted by the National Institute of Allergy and Infectious Diseases.

2.2 Data Sources

Data sources, including all material reviewed, e.g. applicant's study reports, data sets analyzed, are located at FDA's internal server: \\cdsesub1\evsprod\BLA125509.

3 STATISTICAL EVALUATION

3.1 Data and Analysis Quality

In general the submitted data sets were of high quality. All data sets were submitted in AdaM and SEND standard format. However, there are some minor issues. For example, some variables, such as age, were numerical variables in some studies and character variables in other studies. Some important variables, such as challenge time, were available in the SEND data sets, but not available in the analysis data sets in some studies. These problems require detailed modification of analysis programs when analyzing data sets from different studies, thus a longer review time. Nevertheless, in general, we could replicate the primary efficacy analysis results and main study results. The study designs and analyses were relatively simple and straightforward, and usually no separate statistical analysis plans were written.

3.2 Evaluation of Efficacy

3.2.1 Introduction

This section will discuss the results of animal efficacy studies by study type. Section 3.2.2 will review the efficacy from the treatment studies in monkeys and rabbits using IV administration. Section 3.2.3 reviews the efficacy of ETI-204 in the post-exposure prophylaxis studies in rabbits and monkeys. These studies contained both IV and IM administration. The information from the IM administration is considered supportive information. Sections 3.2.4 and 3.2.5 briefly review the pre-exposure prophylaxis and re-challenge studies. An appendix to this document contains detailed reviews of each of the 22 individual studies. Section 6.9 of the appendix provides a table that summarizes all 22 studies.

3.2.2 IV Treatment Studies

The efficacy of ETI-204 as a monotherapy treatment was evaluated in 9 studies in monkeys or rabbits. Table 1 includes all treatment studies covered in this review.

3.2.2.1 Study Design

Six studies of ETI-204 as a monotherapy for the treatment of inhalational anthrax were conducted by the applicant in monkeys (AP201, AP202, AP203, and AP204) and in rabbits (AR021 and AR033). AR021 and AP201 were the first studies with ETI-204. AR033, AP203 and AP204 were conducted to explore the dose-response relationship. AP202 was to confirm the efficacy of the 16 mg/kg dose (Lonza) in monkeys (primary analysis) and to compare the efficacy of ETI-204 from two manufacturers (Lonza and Baxter).

In addition to these 6 monotherapy treatment studies, there were 3 NIAID sponsored studies, NIAID 1030, 1045 and 1056. These 3 NIAID sponsored studies were conducted to assess the combination of ETI-204 and antibacterials but they also included an ETI-204 alone treatment group and an untreated group and those relevant treatment arms will be summarized under the treatment studies in this review. NIAID 1030 and 1045 were exploratory studies in rabbits that were conducted for the development of an animal model to assess the additive benefit of ETI-204 in combination with an antimicrobial in the treatment of inhalational anthrax. NIAID 1056 was an exploratory model development study in monkeys to investigate the feasibility of delaying treatment relative to the onset of toxemia.

In these treatment studies, animals were randomized to receive ETI-204 IV at various doses or to receive placebo or no treatment. Randomization for most studies took place prior to challenge, though in AP202 randomization took place just prior to treatment. Animals were challenged with a target dose of 200 LD_{50} anthrax spores.

Table 1: List of all treatment studies included in analysis

Study and product	Design	Treatment period	Follow-up period	# of Animals per Arm (randomized)
Monkey treatment studies				
AP201 Baxter	Randomized	Single dose IV	30 days	Placebo: 15 ETI-204 4 mg/kg: 14 ETI-204 8 mg/kg: 14
AP202 Baxter vs. Lonza	Randomized, blinded	Single dose IV	28 days	Placebo: 17 Lonza 16 mg/kg ETI-204: 17 Baxter 16 mg/kg ETI-204: 17
AP203 Lonza	Randomized, blinded	Single dose IV	28 days	Placebo: 16 ETI-204 8 mg/kg: 16 ETI-204 32 mg/kg: 16
AP204 Baxter	Randomized	Single dose IV	28 or 56 days	Placebo: 16 ETI-204 4 mg/kg: 16 ETI-204 16 mg/kg: 16
NIAID 1056 Baxter	Randomized, open-label	Single dose IV	28 days	Untreated control: 8 ETI-204 8 mg/kg: 8
Rabbit treatment studies				
AR021 Baxter	Randomized, open-label	Single dose IV	28 days	Placebo: 9 ETI-204 1 mg/kg: 9 ETI-204 4 mg/kg: 17 ETI-204 16 mg/kg: 17
AR033 Baxter	Randomized, blinded	Single dose IV	28 days	Placebo: 14 ETI-204 1 mg/kg: 14 ETI-204 4 mg/kg: 14 ETI-204 8 mg/kg: 14 ETI-204 16 mg/kg: 14
NIAID 1030 Baxter	Randomized, open-label	Single dose IV	28 days	Control: 6 ETI-204 8 mg/kg: 16
NIAID 1045 Baxter	Randomized, open-label	Single dose IV 72 hrs post-median challenge	28 days	Control: 6 ETI-204 8 mg: 16

Treatment studies, as opposed to post-exposure prophylaxis studies, begin randomized treatment after the development of symptoms when the disease is more established in the animal and more difficult to treat. In these studies ETI-204 or placebo was administered to rabbits or monkeys exhibiting clinical signs or symptoms of systemic anthrax. PA-ECL and/or significant increase in body temperature (SIBT) were used as a treatment trigger. SIBT was defined as a temperature reading ≥ a two standard deviation (SD) increase from (daily) baseline temperature either three consecutive times or two consecutive times twice (measured hourly). SIBT was not used in monkeys because of their strong diurnal temperature rhythms. If no trigger was observed, some studies treated remaining animals at a fixed time post challenge, 54 hours in monkey studies and in rabbit study AR033 or 72 hours in AR021. Only one treatment study (NIAID 1045) did not use a treatment trigger. In this rabbit study ETI-204 was administered at 72 hours post challenge

for all animals. This was at a later time point than the development of symptoms in the two rabbit treatment studies conducted by the applicant, AR021 and AR033. So though symptoms were not used as the trigger for treatment, this study is considered as a treatment study.

Animals were monitored and blood collected regularly until the end of the trial.

3.2.2.2 Primary Efficacy Endpoint and Analysis Population

The primary efficacy endpoint was survival at the end of the study (usually 28 days post-challenge).

In the protocols, analysis populations were usually not explicitly or clearly defined. Relevant information is often scattered in the statistical analysis section and/or gathered from the analysis results. If more than one analysis population was used, sometimes it was not clear if the different analyses were ordered (primary, co-primary, or secondary etc). Furthermore, analysis populations varied from study to study. Some studies included all randomized animals, and some included all randomized and treated animals. Another commonly used analysis population included all randomized animals that were positive for bacteremia prior to study treatment (bacteremic population).

In this review the analyses will be presented in all randomized animals that received treatment referred to as the mITT population. Additional analyses will be presented in the population of bacteremic animals who received treatment.

3.2.2.3 Statistical Methods

Sample size calculation
Sample size calculations were usually based on Fisher's exact method using a two-sided type I error rate of 0.05, without considering multiple comparisons in a study.

Analysis methods
Most of the studies used a Fisher's exact test to test if there was a difference between two groups in the individual study reports. Detailed information on the tests used in each study is available in the Appendix. In the applicant's submitted Overview of Efficacy and Safety section, results from Boschloo's test were included. One-sided p-values from Boschloo's exact test with a Berger-Boos correction of gamma=0.001 were presented with statistical significance declared at the 0.025 one-sided level. Some studies used no tests, presenting only survival proportions with 95% confidence intervals for each group.

Boschloo's exact test was recommended by the FDA during the protocol review of AP202 because it is a more powerful test than Fisher's exact for detecting significant differences between groups while controlling the type I error. In this submission p-values from the Boschloo's test and/or Fisher's exact were reported. Because Fisher's exact test is too conservative, to be consistent across all studies, one-sided p-values from Boschloo's tests were

reported for the comparison of survival proportions. P-values from Fisher's exact tests were available in some study reports, but not reported in this review.

In many studies, more than 2 treatment groups were included and there were multiple comparisons among groups. In this review, if multiple comparisons needed to be adjusted for, along with unadjusted p-values, a significance level (0.025 for one-sided test or 0.05 for two-sided) divided by the number of comparisons was reported as the level to use for the determination of significance.

In our analyses of the binary outcome of survival, we also calculated Bonferroni adjusted 95% confidence intervals in order to adjust for the multiple comparisons discussed above. These confidence intervals are two-sided (1-0.05/k) confidence intervals where k is the number of treatment arms being compared to the control in a study. For example, if there are two treatment arms being compared to placebo the confidence intervals will be (1-0.025) or 97.5% confidence intervals. These calculated adjusted 95% confidence intervals allow with 95% confidence that all the adjusted confidence intervals simultaneously cover the true treatment effects.

3.2.2.4 Results and Conclusions

3.2.2.4.1 *Treatment studies in monkeys*

Survival

The following table shows the results of the five monotherapy treatment studies in monkeys in the mITT population. Both the 95% confidence intervals and the adjusted confidence intervals are reported. The p-value and the related significance level are also given. In Study AP202, the primary analysis was the comparison of Lonza ETI-204 with placebo. Therefore, no multiple comparison adjustment is needed.

Table 2: Survival proportions in monotherapy treatment studies in cynomolgus monkeys (mITT population)

Study **Product** **Primary endpoints**	**Dose (mg/kg)**	**Survival n/N (%)**	**Difference in proportion [95% CI] [Adjusted 95% CI]**	**One-sided p-value (significance level)**
AP202 **Lonza vs Baxter** (Day 28 survival)	0	0/17 (0)		
	16 (Lonza)	5/16 (31)	0.31 [0.08, 0.59]	0.0085* (0.025)
	16 (Baxter)	6/17 (35)	0.35 [0.11, 0.62]	0.0046* (0.025)
AP203 Lonza (Day 28 survival)	0	2/16 (12.50)		
	8	1/16 (6.25)	-0.063 [-0.329, 0.194] [-0.358, 0.238]	0.761
	32	6/16 (37.50)	0.25 [-0.065, 0.541] [-0.114, 0.577]	0.064
AP204 Baxter (Day 56 survival)	0	1/16 (6.3)		
	4	4/16 (25.0)	0.188 [-0.090, 0.473] [-0.135, 0.513]	0.1077

Study Product Primary endpoints	Dose (mg/kg)	Survival n/N (%)	Difference in proportion [95% CI] [Adjusted 95% CI]	One-sided p-value (significance level)
	16	8/16 (50.0)	0.438 [0.113, 0.703] [0.070, 0.733]	0.0036* (0.0125)
AP201 Baxter (Day 30 survival)	0	2/14 (14.3)		
	4	11/14 (78.6)	0.643 [0.260, 0.879] [0.206, 0.898]	0.00046* (0.0125)
	8	11/15 (73.3)	0.590 [0.207, 0.841] [0.162, 0.864]	0.00075* (0.0125)
NIAID 1056 Baxter (Day 28 survival)	0	0/8 (0)		
	8	4/8 (50)	0.50 [0.058, 0.843]	0.014* (0.025)

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at the specified significant level with Bonferroni adjustment for multiple comparisons, if needed

Four out of the five studies showed significant results for an 8 mg/kg dose or higher. However, there is large variability across the studies in survival rate. Concern was raised over the lack of significant findings in study AP203, especially because this study used the Lonza product, the proposed commercial product. The applicant hypothesizes that it was due to the variability across studies in the severity of illness of the animals just prior to treatment. This will be explored later in this section.

The concern over the results of AP203 led the applicant to conduct AP202 which contained both the Baxter and the Lonza product at the proposed dose of 16 mg/kg. Numerically, the survival proportions in the Lonza and Baxter groups in AP202 were comparable (31% versus 35%); however, the study was not powered to compare the efficacy of these two products. The 95% confidence interval for the difference between the two products is too wide [-0.365, 0.290] to make a meaningful non-inferiority comparison. Though the study was not powered to statistically compare the two products, they both were found to be superior to placebo. The results from other studies using Lonza ETI-204 (i.e., some post-exposure prophylaxis and pre-exposure prophylaxis studies with IV or IM administration) will provide additional support for the efficacy of ETI-204 and will be discussed further later in the review.

There were only a few differences between the all treated and the bacteremic analysis population. Out of these 5 studies only 3 animals were not bacteremic at the time of treatment. The following table shows the few cases where the survival proportions in the bacteremic analysis population in monkey studies are different than in the all treated analysis population. The conclusions remain the same as in the all treated analysis population.

Table 3: Survival proportions in monotherapy treatment studies in cynomolgus monkeys (bacteremic population)

	Dose (mg/kg)	n/N (%)	Difference in proportion [95% CI] [Adjusted 95% CI]	One signed p-value (significance level)
AP203 Lonza	32	5/15 (33.33)	0.208 [-0.104, 0.510] [-0.148, 0.550]	0.104 (0.0125)
AP204 Baxter	16	7/15 (46.7)	0.404 [0.089, 0.681] [0.048, 0.712]	0.0058* (0.0125)
AP201 Baxter	4	10/13 (76.9)	0.626 [0.226, 0.867] [0.179, 0.888]	0.00078* (0.0125)

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at the specified significant level with Bonferroni adjustment for multiple comparisons

The relationships between challenge dose, bacteremia, and PA-ELISA prior to treatment in monkey monotherapy studies

As discussed above, the applicant hypothesized that the failure of AP203 to find a significant treatment effect was likely due to the severity of illness at the time of treatment. All five studies collected information on challenge dose, bacteremia prior to treatment and PA-ELISA prior to treatment. In this section we explore the relationship between these three variables in these 5 studies. Note that because NIAID 1056 contain untreated controls, these animals did not have a "pre-treatment" bacteremia or PA-ELISA and are not included in the analyses in this section.

The following table shows the Pearson correlation coefficients between challenge dose, $\log_{10}$ bacteremia, and $\log_{10}$ PA-ELISA prior to treatment. The correlation between challenge dose and bacteremia was low, although it was statistically significant. The correlation between challenge dose and PA was even lower and not statistically significant. It means that challenge dose was not strongly linearly correlated with bacteremia or PA levels. The correlation between bacteremia and PA was quite strong at 0.72.

Table 4. Pearson correlation coefficients between challenge dose, $\log_{10}$ bacteremia, and $\log_{10}$ PA-ELISA prior to treatment, including two-sided p-value and sample size for each correlation coefficient

	$\log_{10}$ bacteremia	$\log_{10}$ PA-ELISA
Challenge dose	0.19826 0.0052* n=197	0.03685 0.6147 n=189
$\log_{10}$ bacteremia		0.72450 <0.0001* n=189

*Significant at a two-sided 0.05 significance level

Figure 1 shows no consistent clear linear relationship between challenge dose and bacteremia from the 5 monkey treatment studies. Note that the animals that survived, indicated by the "+" symbol, occur at all challenge doses. However, there does seem to be a pattern with animals

with lower bacteremia being more likely to survive. No animals survived with a $\log_{10}$ bacteremia prior to treatment greater than 5, except for one animal in AP202.

Figure 2 shows no consistent clear linear relationship between challenge dose and log PA-ELISA from the 5 monkey treatment studies. Animals with lower PA-ELISA levels were more likely to survive. No animals survived with a log10 PA-ELISA prior to treatment greater than 2.23. It indicates that PA-ELISA level was an important factor on survival.

Figure 1. Challenge dose and bacteremia prior to treatment by study, dose, and survival status in monkey treatment studies

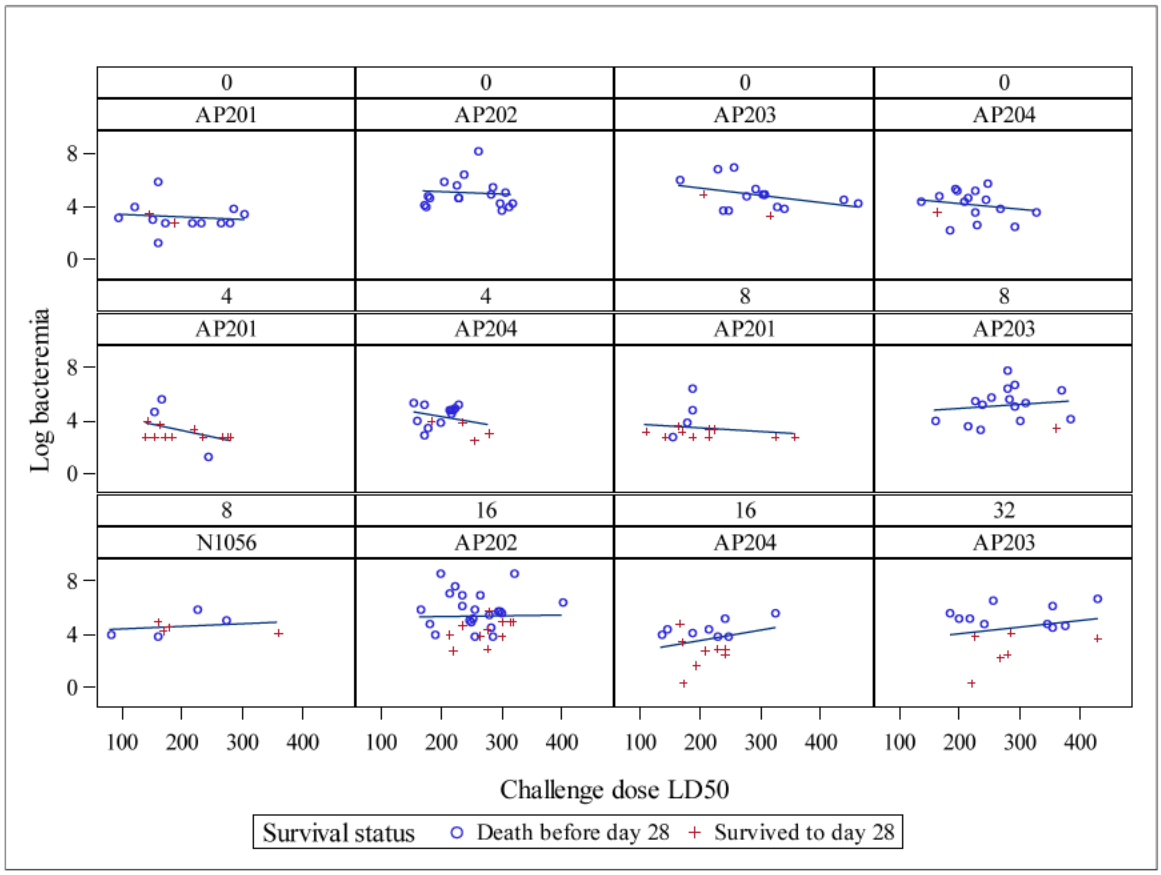


Figure 2. Challenge dose and bacteremia prior to treatment by dose, study, and survival status in monkey treatment studies

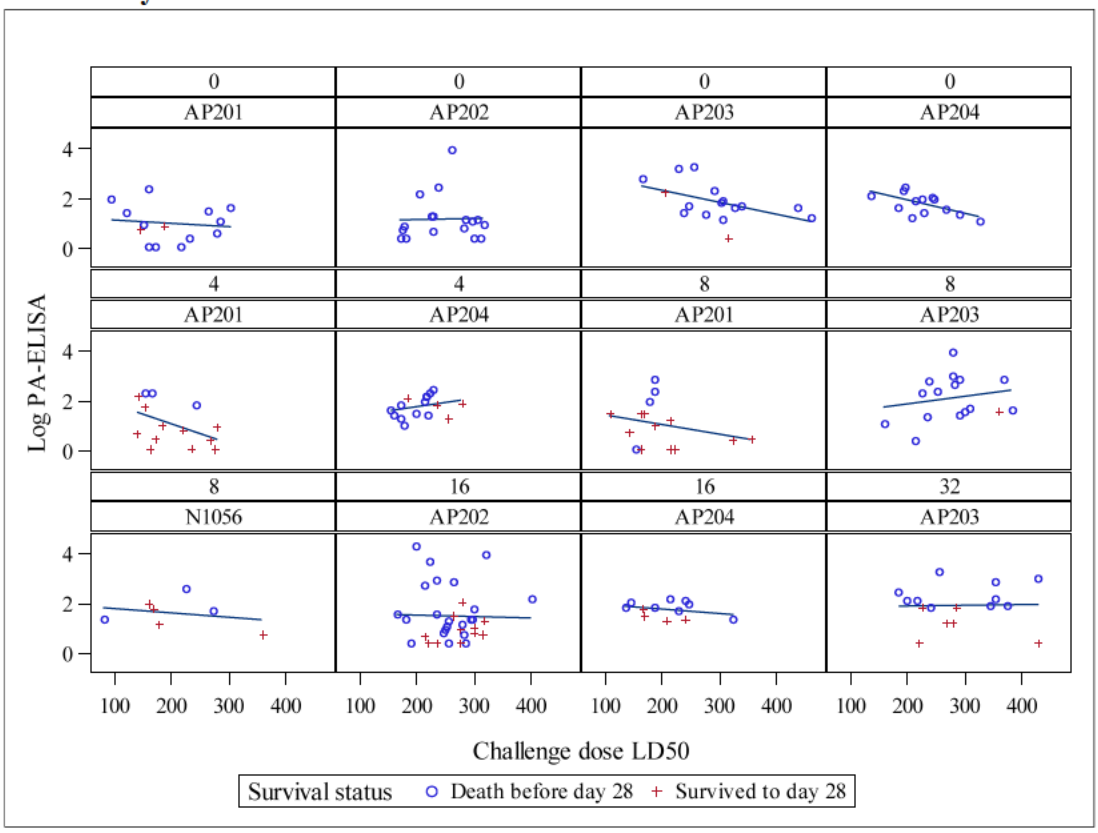
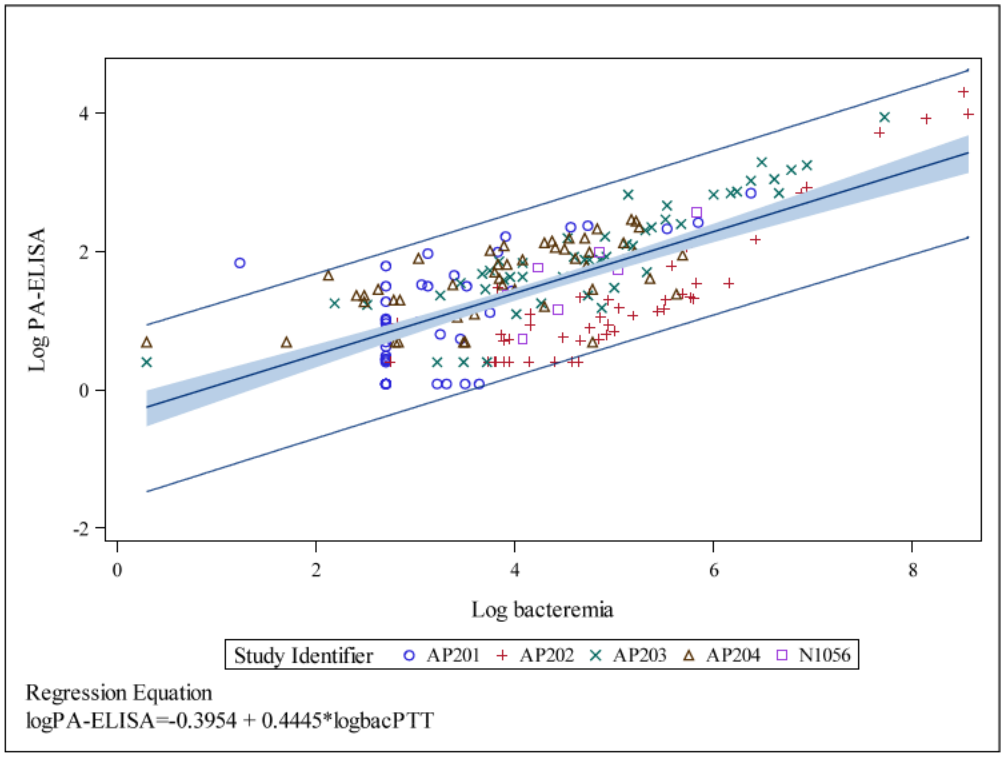


Figure 3 shows the relationship between bacteremia and PA-ELISA prior to treatment in the monkey treatment studies. The solid line in the middle is the regression line, and the dotted line is the 95% upper and lower confidence limits for individual predicted values and the shaded band is the 95% upper and lower confidence limits for the expected values of log PA-ELISA level. The Pearson correlation coefficient was 0.723 (p-value<0.0001), indicting a very strong positive linear relationship. Interestingly, the data points from Study AP202 were more likely to be under the fitted line and data points from AP203 and AP204 were more likely to be above the fitted line. This indicates that given the same bacteremia level, PA levels in AP202 were more likely to be lower than the expected values, and in AP203 and AP204 PA levels were more likely higher than expected from the five studies. Although AP202 and AP203 had similar bacteremia levels, AP203 had a highest PA-ELISA level. The high bacteremia levels and non-proportionally high PA-ELISA levels relative to bacteremia in AP203 may explain the failure of this study. Therefore, in the next section a regression analysis was used to control for the effects of bacteremia and PA-ELISA on survival.

Figure 3. The relationship between bacteremia and PA-ELISA prior to treatment in monkey treatment studies

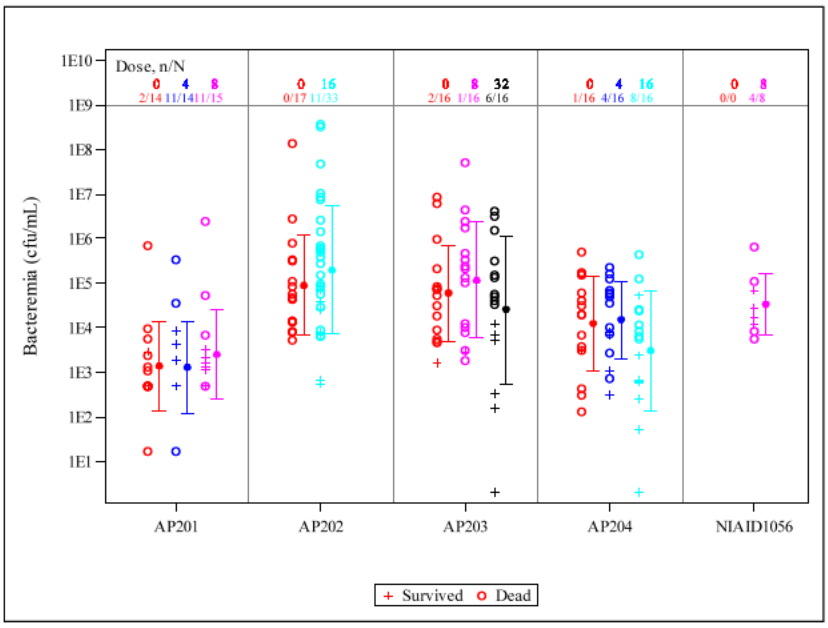


Bacteremia and PA prior to treatment on survival rates across monkey treatment studies

As described previously, AP203, the first monkey treatment study using the to-be-marketed Lonza version of ETI-204, failed to show a treatment effect of either an 8 mg/kg or 32 mg/kg dose. The applicant hypothesized that this was due to the severity of illness at baseline across the studies and not due to the Lonza product. Study AP202 was conducted using both the Lonza and Baxter product at 16 mg/kg and both showed significant effect over placebo. The previous section determined that challenge dose was unlikely to be an adequate measure of baseline disease severity that would explain the different results across studies. Pre-treatment bacteremia and PA-ELISA level might be able to explain the variable survival results. This section will explore this hypothesis.

As the following graph shows, there was considerable variability both within and across studies in bacteremia prior to treatment. Animals with a lower bacteremia level prior to treatment were more likely to survive as shown by a plus sign in the graph. In Study AP201, the bacteremia levels were lower compared with other studies, and AP202 and AP203 had the highest geometric means and the lowest survival proportions in the treated groups.

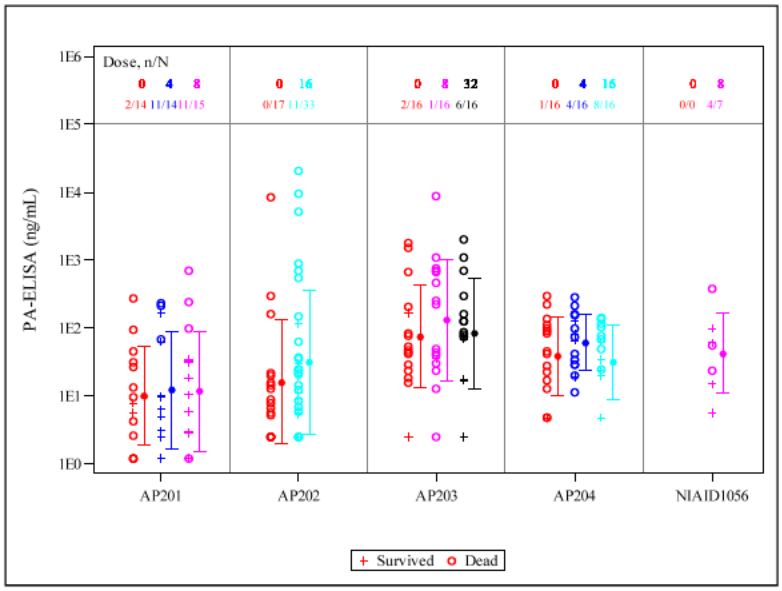
Figure 4. Bacteremia prior to treatment by study and treatment in monkey treatment studies



n/N: survival proportion

As the following graph shows, there was considerable variability in PA-ELISA prior to treatment. Animals with a lower PA-ELISA level prior to treatment were more likely to survive as shown by a plus sign in the graph. In Study AP201, the PA-ELISA levels were the lowest compared with other studies. AP203, the failed trial, had the highest geometric means and the lowest survival proportions in the treated groups.

Figure 5. PA-ELISA prior to treatment by study and treatment in monkey treatment studies



n/N: survival proportion

Bivariate analyses of survival with bacteremia and PA-ELISA in monkey treatment studies were conducted to explore how these variables might help predict survival. The following table shows the survival status by bacteremia prior to treatment. It is clear that within each dose group, as the bacteremia levels increased, survival proportions decreased.

Table 5. Survival by bacteremia prior to treatment in monkey treatment studies

Bacteremia	0 mg/kg N=63	4 mg/kg N=30	8 mg/kg N=39	16 mg/kg N=49	32 mg/kg
$<10^4$	4/28 (14.3%)	15/20 (75%)	12/19 (63.2%)	12/18 (66.7%)	5/5 (100%)
10^4 - $<10^6$	1/31 (3.2%)	0/10 (0)	4/15 (26.7%)	7/23 (30.4%)	1/8 (12.5%)
10^6 or higher	0/4	0	0/5	0/8	0/3

The following table shows the survival status by PA-ELISA prior to treatment. It is clear that there was a relationship between PA-ELISA levels and survival in the 8 and 16 mg/kg groups: as the PA-ELISA levels increased, survival proportions decreased.

Table 6. Survival by PA-ELISA prior to treatment in monkey treament studies

PA-ELISA	**0 mg/kg N=60**	**4 mg/kg N=30**	**8 mg/kg N=38**	**16 mg/kg N=45**	**32 mg/kg N=16**
<10	3/18 (16.67%)	8/8 (100%)	7/9 (77.8%)	7/13 (53.9%)	2/2 (100%)
10 - <50	0/22 (0%)	2/8 (25%)	7/14 (50%)	6/16 (37.5%)	2/2 (100%)
50 or higher	1/20 (5%)	5/14 (35.7%)	2/15 (13.3)	2/16 (12.5%)	2/12 (16.7%)

Regression analyses of survival with covariates of bacteremia and PA-ELISA were conducted to help further explore the relationship between pretreatment severity of illness and survival. Analyses from individual treatment studies showed that bacteremia level and/or PA-ELISA were an important factor for survival (see Appendix). However, there was considerable variability in bacteremia and PA-ELISA across five treatment studies in monkeys. Therefore, as an exploratory analysis, a GEE regression with study as a cluster was used to adjust for bacteremia and PA-ELISA prior to treatment. The following table shows the regression results (the reference groups were AP204 and placebo for study and dose, respectively). Bacteremia was a significant variable in the regression. A higher bacteremia level was associated with a lower survival probability. All dose levels were statistically significant, and the largest treatment effect was seen with the 16 mg/kg dose (reference group was the control group). After controlling for bacteremia and dose, study variable was not statistically significant. Due to correlation of bacteremia and PA-ELISA, only one of them can be included in the model.

In AP202 the two products (Lonza and Baxter ETI-204) were used. This provided an opportunity to directly compare them in one study. In all other monkey treatment studies, either the Lonza product was used (AP203) or the Baxter ETI-204 product was used (AP201, AP204, and NIAID1056). The following GEE model included product as an additional covariate. The log odds ratio for Lonza was positive, which means that the Lonza product had a higher survival probability compared to Baxter after controlling for bacteremia and dose. However given that its 95% confidence interval included 0, this effect was not statistically significant. The observed

survival proportion in the Lonza group was lower, but after adjusting for bacteremia, the Lonza product appeared to have a numerically better treatment effect (but not statistically significant). The effects for other covariates are similar as in the previous model.

Table 7. Monkey monotherapy studies: Log odds ratios from GEE regression analyses controlling for bacteremia and dose, including all studies and all doses

Parameter	Estimate	Standard Error	95% Confidence Limits		Z	Pr > |Z|
Intercept	1.4853	1.0882	-0.6476	3.6182	1.36	0.1723
4 mg/kg	2.9736	0.9234	1.1639	4.7834	3.22	0.0013
8 mg/kg	2.1936	0.9361	0.3589	4.0283	2.34	0.0191
16 mg/kg	3.6739	0.9239	1.8631	5.4848	3.98	<0.0001
32 mg/kg	3.1420	1.4303	0.3387	5.9454	2.20	0.0280
Log_{10}Bacteremia	-1.4008	0.2838	-1.9570	-0.8446	-4.94	<0.0001
AP201	1.5517	0.8038	-0.0237	3.1272	1.93	0.0536
AP202	0.9708	0.7364	-0.4725	2.4140	1.32	0.1874
AP203	0.9151	1.3858	-1.8011	3.6312	0.66	0.5091
NIAID 1056	2.6061	1.3939	-0.1258	5.3381	1.87	0.0615

*Statistically significant at a two-sided 0.05 significance level

Table 8. Monkey monotherapy studies: Log odds ratios from GEE regression analyses controlling for bacteremia, dose and product, including all studies and all doses

Parameter	Estimate	Standard Error	95% Confidence Limits		Z	Pr > |Z|
Intercept	1.7086	1.1977	-0.6388	4.0560	1.43	0.1537
4 mg/kg	2.9637	0.9407	1.1200	4.8075	3.15	0.0016*
8 mg/kg	2.1972	0.9433	0.3485	4.0460	2.33	0.0198*
16 mg/kg	3.6097	0.9707	1.7071	5.5122	3.72	0.0002*
32 mg/kg	3.1642	1.4435	0.3350	5.9934	2.19	0.0284*
Log_{10} bacteremia	-1.4524	0.3161	-2.0718	-0.8329	-4.60	<0.0001*
AP201	1.4928	0.8196	-0.1136	3.0991	1.82	0.0686
AP202	0.6900	0.7722	-0.8235	2.2035	0.89	0.3716
AP203	0.8944	1.3996	-1.8488	3.6376	0.64	0.5228
NIAID 1056	2.6080	1.4166	-0.1685	5.3846	1.84	0.0656
Lonza	0.8783	0.9368	-0.9577	2.7143	0.94	0.3484

*Statistically significant at a two-sided 0.05 significance level

The model with PA-ELISA but not with bacteremia yields similar results, and PA-ELISA was statistically significant (p-value<0.0001). These GEE regression models demonstrate that bacteremia and PA-ELISA were associated with survival and after adjusting for one of them, the 16 mg/kg ETI-204 had the strongest treatment effect among all dose groups. It is noted that after adjusting for bacteremia, the 32 mg/kg dose had the second strongest treatment effect, followed by the 4 mg/kg and 8 mg/kg groups. As discussed previously, the applicant claims that the monkey studies demonstrated that 16 mg/kg ETI-204 was the maximally efficacious dose. Our

analysis yielded a similar conclusion that of the doses studied, 16 mg/kg was the most effective; however, only limited information was available on the 32 mg/kg dose.

3.2.2.4.2 *Treatment studies in rabbits*

The study results from the treatment studies in rabbits in the mITT population are shown in the following table. In Study AR021, two animals (one in the placebo group and one in the 1 mg/kg group) that were inadvertently dosed with levofloxacin and survived were included in the reviewer's modified intend-to-treat (mITT) analysis, because they were randomized and received a treatment. This potentially leads to slightly conservative results in the 4 mg and 16 mg comparisons to placebo because of the inclusion of the one placebo survivor.

Table 9. Reviewer's analysis: 28-day survival rates in IV monotherapy treatment studies in NZW Rabbit (mITT population)

	ETI-204 IV (mg/kg) Baxter	**Survival n/N (%)**	**Difference [95% CI] [Adjusted 95% CI]**	**One-sided p-value (significance level)**
AR021 Baxter	0 (placebo)	1/10 (10)		
	1	4/10 (40.0)	0.3 [-0.107, 0.659] [-0.219, 0.732]	0.059 (0.0083)
	4	13/17 (76.5)	0.665 [0.249, 0.878] [0.155, 0.918]	0.0005* (0.0083)
	16	16/17 (94.1)	0.841 [0.443, 0.978] [0.352, 0.989]	<0.0001* (0.0083)
AR033 Baxter	0	0/14		
	1	4/14 (28.6)	0.286 [0.012, 0.581] [-0.077, 0.649]	0.02081 (0.0063)
	4	6/14(42.9)	0.429 [0.135, 0.711] [0.044, 0.769]	0.003* (0.0063)
	8	10/14 (71.4)	0.714 [0.406, 0.916] [0.312, 0.944]	<0.001 (0.0063)
	16	9/14 (64.3)	0.643 [0.334, 0.872] [0.237, 0.909]	0.001* (0.0063)
NIAID 1030 Baxter	0	0/6 (0)		
	8	12/16 (75)	0.75 [0.221, 0.927] [0.174, 0.941]	0.0008* (0.0125)
NIAID 1045 Baxter	0	0/6 (0)		
	8	7/11 (63.6)	0.636 [0.078, 0.891] [0.022, 0.911]	0.0052* (0.0125)

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at the specified significant level with Bonferroni adjustment for multiple comparisons

In these 4 studies, the dose groups of 4, 8, and 16 mg/kg ETI-204 demonstrated significant treatment effects. Note all products were Baxter products.

In rabbit treatment studies, only AR033 had bacteremia and PA-ELISA data and only 6 animals had a positive PA-ELISA result (2, 1, 2, 1 in the 1, 4, 8, 16 mg/kg group, respectively). As was seen in the monkey treatment studies, there was little correlation between challenge dose and bacteremia or PA-ELISA. The Pearson correlation coefficient between challenge dose and $\log_{10}$ bacteremia or $\log_{10}$ PA-ELISA was -0.113 and -0.115 (p-value=0.35 and 0.34), respectively. All rabbits with positive PA levels succumbed to anthrax infection.

Due to the scarcity of data it was not useful to conduct further bivariate or regression analyses for rabbit treatment studies.

3.2.3 Post-exposure Prophylaxis Studies

Three studies in monkeys (AP107, AP301, and AP307) and six studies in rabbits (AR004, AR007, AR012, AR035, AR037, and AR0315) were conducted to evaluate the efficacy of ETI-204 for post-exposure prophylaxis (PEP). In addition, in Phase 1 of the re-challenge rabbit study, AR034, treatment was administered at 30 hours post challenge. Because in rabbits the mean time to trigger was around 28 hours and the mean time from trigger to treatment was 1.7 hours, it is not possible to know if all animals in AR034 would have had clinical signs of disease at the time of treatment so this study was classified as a PEP study.

Five studies included IV doses, the remaining 5 only contained IM dosing. A list of all 10 post-exposure prophylaxis studies is as follows:

Table 10: List of all post-exposure prophylaxis studies included in analysis

Study	Design	Treatment Period	Follow-up Period	# of Animals per Arm (randomized)
Monkey PEP				
AP107 Baxter	Randomized, open-label	Single dose 24 hrs post-challenge IM or IV	30 days	Placebo: 6 ETI-204 2 mg/kg, IV: 9 ETI-204 4 mg/kg, IM: 8 ETI-204 8 mg/kg, IV: 9 ETI-204 8 mg/kg, IM: 9
AP301 Lonza	Randomized, blinded	Single dose IM	28 days	Control/vehicle 18 hrs post challenge: 6 ETI-204 8 mg/kg 18 hrs post challenge: 6 ETI-204 8 mg/kg 24 hrs post challenge: 6 ETI-204 8 mg/kg 36 hrs post challenge: 6 ETI-204 16 mg/kg 18 hrs post challenge: 6 ETI-204 16 mg/kg 24 hrs post challenge: 6 ETI-204 16 mg/kg 36 hrs post challenge: 6
AP307 Lonza	Randomized, open-label	Single dose 16 mg IM	28 days	Placebo, 24 hrs post mean challenge: 10 ETI-204 24 hrs post mean challenge: 14 ETI-204 36 hrs post mean challenge: 14 ETI-204 48 hrs post mean challenge[1]: 16

Study	Design	Treatment Period	Follow-up Period	# of Animals per Arm (randomized)
Rabbit PEP				
AR004 Elusys	Randomized	Single dose 10 mg IV	28 days	Placebo: 10 ETI-204 24 hrs post challenge: 10 ETI-204 36 hrs post challenge: 10 ETI-204 48 hrs post challenge[3]: 10
AR007 (b) (4)	Randomized, open-label	Single dose IM or IV	34 days	Placebo: 9 ETI-204 10 mg IV: 9 ETI-204 20 mg IM: 9
AR012 Elusys	Randomized, open-label	Single dose IV or IM	14 days	Placebo: 9 ETI-204 2.5 mg, IV: 9 ETI-204 5 mg, IM: 9 ETI-204 10 mg, IV: 12 ETI-204 10 mg, IM: 9 ETI-204 20 mg, IV:12 ETI-204 20 mg, IM: 12 ETI-204 40 mg, IM: 12
AR0315 Baxter	Randomized open-label	Single dose IM	28 days	Placebo, 24 hrs: 10 ETI-204 4 mg/kg IM, 18 hrs: 12 ETI-204 16 mg/kg IM, 18 hrs: 12 ETI-204 4 mg/kg IM, 24 hrs: 12 ETI-204 16 mg/kg, 24 hrs: 12
AR035 Lonza	Randomized, open-label	Single dose 16 mg/kg IM	28 days	Placebo (vehicle): 10 ETI-204 18 hrs post-challenge: 10 ETI-204 24 hrs post-challenge: 10 ETI-204 30 hrs post-challenge[4]: 10
AR037 Lonza	Randomized, open-label	Single dose IM, 24 hrs post challenge	28 days	Placebo (vehicle): 10 ETI-204 8 mg/kg: 16 ETI-204 16 mg/kg: 16 ETI-204 32 mg/kg: 16
AR034 Phase 1 Lonza	open-label	Single dose IV, 30 hours post challenge	9 months	Placebo (vehicle): 8 ETI-204 16 mg/kg: 20

3.2.3.1 Study Design

The post-exposure prophylaxis studies had almost the same design as the treatment studies. The only difference was that treatment was started at a pre-specified fixed time point (9, 18, 24, 36, or 48 hours) post challenge, typically at a time before (except for the last time point) clinical signs and/or symptoms would have developed (which would have been approximately 37-40 hours post challenge in monkeys and 28 hours post challenge in rabbits). The PEP studies with ETI-204 administered before the development of clinical signs/symptoms were expected to have a higher survival probability than in the treatment studies. All of the studies except AR034 Phase 1 contained either multiple arms containing different doses of ETI-204 (1, 2, 4, 8, 16, 32 mg/kg), or multiple arms that dosed ETI-204 at various time points. Only Study AP301 was blinded.

3.2.3.2 Primary Efficacy Endpoint and Analysis Population

The primary efficacy endpoint was survival at the end of the study (usually 28 days post-challenge, unless stated otherwise).

The analysis population included all challenged animals, except for AP301, which used all animals that received treatment.

3.2.3.3 Statistical Methods

The statistical methods were the same as in the treatment studies.

3.2.3.4 Study Results and Conclusions

PEP studies in monkeys

The following table shows the results from monkey PEP studies with IM or IV administration at 18, 24, or 36 hours post challenge. In AP107, after Bonferroni's adjustment, there were no statistically significant differences between any ETI-204 treatment group and the placebo group. However, a dose-response relationship trend was observed in the IV groups, but not in the IM groups.

In the next two monkey studies (AP301 and AP307), the 8 mg/kg and 16 mg/kg groups administered IM at 18 hours or 24 hours post-challenge demonstrated significant treatment effects. Treatment started at or after 36 hours did not show any statistically significant treatment effect. These two studies used Lonza ETI-204. These significant results were supportive of the efficacy of the Lonza product.

In monkeys, 8 mg/kg IV administered 24 hours post challenge was not statistically significant after multiple comparison adjustment, but the treatment effect was numerically high (0.583). There was no 16 mg/kg IV administered 24 hours post challenge studied. However, the 16 mg/kg IM administration was effective when given at either 18 or 24 hours post-exposure. This can be considered supportive evidence of efficacy of at 16 mg/kg IV dose given the likely better availability of the IV administration than the IM administration.

Table 11. Survival rates in post-exposure prophylaxis studies in cynomolgus monkeys

Study	Route	Hours post challenge	ETI-204 mg/kg	n/N(%) Survival	Difference [95% CI] [Adjusted 95% CI]	One-sided p-value (sig. level)
AP107 Baxter Day 30 survival	IV or IM	24	0	1/6 (16.7)		
	IV	24	2	4/9 (44.4)	0.278 [-0.295, 0.641] [-0.391, 0.765]	0.210 (0.0063)
	IV	24	8	6/8 (75.0)	0.583 [0.018, 0.902] [-0.130, 0.941]	0.020 (0.0063)
	IM	24	4	6/8 (75.0)	0.583 [0.018, 0.902] [-0.130 0.941]	0.020 (0.0063)

Study	Route	Hours post challenge	ETI-204 mg/kg	n/N(%) Survival	Difference [95% CI] [Adjusted 95% CI]	One-sided p-value (sig. level)
	IM	24	8	5/9 (55.6)	0.389 [-0.158, 0.777] [-0.292, 0.835]	0.087 (0.0063)
AP301 Lonza Day 28 or 56 survival[1]	IM	18	0	0/6 (0)		
	IM	18	8	6/6 (100)	1 [0.471, 1] [0.438, 1]	0.0012* (0.0042)
	IM	18	16	6/6 (100)	1 [0.471, 1] [0.438, 1]	0.0012* (0.0042)
	IM	24	8	5/6 (83)	0.83 [0.230, 0.996] [0.196, 0.998]	0.0032* (0.0042)
	IM	24	16	5/6 (83)	0.83 [0.230, 0.996] [0.196, 0.998]	0.0032* (0.0042)
	IM	36	8	0/6 (0)	0	1.0000 (0.0042)
	IM	36	16	3/6 (50)	0.5 [-0.037, 0.882] [-0.069, 0.893]	0.0345 (0.0042)
AP307 Lonza Day 28 survival	IM	24	0	1/10 (10)		
	IM	24	16	13/14 (93)	0.83 [0.431, 0.976] [0.347, 0.987]	0.001* (0.0083)

[1] Survival assessed after spore challenge (28 days) except for the 16 mg/kg IM dose in AP301 which was assessed at 56 days after spore challenge
Adapted from Table 9 from Clinical Overview. Sig.: Significance.
Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at the specified significant level with Bonferroni adjustment for multiple comparisons

PEP studies in rabbits

As the following table shows, with IV administration at 9 or 24 hours post challenge in rabbits, the dose of 10 to 20 mg/animal (approximately 4 mg/kg to 8 mg/kg), 3 of 4 comparisons in 3 studies (AR004, AR007, and AR012) demonstrated a statistically significant result. AR012 10 mg with a 50% survival rate was not statistically significant after adjusting for many multiple comparisons. It appeared that these doses were effective if administered by 24 hours post challenge. Further delay of treatment reduced the treatment effect.

AR034 Phase I also demonstrated a significant treatment effect of 16 mg/kg dose administered IV 30 hours post challenge.

With IM administration, the dose of 16 mg/kg at 18 or 24 hours post challenge showed a statistically significant result in AR0315 and AR035 (2 groups per dose), but not in AR037 or AR012 (40 mg is approximately 16 mg/kg).

Table 12. Survival in post-exposure prophylaxis studies in rabbits

Study	Route	Hours post challenge	ETI-204 mg	n/N(%) Survival	Difference [95% CI] [Adjusted 95% CI]	One-sided p-value (Significance level)
AR004 Elusys Day 28	IV	48	0	0/9 (0)		
		24	10 mg/animal	8/10 (80.0)	0.80 [0.402, 0.975] [0.303, 0.986]	0.0001* (0.0083)
		36	10 mg/animal	5/10 (50.0)	0.50 [0.084, 0.813] [-0.017, 0.856]	0.010 (0.0083)
		48	10 mg/animal	3/7 (42.9)	0.429 [0.012, 0.816] [-0.084, 0.865]	0.0226 (0.0083)
AR007 (b) (4) Day 34	IV	9	0	0/9 (0)		
	IV		10 mg/animal	9/9 (100)	1 [0.629, 1]	<0.0001* (0.0125)
	IM		20 mg/animal	9/9 (100)	1 [0.629, 1]	<0.0001* (0.0125)
AR012 Elusys Day 14	IM	24	0	0/9 (0)		
	IV		2.5 mg/animal	1/9 (11.1)	0.111 [-0.224, 0.483] [-0.436, 0.610]	0.4073 (0.0036)
			10 mg/animal	6/12 (50)	0.50 [0.094, 0.789] [-0.057, 0.859]	0.0074 (0.0036)
			20 mg/animal	7/12 (58.3)	0.583 [0.187, 0.848] [-0.018, 0.904]	0.0026* (0.0036)
	IM		5 mg/animal	1/9 (11.1)	0.111 [-0.224, 0.483] [-0.436, 0.610]	0.4073 (0.0036)
			10 mg/animal	3/9 (33.3)	0.333 [-0.071, 0.701] [-0.238, 0.794]	0.049 (0.0036)
			20 mg/animal	5/12 (41.7)	0.417 [0.034, 0.725] [-0.134, 0.806]	0.0186 (0.0036)
			40 mg/animal	4/12 (33.3)	0.333 [-0.066, 0.655] [-0.217, 0.749]	0.051 (0.0036)

Study	Route	Hours post challenge	ETI-204 mg	n/N(%) Survival	Difference [95% CI] [Adjusted 95% CI]	One-sided p-value (Significance level)
AR0315 Baxter Day 28	IM	24	0	0/10 (0)		
		18	4 mg/kg	11/12 (91.7)	0.917 [0.535, 0.998] [0.425, 1]	<0.0001* (0.0063)
		24	4 mg/kg	5/12 (41.7)	0.417 [0.065, 0.723] [-0.058, 0.786]	0.0131 (0.0063)
		18	16 mg/kg	11/12 (91.7)	0.917 [0.535, 0.998] [0.425, 1]	<0.0001* (0.0063)
		24	16 mg/kg	8/12 (66.7)	0.667 [0.290, 0.901] [0.172, 0.934]	0.0005* (0.0063)
AR034 Phase I Lonza Day 28	IV	30	0	0/8		
			16 mg/kg	13/20 (65)	0.65 [0.156, 0.846] [0.300, 0.969]	0.0008* (0.0125)
AR035 Lonza Day 28	IM	18	0	0/10 (0)		
		18	16 mg/kg	6/10 (60)	0.60 [0.213, 0.878] [0.119, 0.912]	0.0018* (0.0083)
		24	16 mg/kg	6/10 (60)	0.60 [0.213, 0.878] [0.119, 0.912]	0.0018* (0.0083)
		36	16 mg/kg	0/8 (0)	0 [-0.309, 0.369] [-0.387, 0.480]	0.5 (0.0083)
AR037 Lonza Day 28	IM	24	0	0/10		
			8 mg/kg	5/16 (31.3)	0.313 [-0.019, 0.587]	0.33 (0.0083)
			16 mg/kg	5/16 (31.3)	0.313 [-0.019, 0.587]	0.33 (0.0083)
			32 mg/kg	5/16 (31.3)	0.303 [-0.019, 0.587]	0.33 (0.0083)

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at the specified significant level with Bonferroni adjustment for multiple comparisons

These studies showed that if ETI-204 was administered IM with a dose of 16 mg/kg at 18 or 24 hours post challenge, or administered IV with a dose of 10 to 20 mg/animal (approximately 4 mg/kg to 8 mg/kg) at 9 or 24 hours post challenge, most of these studies (6 out of 7) provided supportive evidence for the efficacy of ETI-204. Given each study was usually designed with a statistical power of 0.8 and a 0.05 one-sided type I error, without any consideration of multiple comparisons, it was expected that some studies would not demonstrate a significant treatment effect if the treatment was indeed effective, based on a binomial distribution of the success trials out of all the trials conducted.

There were three studies using the Lonza product (AR034 Phase I, AR035 and AR037). In AR034 Phase I, survival in the 16 mg/kg IV administered 30 hours post challenge was statistically significantly improved compared with the placebo group. In AR035, the dose of 16 mg/kg IM administered 18 or 24 hours did show a statistically significant treatment effect. However this dose in AR037 failed to replicate this significant treatment effect. The reason was not clear. If there were no treatment effect, based on a binomial distribution with a one-sided type I error of 0.025 for each study, the probability of observing two or more successful studies out of three was 1.6E-5, very small. The observed significant treatment results were very unlikely due to chance. The significant results from the two studies out of these three studies using the Lonza product support the efficacy of this product.

3.2.4 Pre-exposure Prophylaxis Studies

3.2.4.1 Summary of Pre-exposure Prophylaxis Studies

The efficacy of ETI-204 as a monotherapy for the pre-exposure prophylaxis (PrEP) for inhalational anthrax was evaluated in one study in monkeys and two studies in rabbits (AP305, AR001, and AR003) to define the dose, time, and window of protection.

Table 13. List of all pre-exposure prophylaxis studies in monkeys and rabbits

Study	Design	Treatment Period	Follow-up Period	# of Animals per Arm (randomized)
Monkey PrEP				
AP305 Lonza	Randomized, blinded	Single dose 16 mg/kg IM	56 days	Placebo IM, Day -3, Day -2, and Day -1: 10 ETI-204 IM, Day -1: 15 ETI-204 IM, Day -2: 14 ETI-204 IM, Day -3: 14
Rabbits PrEP				
AR001 Elusys	Randomized, open-label	Single dose IV 30-45 min prior to exposure	28 days	Placebo: 5 ETI-204 10 mg: 9
AR003 Elusys	Randomized Open-label	Single dose IV or IM within 35 min prior to exposure	28 days	Placebo (PBS): 8 ETI-204 1.25 mg IV: 8 ETI-204 2.5 mg IV: 8 ETI-204 5 mg IV: 8 ETI-204 10 mg IV: 8 ETI-204 20 mg IM 8

3.2.4.2 Study Design

In these studies, animals received treatment (IM or IV) first and then were challenged with anthrax spores. Only the monkey study, AP305, was a blinded study. The target challenge dose was 100 LD_{50} spores in AP305 and AR001 and 200 LD_{50} spores in AR003. As seen below, there was a low survival rate in the untreated animals challenged with a lower dose (10% and 0%). So

although the challenge dose was low, the high mortality in the control group demonstrated its lethality, and is not a concern for this reviewer.

In AP305, a 16 mg/kg IM dose was tested when given at 3 different time points before challenge, 1 day, 2 days and 3 days. AR001 considered only a 10 mg/animal IV dose (approximately 4 mg/kg) given 30-45 minutes before challenge. AR003 looked at four IV doses ranging from 1.25 mg/animal to 10 mg/animal (approximately 0.5 mg/kg to 4 mg/kg) and 1 IM dose of 20 mg/animal (approximately 8 mg/kg) all given within 35 minutes prior to challenge.

3.2.4.3 Primary Efficacy Endpoint and Analysis Population

The primary endpoint was survival to the end of study.
The analysis population included all randomized animals that received treatment and were challenged.

3.2.4.4 Statistical Methods

The same methods were used as in the treatment studies. In AP305, a closed testing procedure was used. The null hypotheses testing the differences between ETI-204 given 2 days, 1 day or 3 days prior to challenge were ordered. The second one would be tested if the first one was significant; and the third one would be tested if the second one was significant. Therefore, there was no need to control for multiple comparisons in this study.

3.2.4.5 Results and Conclusions

As shown in the following table, Study AP305 demonstrated that a dose of 16 mg/kg administered IM 1, 2, or 3 days prior to challenge statistically significantly increased survival rates in monkeys. The results from this study lend support to the efficacy of Lonza ETI-204.

Table 14. Survival at Day 56 in pre-exposure prophylaxis monkey Study AP305

ETI-204 Lonza mg/kg IM	Days before challenge	n/N(%) Survival	Difference [95% CI]	One-sided P-value (significance level)
0		1/10 (10)		
16	3	15/15(100)	0.9 [0.554, 0.998]	<0.0001* (0.025)
	2	14/14(100)	0.9 [0.554, 0.998]	<0.0001* (0.025)
	1	14/14(100)	0.9 [0.554, 0.998]	<0.0001* (0.025)

Closed comparison procedure was used. Therefore, no additional adjustment for multiple comparisons is needed.
*Statistically significant at a one-sided 0.025 significance level
Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer

The results from the two rabbit studies are listed Table 15. There were two routes of administration: IV and IM. The IM data were considered by the applicant to provide additional

supportive evidence for the efficacy of IV ETI-204 for the pre-exposure prevention of inhalational anthrax.

These two rabbit studies showed that doses of at least 2.5 mg/animal (approximately 1 mg/kg) in rabbits administered IV and 20 mg/animal (approximately 8 mg/kg) administered IM about 30 minutes prior to challenge provided statistically significant protection against anthrax exposure.

Table 15. Survival at Day 28 in pre-exposure prophylaxes studies in rabbits

ETI-204 mg	Route	n/N(%) Survival	Difference 95% CI Adjusted 95% CI	One-sided p-value (significance level)
AR001 Elusys, 30-45 minutes prior to a targeted 100 LD_{50} exposure				
0	IV	0/5 (0)		
10		9/9 (100)	1 [0.474, 1]	0.0001* (0.025)
AR003 Elusys, within 35 minutes prior to a targeted 200 LD_{50} exposure				
0	IV	0/8 (0)		
1.25		1/8 (12.5)	0.125 [-0.292, 0.527] [-0.427, 0.632]	0.402 (0.005)
2.5		5/8 (62.5)	0.625 [0.173, 0.915] [0.019, 0.953]	0.004* (0.005)
5		5/8 (62.5)	0.625 [0.173, 0.915] [0.019, 0.953]	0.004* (0.005)
10		7/8 (87.5)	0.875 [0.395, 0.997] [0.237, 0.999]	0.0003* (0.005)
20	IM	8/8 (100)	1 [0.588, 1] [0.436, 1]	<0.0001* (0.005)

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at the specified significant level with Bonferroni adjustment for multiple comparisons if needed

In conclusion, 16 mg/kg ETI-204 IM 1 to 3 days prior to challenge in monkeys or 2.5, 5, 10 mg/animal IV, or 20 mg/animal IM (or approximately 1, 2, 4, 8 mg/kg) about 30 minutes prior to challenge in rabbits provided statistically significant protection against inhalational anthrax.

3.2.5 Re-challenge Study

One rabbit study, AR034, was conducted to investigate the effect of ETI-204 on survival after re-challenge of anthrax spores. In Phase I, animals were challenged and treated with ETI-204 16 mg/kg (IV), levofloxacin (50 mg/kg/day for 3 days), ETI-204 and levofloxacin, or placebo.

Surviving animals were re-challenged and new control animals were challenged 9 months later in Phase II. No treatment was administered in Phase 2.

The analysis population included all animals that were spore challenged in Phase II.

The primary endpoint was survival to day 21 in phase II.

The study results from this study by Phase are as follows:

Table 16. Study AR034: Survival at the end of each phase by treatment group

	Control n/N(%)	**ETI-204** n/N(%)	**Levo** n/N(%)	**ETI-204 and Levo** n/N(%)
Phase I	0/8 (phase I controls)	13/20	20/20	19/20
Phase II	0/12 (phase II controls)	13/13 (100%)	19/20 (95%)	17/19 (89%)
Phase II analysis (treatment – phase 2 control) p-value and 95% CI		<0.0001* (0.025) [0.724, 1]	<0.0001* [0.695, 0.999]	<0.0001* [0.615, 0.987]

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at the specified significant level

The survival proportions in Phase II were 100% in the ETI-204 alone re-challenged group, 89% in the ETI-204 and levofloxacin re-challenged group, and 95% in the levofloxacin-alone re-challenged group. All were statistically significantly different than the Phase II control group with no surviving animals. This demonstrated that ETI-204 with or without co-administration with levofloxacin provided a statistically significant post-exposure prophylactic effect after first exposure to anthrax spores and the ETI-204 treated animals in Phase I could develop protective immunity after a secondary exposure to anthrax spores.

3.3 Evaluation of Safety

Tissue bacterial assessments were included in most studies. Please see the review of individual studies in the Appendix for detailed evaluation.

In the Nonclinical Overview, Toxicology section, it is stated that because administration of the first in class raxibacumab has been associated with greater incidence and/or severity of CNS lesions in anthrax-challenged animals that did not survive following treatment, neuropathological examinations of brain tissues from monkeys and rabbits were conducted in several studies. This reviewer checked all treatment studies contained in this review and focused on the microscopic pathological effect of ETI-204 on the brain only. Table 17 shows the proportions of positive pathological findings in the brain among non-survivors in the monkey treatment studies. Overall, 16/19 (84.2%) from ETI-204 8 mg/kg, 11/29 (37.9%) from the ETI-204 16 mg/kg, and 8/52 (15.4%) from placebo had a positive pathological finding (discolorations, etc.). The mechanism for these differences was not clear. The applicant provided an explanation that the abnormalities

are consistent with the ETI-204-treated animals attempting to mount an immune response to the bacteria/bacterial products.

All survivors, except for one from the 32 mg/kg group in AP203, had no reported positive pathological findings in the brain.

Table 17. Proportion of non-survivors with positive microscopic pathological findings in the brain in the treatment studies in monkeys

Study and dose (mg/kg)	# of animals with positive findings in the brain	# Tested (# of non-survivors, if different)	Proportion of positive findings out of tested animals
AP201			
0	2	12	16.7%
4	2	3	66.7%
16	2	4	50.0%
AP202			
0	1	17	5.9%
16	3	11	27.3%
16	3	11	27.3%
AP203			
0	3	14	21.4%
8	13	15	86.7%
32	5	10	50.0%
AP204			
0	1	1 (15)	100%
4	5	5 (12)	100%
16	3	3 (8)	100%
NIAID 1056			
0	1	8	12.5%
8	3	4	75%

The following table shows the proportion of non-survivors with positive pathological findings in the tested brain in rabbit treatment studies. Overall, 13/23 (56.5%), 5/8 (62.5%), and 2/3 (66.7%) of animals from the placebo, 8 mg/kg, and 16 mg/kg had positive pathological findings in the brain, respectively. However, the numbers of animals in the ETI-204 treated groups were too small to be conclusive.

There were no positive pathological findings among survivors.

Table 18. Proportion of non-survivors with positive microscopic pathological findings in the brain in the IV treatment studies in rabbits

Study and dose (mg/kg)	# of animals with positive findings in the brain	# Tested (# of non-survivors, if different)	Proportion of positive findings out of tested animals
AR021			
0	9	9 (14)	100%
1	5	6	83.3%
4	2	4	50%
16	0	1	0
AR033			
0	2	2 (14)	100%
1	1	1 (10)	100%
8	1	1 (4)	100%
16	2	2 (5)	100%
NIAID 1030			
0	1	6	16.7%
8	1	3 (4)	33%
NIAID 1045			
0	1	6	16.7%
8	3	4 (5)	75%

For safety information from human trials, please see the medical safety review.

4 FINDINGS IN SPEAL/SUBGROUP POPULATIONS

4.1 Gender, Race, Age, and Geographic Region

Since these studies were animal studies, race and region were not applicable. Age was either reported in a range, unknown, or if an actual age was given, there was a very narrow range across the animals. Therefore, subgroup analyses by age were not performed by the reviewer. There were no concerns about the treatment effects between male and female animals, although in a few studies a gender effect was statistically significant; however, the effect between genders was not consistent across the studies. Given the many studies that were conducted it would not be surprising to observe a few significant results even if there was no gender effect. The sample sizes were usually too small to have a definite conclusion for the gender effect.

4.2 Other Special/Subgroup Populations

No other special populations or subgroups were considered in this review.

5 SUMMARY AND CONCLUSIONS

5.1 Statistical Issues

The main issue is that the applicant conducted many studies all with slightly different designs and that the results are highly variable across studies. Given the differences in study designs, including the time of ETI-204 administration, we needed to explore the relationship between the pre-treatment bacteremia and survival in the treatment studies in order to better understand the treatment effect of ETI-204.

During the development of this product, many studies were conducted without discussion with the FDA. Therefore there were some deficiencies in study design: one is blinding and one is lack of adjustment for multiple comparisons in a study.

Some studies were open-label. Some studies were labeled as "blinded", but are not considered as blinded in this review. For example, one study is stated to be a blinded study, but treatment vials were labeled as Groups "X", "Y", "Z". Because it would be easy to single the control group out within the first few days of treatment due to the extremely high mortality, this study could not be considered as fully blinded. In some cases, it is difficult to know if a study was fully blinded. Nevertheless, because of the large number of studies and the fact that the survival status (survival/death) was objective, we are assuming that potential knowledge of treatment assignment by study staff did not affect the study results overtly.

Only a few studies considered multiple comparisons in the study design and analysis. In one study, a procedure to control for multiple comparisons was only mentioned in the study result section, but not the protocol. It is not clear if it was pre-specified or post-hoc after the data was locked. However, we used a Bonferroni adjustment for all studies with multiple comparisons. Bonferroni's adjustment is a conservative method. Despite the conservative nature of this method, many study results remained statistically significant. Therefore, the lack of adjustment for multiple comparisons in many studies does not have an influence on the overall conclusions of the efficacy of ETI-204.

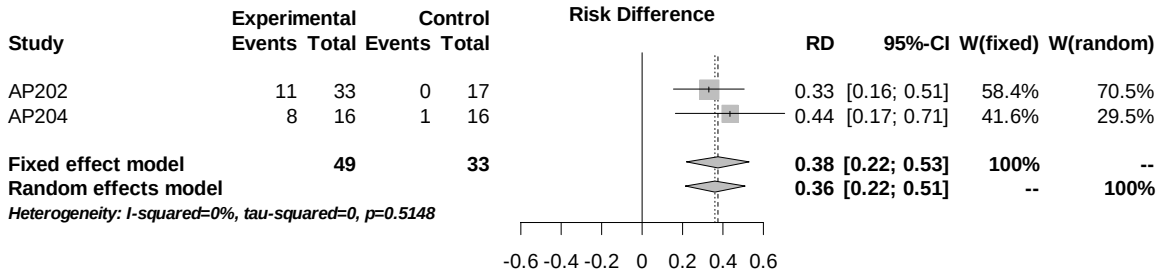
5.2 Collective Evidence

In the following sections, we present the results of some exploratory meta-analyses for the monotherapy studies in monkeys and rabbits and a summary of the treatment effects of the Lonza product. Note the meta-analyses are based on large-sample or asymptotic theories. Given the small sample sizes in these studies the results, including 95% confidence intervals, are merely exploratory.

5.2.1 Meta-analysis of monkey and rabbits monotherapy studies

16 mg/kg IV in monkeys

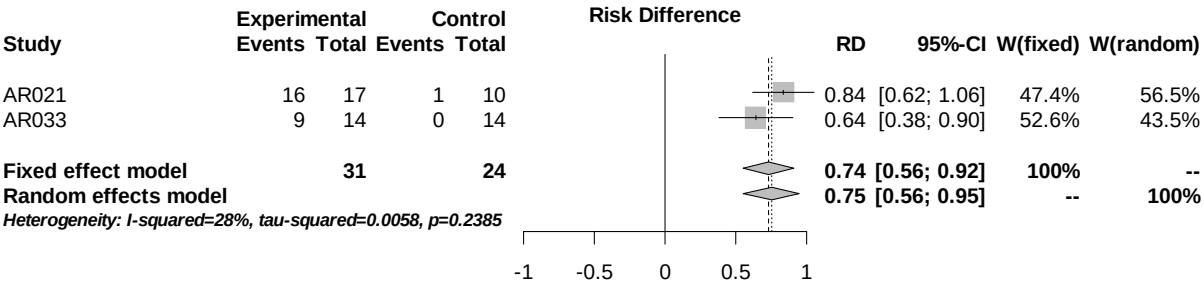
Because the 16 mg/kg dose is the proposed dose, this review will only include the meta-analysis results for this dose. Meta-analysis of 16 mg/kg IV in monkey studies is shown in the following graph.



The results from both fixed effect model and random effects model showed consistent and almost identical study results. This meta-analysis demonstrated that this dose did show significant differences in survival proportions from the fixed effect and random effects models.

16 mg/kg IV in rabbits

The following graph shows the meta-analysis results for the 16 mg/kg dose. The analysis shows significant treatment effect from these two studies. Both fixed effect model and random effects model provided consistent estimates for the difference in survival proportion.

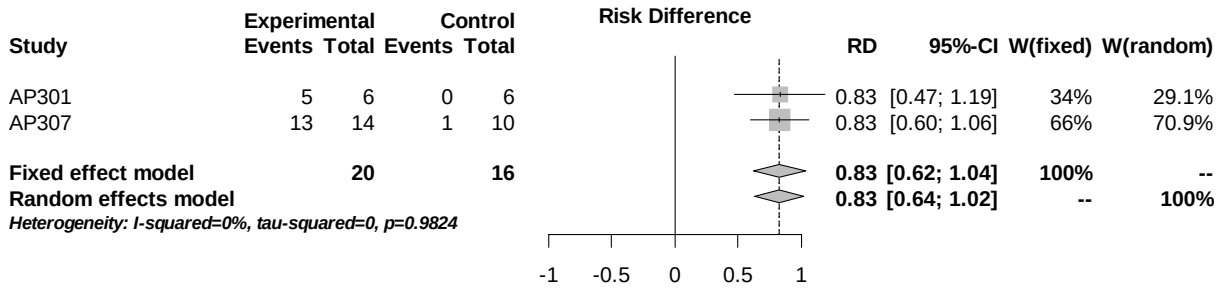


5.2.2 Meta-analysis of PEP studies in monkeys and rabbits

The following meta-analysis analyzed the 16 mg/kg IM at the most common time points (18 and 24 hours post challenge) in PEP studies in rabbits and monkeys.

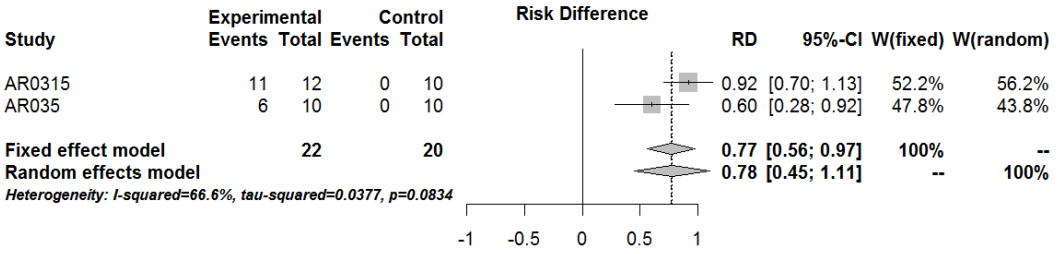
16 mg/kg IM 24 hours post challenge in monkeys

There were 2 studies (AP301 and AP307) conducted in monkeys to evaluate the efficacy of 16 mg/kg IM 24 hours post challenge. The two studies showed a statistically significant treatment effect. Both fixed effect model and random effects model yielded almost identical results.



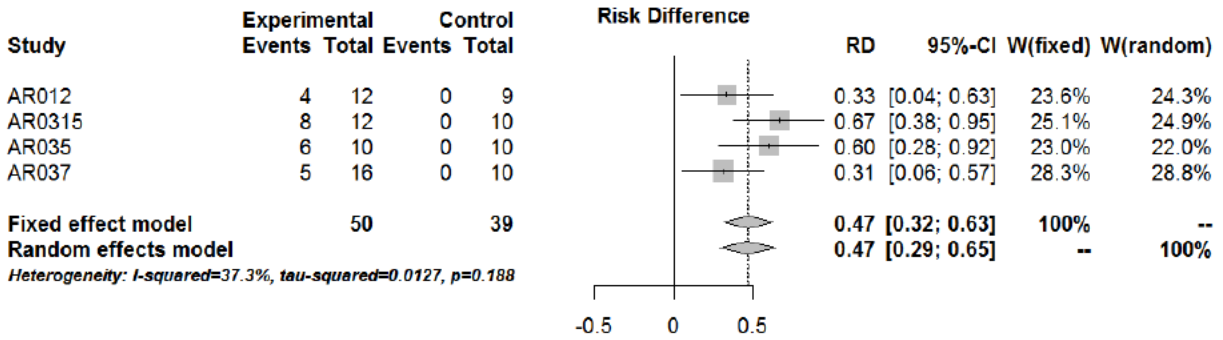
16 mg/kg IM 18 hours post challenge in rabbits

There were two studies using the 16 mg/kg IM 18 hours post challenge (AR0315 and AR035). The meta-analysis below shows that this dose had a significant treatment effect in rabbits.



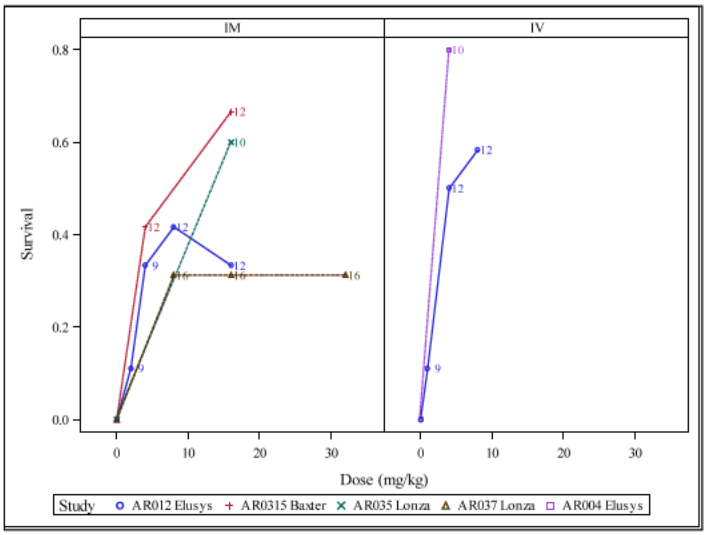
16 mg/kg IM 24 hours post challenge in rabbits

There were 4 rabbit studies for post exposure prophylaxis including 16 mg/kg group IM 24 hours post challenge. In the analysis of each individual study, two studies (AR0315 and AR035) showed significant treatment effect, and the remaining two studies (AR012 and AR037) did not, using an exact confidence interval with Bonferroni's adjustment. Note 40 mg/animal in AR012 is approximately equivalent to 16 mg/kg. In the following graph, meta-analysis indicates that the overall treatment effect was statistically significant, because the fixed effect model and random effects model showed consistent results. However because the sample size was small, this exploratory meta-analysis should be interpreted cautiously.



The following graph shows a summary of the PEP studies with ETI-204 administered 24 hours post challenge. The numbers in the graph indicate the group sample sizes. The survival proportions varied across studies and products. Note that the Lonza product administered via IM in study AR037 had the smallest treatment effect; however, study AR035 showed an effect similar to the other studies and also used the Lonza product. The reason for the difference between AR035 and AR037 is not clear. The efficacy of the IV product seems strong even at the low doses given in studies AR004 and AR012.

Figure 6. The survival proportions by study and dose in rabbits treated 24 hours post-exposure



5.2.3 Efficacy of Lonza ETI-204

As discussed above, the majority of the animal efficacy studies were conducted using the product manufactured at sites other than the Lonza site, the site planned to be used for the marketed product. Later studies were conducted using product from the Lonza manufacturing site. Due to the failure of ETI-204 in the first monkey treatment study (AP203) using the Lonza product, concern arose as to if it signified a problem with the product. The applicant explored possible reasons for the failed study and hypothesized that the failure was due to the increased severity of illness just prior to treatment as measured by both pre-treatment bacteremia and PA levels. The

applicant also conducted an additional monkey treatment study (AP202) under a special protocol assessment. This section of the review will assess the evidence of efficacy of the Lonza product and discuss the differences seen between the Lonza product and products manufactured at other sites. Note that only study AP202 studied two different products in one study. All of the comparisons given here of Lonza versus other products are based on cross-study comparisons and should be considered cautiously.

Among the 22 studies evaluated in this review, the Lonza product was used in 8 studies. Two of these studies contained unexpectedly low survival rates. One was a treatment study in monkeys (AP203) and one was a post-exposure prophylaxis study in rabbits (AR037).

Regarding the monkey treatment studies, we conducted an analysis taking into account severity of disease at the time of treatment based on either PA-ELISA or bacteremia. The analysis showed that the treatment effect for Lonza was not significantly different than the effect for Baxter and that its effect was positive, meaning that the point estimate of its effect was larger than Baxter's.

AR037, a post-exposure prophylactic study in rabbits using the Lonza product did have lower than expected survival rates. Doses of 8, 16 and 32 mg/kg IM all given at 24 hours had survival rates of 31%. This was lower than AR035, another study using the Lonza product that had a 60% survival rate when dosed at 16 mg/kg IM at 24 hours. To compare with other products, two other studies looked at 16 mg/kg IM at 24 hours, one using the Elusys product with a 33% survival rate and one using the Baxter product with a 67% survival rate. The reason for the inconsistency of these results is not clear, but does not appear to be limited to the Lonza product.

There is not strong evidence that the Lonza product is any less effective than the product manufactured at other facilities. Though we cannot say conclusively that the Lonza product is identical in efficacy to the previous manufactured product, we can say that there is adequate evidence of the efficacy of the Lonza product in both the treatment and prophylaxis of anthrax using data from rabbits and monkeys.

5.2.4 Summary of collective evidence

This BLA submission contains studies for treatment, post-exposure prophylaxis, pre-exposure prophylaxis, and re-challenge. The following is a summary of the treatment effects by administration time. Since the proposed dose of 16 mg/kg IV was not available for all administration times, doses closest to 16 mg/kg IV are reported.

Animal and administration time	Difference in survival proportion compared with controls	Doses studied	Study
Cynomolgus monkeys			
3, 2, 1 days pre-exposure	90%	16 mg/kg IM	AP305
18 hours post-challenge	100%	16 mg/kg IM	AP301
24 hours post-challenge	58-83%	8 mg/kg IV or 16 mg/kg IM	AP107, AP301, AP307
39–44 hours post-challenge	31-44%	16 mg/kg IV	AP202, AP204
New Zealand White rabbits			
30-45 minutes pre-challenge	88-100%	4 mg/kg IV or 8 mg/kg IM	AR001, AR003
9 hours post-challenge	100%	4 mg/kg IV or 8 mg/kg IM	AR007
18 hours post-challenge	60-92%	16 mg/kg IM	AR035, AR0315
24 hours post-challenge	31-67%	8 mg/kg IV or 16 mg/kg IM	AR035, AR012, AR037, AR0315
28-30 hours post-challenge	64-84%	16 mg/kg IV	AR021 AR033 AR034

The study results as discussed in this review are briefly summarized as follows:

- In treatment studies, 16 mg/kg IV dose showed a significant treatment effect in both the monkey and rabbit treatment studies.
- In post-exposure prophylaxis studies, doses given closer to the time of challenge gave higher survival rates. The majority of the prophylaxis studies used IM dosing. A 16 mg/kg IM dose given to monkeys and rabbits by 24 hours was effective. We can extrapolate that the IV dose would also be effective.
- In pre-exposure studies, a 16 mg/kg IM dose was effective when treatment was given 30 minutes to 3 days prior to challenge. Again, we can extrapolate that the IV dose would also be effective.
- In a re-challenge trial, 100% of the animals who were previously treated with ETI-204 16 mg/kg IV survived after a second challenge and 89% of the animals who were previously treated with ETI-204 16 mg/kg IV and levofloxacin survived after a second challenge.
- Lonza ETI-204 showed a significant treatment effect in AP202. The efficacy was supported by prophylaxis studies using this product. A failure of in AP203 may be explained by the high bacteremia levels and PA-ELISA levels prior to treatment.

Overall, these studies demonstrated that 16 mg/kg IV of ETI-204 was effective in the treatment, post-exposure prophylaxis and pre-exposure prophylaxis of inhalational anthrax.

For safety, among non-survivors, there was an increased risk of microscopic pathological changes in the brain. However, among survivors, there were no positive pathological changes. The applicant explained that the abnormalities are consistent with the ETI-204-treated animals attempting to mount an immune response to the bacteria/bacterial products. The exact mechanism behind these pathological changes in the brain remains to be further studied.

5.3 Conclusions and Recommendations

The animal studies demonstrated the efficacy of ETI-204 in the treatment and prophylaxis of inhalational anthrax. From a statistical perspective, the 16 mg/kg IV dose was the most appropriate dose among all doses studied.

5.4 Labeling Recommendations

For Section 16 in the labeling, we have the following recommendations:

Overview
Because it is not feasible or ethical to conduct controlled clinical trials in humans with inhalational anthrax, the efficacy of Anthim for the treatment of inhalational anthrax is based on efficacy studies in New Zealand White (NZW) rabbits and cynomolgus macaques. The animal efficacy studies are conducted under widely varying conditions, such that the survival rates observed in the animal studies cannot be directly compared between studies and may not reflect the rates observed in clinical practice.

Types of Studies
The efficacy of Anthim for treatment and prophylaxis of inhalational anthrax was studied in multiple studies in the cynomolgus macaques and NZW rabbit models of inhalational anthrax. These studies tested the efficacy of Anthim compared to placebo and the efficacy of Anthim in combination with antibacterial drugs relative to the antibacterial drugs alone.

Study Design
The animals were challenged with aerosolized *B. anthracis* spores (Ames strain) at approximately 200xLD_{50} to achieve 100% mortality if untreated. Animals in prophylaxis of inhalational anthrax studies were treated prior to the development of symptoms. In treatment studies, animals were administered treatment after exhibiting clinical signs or symptoms of systemic anthrax. Monkeys were treated at the time of a positive serum electrochemiluminescence (ECL) assay for *B. anthracis* PA at a mean time of approximately 40 hours post-challenge with *B. anthracis*. In most NZW rabbit treatment studies, animals were treated after sustained elevation of body temperature above baseline, at a mean time of approximately 30 hours post-challenge. In some of the treatment studies assessing the effect of Anthim in combination with antibacterial drugs, treatment was delayed to 72 to 96 hours post challenge. Most study animals were bacteremic and had a positive ECL assay for PA prior to treatment. Survival was assessed at 28 days post-challenge with *B. anthracis* in most studies.

Results
Rabbit studies 1 and 2 and cynomolgus macaque studies 3, and 4 evaluated treatment with Anthim 16 mg/kg IV single dose compared to placebo in animals with systemic anthrax. Treatment with Anthim alone resulted in statistically significant improvement in survival relative to placebo (Table X).

Table X: Survival Proportions in Monotherapy Treatment Studies of 16 mg/kg IV, All Randomized Animals Positive for Bacteremia Prior to Treatment

	Proportion of Survival at Day 28[1] (# survived/n)		**95% CI**[2]
	Placebo	**Anthim 16 mg/kg IV**	
NZW Rabbits			
Study 1	0 (0/9)	93% (13/14)	(0.59, 1.00)
Study 2	0 (0/13)	62% (8/13)	(0.29, 0.86)
Cynomolgus Monkey			
Study 3	6 % (1/16)	47% (7/15)	(0.09, 0.68)
Study 4[3]	0 (0/17)	31% (5/16) 35% (6/17)	(0.08, 0.59) (0.11, 0.62)

IV: intravenous, CI: Confidence Interval
[1] Survival assessed 28 days after spore challenge
All p-values from 1-sided Boschloo Test (with Berger-Boos modification of gamma=0.001) compared to placebo were <0.01
[2]Exact 95% confidence interval of difference in survival rates
[3]Anthim products manufactured at two different facilities were tested in two separate treatment arms.

Anthim administered in combination with antibacterial drugs (levofloxacin, ciprofloxacin, doxycycline) for the treatment of systemic inhalational anthrax disease did not interfere with the efficacy of antibacterial drugs and resulted in higher survival outcomes than antibacterial therapy alone in multiple studies where Anthim and antibacterial therapy was given at various doses and treatment times. Anthim treatment administered as prophylaxis resulted in higher survival outcomes compared to placebo in multiple studies where treatment was given at various doses and treatment times. After treatment with Anthim, there was a decrease in bacteremia and PA levels and a majority of the surviving animals had negative blood cultures and PA levels below the limit of detection at the end of the studies.

6 APPENDICES

6.1 Overview

As discussed in the body of this review, this BLA submission contains a large number of animal efficacy studies. This appendix contains the detailed review of each of the monotherapy studies covered in this statistical review. The appendix is broken down by type of study, IV monkey treatment studies, IV rabbit treatment studies, monkey post-exposure prophylactic studies, rabbit post-exposure prophylactic studies, monkey pre-exposure prophylactic studies, rabbit pre-exposure prophylactic studies, and the re-challenge study. The following table contains a listing of the sections and the studies reviewed within each section.

Table 19. List of studies in monkeys and rabbits by study type

Section	Study type	Study number
6.2	IV monkey treatment studies	AP201, AP202, AP203, AP204, NIAID 1056
6.3	IV rabbit treatment studies	AR021, AR033, NIAID 1030, NIAID 1045
6.4	Monkey post-exposure prophylactic studies	AP107, AP301, AP307
6.5	Rabbit post-exposure prophylactic studies	AR004, AR007, AR012, AR034 (Phase 1), AR035, AR037, AR0315
6.6	Monkey pre-exposure prophylactic studies	AP305
6.7	Rabbit pre-exposure prophylactic studies	AR001, AR003
6.8	Re-challenge study	AR034 (Phase 2)

In all analyses of quantitative bacteremia data, values less than the LOD will be replaced with1/2 the established LOD for the assay. Quantitative bacteremia values reported as less than the LLOQ will be replaced with 1/2 the LLOQ for analysis. In all analyses of serum PA measured by ELISA, values less than the LLOQ will be replaced with 1/2 the established LLOQ.

In the original study reports, the statistical method for comparing two survival proportions between a treatment and a control group was a Fisher's exact test. However in the applicant's clinical overview section, all p-values reported were from Boschloo's tests. To be consistent and to avoid Fisher's exact test's over-conservativeness, only one-sided p-values from the Boschloo's test will be reported for each study, although the specified statistical method in a protocol was a Fisher's exact test. When there were multiple comparisons in a study to adjusted, an exact (1-0.05/m)×100% confidence interval was calculated and called adjusted 95% confidence interval. For detailed description of statistical analysis methods, please see Section 3.2.2.3.

6.2 IV Monkey Treatment Studies

6.2.1 Summary of IV monkey treatment studies

There were five monkey studies that assessed the efficacy of ETI-204 IV as monotherapy, 4 were conducted by the applicant and one by NIH. Three used the Baxter product only, one used the Lonza product only, and one assessed both products. These studies varied the doses of ETI-204,

typically based on the results of the previous studies. The survival results were very variable across the studies, which was likely due to the severity of disease at the time of therapy.

Table 20. Survival results in monkey treatment IV studies testing mono-therapy

Study manufacturer year	**Blinded**	**Average challenge dose (LD_{50}) mean (SD)**	**Average time to treatment (hrs) mean (SD)**	**Pre-Bacter-emia (log10) mean**	**ETI-204 Dose (mg/kg)**	**Survival %**	**One sided P-value**
AP201 Baxter 2009	Not fully blinded	198.7 (65.8) 200.7 (51.9) 198.8 (64.9)	44.49 (8.49) 41.35 (9.54) 42.54 (7.22)	3.14 3.12 3.39	0 4 8	14% (2/14) 79% (11/14) 73% (11/15)	 0.00046* 0.00075*
AP204 Baxter 2010	Not fully blinded	220.1 (49.2) 207.4 (34.7) 209.2 (47.0)	39.18 (4.96) 40.42 (5.97) 44.41 (8.70)	4.09 4.17 3.50	0 4 16	6% (1/16) 25% (4/16) 50% (8/16)	 0.1077 0.0036*
AP203 Lonza 2012	Yes	294.6 (76.7) 279.4 (59.2) 291.8 (79.7)	37.1 (4.2) 36.2 (5.2) 37.5 (4.0)	4.77 5.07 4.67	0 8 32	13% (2/16) 6% (1/16) 38% (6/16)	 0.761 0.064
AP202 Baxter/Lonza 2014	Yes	247.6 (52.6) 270.2 (54.8) 254.4 (41.0)	38.9 (5.4) 39.3 (5.6) 39.3 (4.3)	4.95 5.52 5.08	0 16 (Lonza) 16 (Baxter)	0 (0/17) 31% (5/16) 35% (6/17)	 0.0085* 0.0046*
1056 Baxter 2010	No	187.3 (28.0) 201.6 (84.4)	n/a 35.8 (5.0)	n/a 4.51	0 8	0 (0/8) 50% (4/8)	 0.014*

*Statistically significant at an overall one-sided significance level of 0.025 with Bonferroni adjustment for multiple comparisons

6.2.2 AP201

6.2.2.1 Study Design and Endpoints

Primary Objective
The primary objective of this study was to evaluate the efficacy of ETI-204, when administered therapeutically against lethality due to inhalational anthrax.

Secondary Objective
The secondary objective was to include expanded microscopic evaluation of brain and meninges of surviving and non-surviving NHPs was well as neurological examinations pre-study and at 28 and 56 days post challenge.

Study Design
This was a randomized, blinded, placebo-controlled, trigger-to-treat (dosing upon positive PA), dose ranging study in anthrax challenged animals, conducted at the (b) (4) in 2009.

Animals were randomized to one of the following 3 groups:

- Saline (placebo)
- ETI-204 4 mg/kg
- ETI-204 8 mg/kg

The test product was manufactured at Baxter Bioscience.

Treatment vials were labeled as "Y", "X" and "Z" for saline, ETI-204 4 mg and ETI-204 8 mg. Because of this, this study is not considered a blinded study because those involved in the study had knowledge of masked treatment group assignment.

Randomization was conducted in three steps: 1) 45 animals were randomized by weight into one of three treatment groups of 15 animals (with each group containing ~50% male, ~50 female), 2) they were randomized to one of three challenge days, and 3) a challenge order per day. Another staff member, not associated with the conduct of this study, randomly assigned a vial identification to each of the three groups.

Animals were challenged with a target inhaled dose of 200 median LD_{50}s. Treatment was started once an animal reached the treatment trigger. Treatment trigger was a positive serum PA-ECL assay result on or before 54 hours post-challenge time point, or 54-hours post-challenge if PA-ECL results had not become positive.

Animals were monitored and blood collected regularly post challenge up to Day 30 when all surviving animals were euthanized. Blood was collected to measure bacteremia and serum PA levels.

Primary Endpoint

The primary endpoint was survival to 30 days post anthrax spore challenge.

6.2.2.2 Statistical Methodologies

Sample Size Calculation
Assuming the true probability of survival in the control and treated group was 10% and 65%, respectively, 15 animals per group would provide 82.5% power to detect a difference in survival proportions for Fisher's exact test with a one-sided 0.05 level, taking into account a Bonferroni adjustment to control for multiple comparisons across the two tests.

Comment: Using an overall one-sided type I error of 0.05, with 0.025 one-sided type I error for each test with a Bonferroni correction, we agree with the protocol's statistical power. However, we will assess the study with an overall one-sided type I error fixed at 0.025. Using a Bonferroni adjustment, the one-sided type I error for each test should be 0.0125. This leads to a statistical power of 76.25%.

Analysis Populations
The protocol planned to analyze all challenged animals, all challenged animals that had positive bacteremia prior to treatment, and all challenged and treated animals. All challenged animals were included in the analysis, since 100% of the monkeys were treated and were bacteremic prior to treatment.

Primary Analysis
The survival data from each treatment group were compared to the control group using a one-sided Fisher's exact test. The analysis was adjusted for multiple comparisons using a Bonferroni adjustment.

Secondary Analyses
Time to death was plotted using Kaplan-Meier estimate. In addition, log-rank test was used to test for significant differences in survival.

6.2.2.3 Animal Disposition, Demographic and Baseline Characteristics

Two of the 45 animals planned for this study were removed prior to challenge, one of them died prior to telemetry implantation and the other one had abnormal lab results. Demographic variables and baseline characteristics are listed in Table 21. All variables appeared to be comparable, except that PA-ELISA arithmetic means was higher in the 8 mg/kg group, which was due to an individual high value. Only one animal (C36423) in the 4 mg/kg IV group had a missing value in qualitative direct bacteremia but it was positive in quantitative bacteremia. Therefore, all animals were considered as bacteremic by the applicant. Sixty percent (60%) of monkeys received a less and 40% received more than 200 LD_{50} target dose. The average challenge dose varied across days with the highest occurring on challenge day A (251) and the lowest on challenge day C (168), as reported by the applicant.

Table 21. Study AP201: Demographic variables and baseline characteristics by treatment group

	Placebo (N=14)	**ETI-204 4 mg/kg IV (N=14)**	**ETI-204 8 mg/kg IV (N=15)**	**All (N=43)**
Age (years)				
Mean (SD)	3.6 (0.6)	3.6 (0.6)	3.7 (0.6)	3.7 (0.6)
Range	2.9, 5.1	2.6, 4.9	2.9, 5.1	2.6, 5.1
Gender [n (%)]				
Male	8 (57.1)	7 (50.0)	7 (46.7)	22 (51.2)
Female	6 (42.9)	7 (50.0)	8 (53.3)	21 (48.8)
Body weight (kg)				
Mean (SD)	3.4 (0.8)	3.3 (0.6)	3.3 (0.5)	3.4 (0.6)
Range	2.5, 5.3	2.6, 4.6	2.6, 4.7	2.5, 5.3
Challenge dose (LD_{50})				
Mean (SD)	198.7 (65.8)	200.7 (51.9)	198.8 (64.9)	199.4 (59.8)
Range	96.0, 305.0	140.0, 280.0	109.0, 356.0	96.0, 356.0
Challenge dose (LD_{50}) (n(%))				
<200	8 (57.1)	8 (57.1)	10 (66.7)	26 (60.5)
200 or higher	6 (42.9)	6 (42.9)	5 (33.3)	17 (39.5)
Challenge dose (x 10^7 cfu)				
Mean (SD)	1.227 (0.406)	1.240 (0.321)	1.229 (0.401)	1.232 (0.369)
Range	0.591, 1.880	0.865, 1.730	0.676, 2.200	0.591, 2.200
Bacteremia prior to treatment (direct qualitative) [n (%)]	14 (100)	13 (92.9)	15 (100.0)	42 (97.7)
Bacteremia prior to treatment (cfu/mL)*				
Geometric mean	1383.9	1323.7	2461.2	1667.4
95% confidence interval	359.6, 5324.8	337.5, 5191.4	686.5, 8824.5	822.3, 3380.8
Range	17, 700000	17, 333000	500, 2400000	17, 2400000
Log_{10} bacteremia , Mean (SD)	3.14 (1.01)	3.12 (1.03)	3.39 (1.00)	3.22 (1.00)
PA-ECL positivity at trigger (n(%))	13 (92.9)	12 (85.7)	15 (100.0)	40 (93.0)
PA-ELISA prior to treatment* (ng/mL)				
Geometric mean	10.0	12.1	11.7	11.2
95% confidence interval	3.8, 26.4	3.8, 37.9	3.8, 36.4	6.3, 20
Log_{10} PA-ELISA	1.00 (0.73)	1.08 (0.86)	1.07 (0.89)	1.05 (0.81)
PA-ELISA prior to treatment (ng/mL)				
Mean (SD)	36.5 (71.0)	55.4 (83.2)	78.3 (181.6)	57.2 (122.5)
Range	1.2, 266.4	1.2, 228.2	1.2, 695.3	1.2, 695.3

Time to bacteremia, treatment trigger, and treatment

These variables were comparable across different groups, as shown in the following table.

Table 22. Study AP201: Time between challenge, trigger, and treatment

	Placebo (N=14)	ETI-204 4 mg/kg IV (N=14)	ETI-204 8 mg/kg IV (N=15)	All (N=43)
Time to bacteremia (hours)				
Mean (SD)	37.7 (7.8)	34.4 (7.7)	35.6 (4.4)	35.9 (6.7)
Range	28.6, 55.4	25.5, 52.1	25.4, 41.8	25.4, 55.4
Time to trigger (hours)				
N	13*	13*	15	41
Mean (SD)	39.49 (8.05)	37.96 (10.12)	38.65 (8.00)	38.70 (8.54)
Range	28.58, 52.57	25.53, 55.92	25.43, 54.83	25.43, 55.92
Time to randomized treatment (hours)				
Mean (SD)	44.49 (8.49)	41.35 (9.54)	42.54 (7.22)	42.78 (8.34)
Range	31.80, 58.73	29.10, 59.07	29.35, 57.98	29.10, 59.07
Time from trigger to treatment (hours)				
N	13*	13*	15	41
Mean (SD)	3.90 (1.00)	3.14 (1.47)	3.89 (1.41)	3.65 (1.33)
Range	2.87, 5.62	0.07, 4.80	0.07, 5.93	0.07, 5.93

*One animal in the placebo group (C38277) and one in the ETI-204 4 mg/kg group (C37686) were triggered for treatment based on time and had missing values in trigger time so they were not included in this calculation.

6.2.2.4 Results

Survival

In this study, survival to Day 30 was the same as to Day 28. So in figures, survival to Day 28 was used to compare survival across studies. As Table 23 shows, there was a statistically significant difference between both ETI-204 groups and the placebo group, comparing the one-sided p-values to 0.0125 to account for multiple comparisons. This is true for both the primary analysis which includes all animals and a sensitivity analysis which excludes one animal without qualitative direct bacteremia (this animal was positive in quantitative bacteremia) prior to treatment. There was no difference seen between the 4 and 8 mg/kg doses.

Table 23. Study AP201: Survival at Day 30 by treatment group

	Placebo (N=14)	ETI-204 4 mg/kg IV (N=14)	ETI-204 8 mg/kg IV (N=15)
Including all animals			
n (%)	2 (14.3)	11 (78.6)	11 (73.3)
Difference in survival proportion		0.643 [0.260, 0.879] 0.00046	0.590 [0.207, 0.841] 0.00075
Adjusted confidence interval		0.206, 0.898	0.162, 0.864
Excluding one animal without qualitative direct bacteremia			
n (%)	Same as	10/13 (76.9)	Same as above
Difference in survival proportion	above	0.644 [0.271, 0.871] 0.00032	Same as above
Adjusted confidence interval		0.179, 0.888	Same as above

As the following graph and table show, there were statistically significant differences in survival time between the ETI-204 groups and the placebo group, even with a Bonferroni adjustment for multiple comparisons (using a two-sided significance level of 0.05/2=0.025).

Figure 7. Study 201: Kaplan-Meier curve and 95% confidence band by treatment group

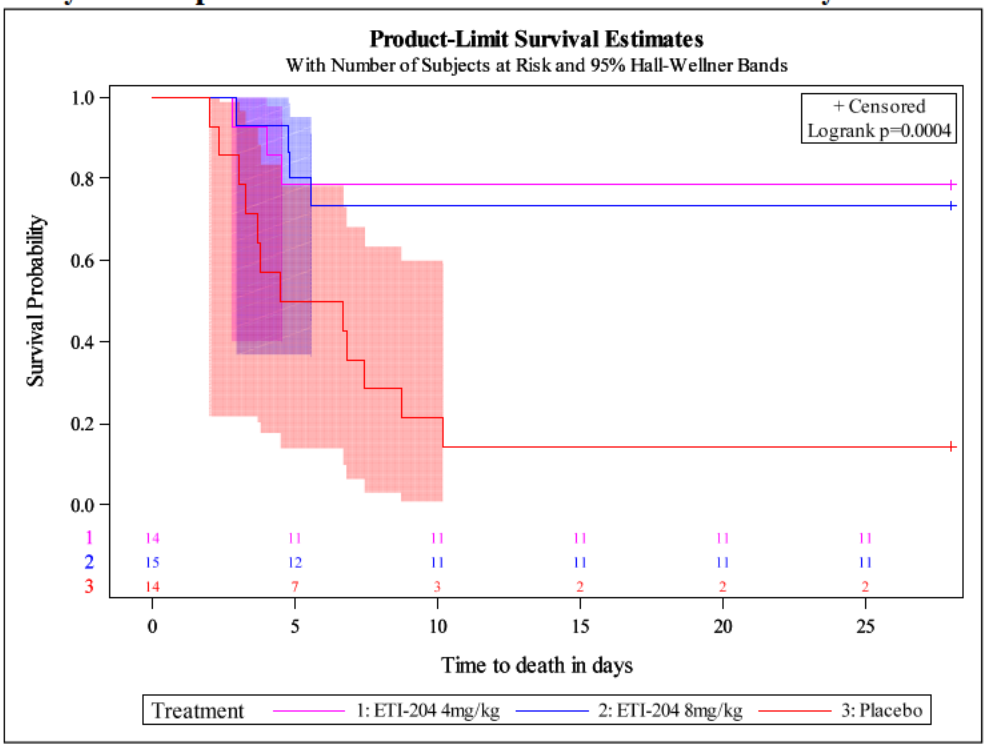


Table 24. Study AP201: two-sided p-values of pairwise log-rank tests comparing time from challenge to death among groups

	ETI-204 4 mg/kg IV (N=14)	ETI-204 8 mg/kg IV (N=15)
Placebo (N=14)	0.0011*	0.0068*
ETI-204 4 mg/kg		0.84

*Statistically significant at a one-sided significance level of 0.025

The following two figures show that animals with lower bacteremia or PA levels prior to treatment were more likely to survive. No animals with a bacteremia greater than 10000 (1E4) cfu/mL survived to Day 30.

Figure 8. Study AP201: Time to death versus bacteremia prior to treatment by survival status at Day 30

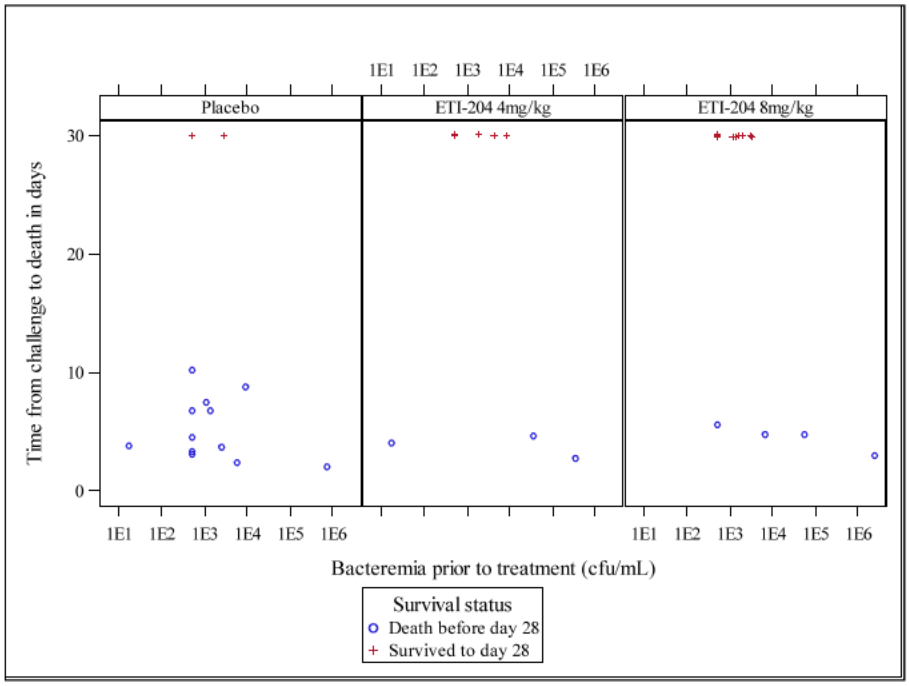
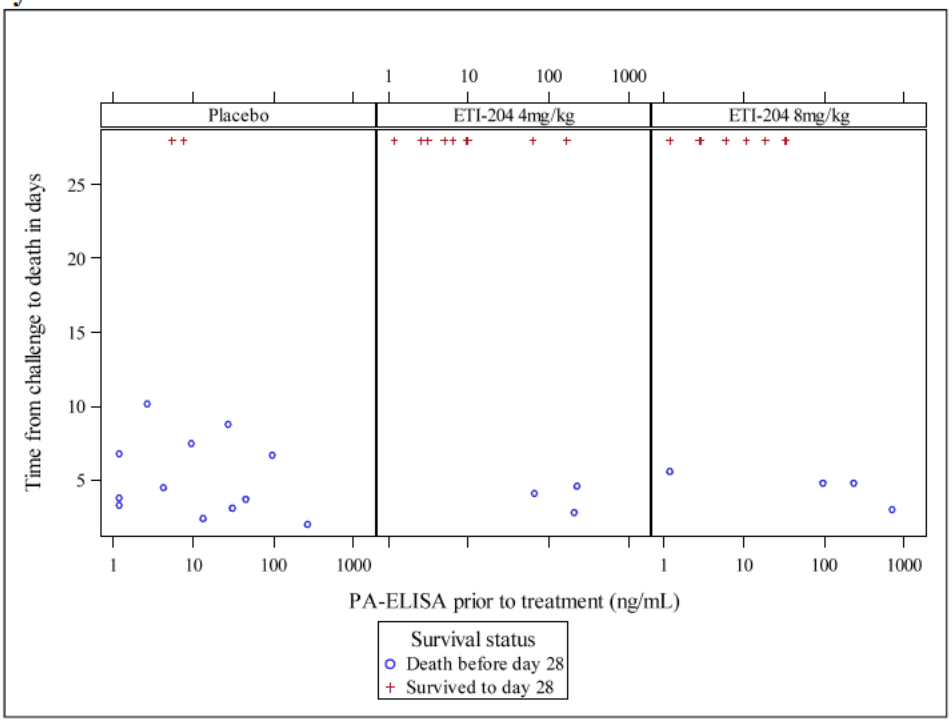


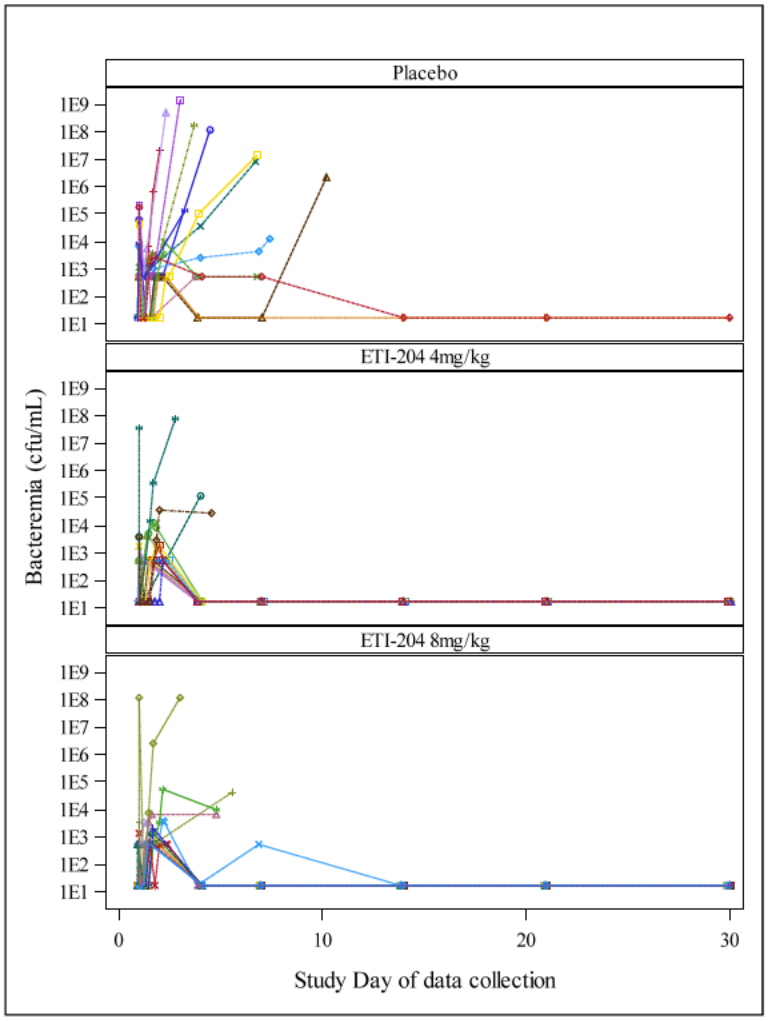
Figure 9. Study AP201: Time to death versus PA-ELISA prior to treatment by survival status at Day 28



Bacteremia over time

As the following graph shows, at 24 hours post-treatment (around Day 3) bacteremia levels were reduced in the two treated groups; at 96 hours post-treatment, all surviving animals reached a level below the limit of detection (LOD).

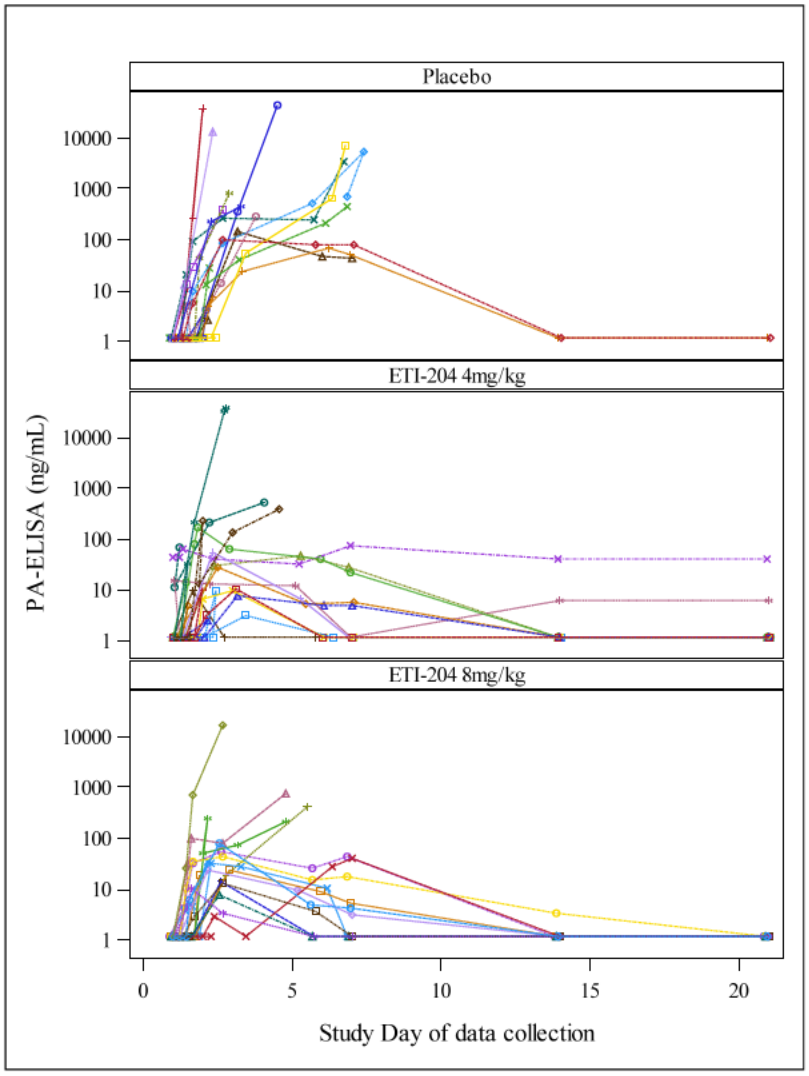
Figure 10. Study AP201: Bacteremia over time by animal



PA-ELISA over time

The following figure shows PA-ELISA levels over time by animals and by treatment. Prior to treatment PA level in each group increased. After 96 hours post-treatment the levels decreased in the treated groups. The two survivors in the placebo group also had a PA level below the LLOQ at and after Day 14.

Figure 11. Study AP201: PA-ELISA over time by animal and treatment



Subgroup Analysis Results

The following table shows the results of subgroup analyses. The survival proportions were comparable between male and female monkeys. For other variables, the sample sizes were too small to make any valid conclusions. The two surviving animals in the placebo groups were one female, with a challenge dose of 187 LD_{50}s, bacteremia of 500 cfu/mL and PA-ELISA of 7.7 ng/mL prior to treatment; one male, with a challenge dose of 145 LD_{50}s, bacteremia of 2830 cfu/mL and PA-ELISA of 5.54 ng/mL, prior to treatment.

Tissue bacterial assessments and pathological findings in the brain

No surviving animals had positive bacterial load in bronchial lymph node and spleen. No data were available for bacterial load in other issues. Only 2 dead animals in each group (16.7%, 66.7%, and 50.0% in the 0, 4, 8 mg/kg group, respectively) had positive pathological findings (discolorations) in the brain. No surviving animals had pathological findings in the brain.

Table 25. Study AP201: Survival at Day 28 by gender, challenge dose, log_{10} bacteremia, PA prior to treatment

	Placebo (N=14)	**ETI-204 4 mg/kg IV (N=14)**	**ETI-204 8 mg/kg IV (N=15)**	**All (N=43)**
Gender				
Female	1/8 (12.5%)	6/7 (85.7%)	5/7 (71.4%)	12/22 (54.6%)
Male	1/6 (16.67%)	5/7 (71.4%)	6/8 (75%)	12/21 (57.1%)
Challenge dose (LD_{50})				
<250	2/10 (20%)	8/11 (72.7%)	9/13 (69.2%)	19/34 (55.9%)
250 or higher	0/4	3/3 (100%)	2/2 (100%)	5/9 (55.6%)
Bacteremia prior to treatment (cfu/mL)				
$<10^2$	0/1	0/1	0	0/2
10^2 - 10^4	2/12 (16.7%)	11/11 (100%)	11/13 (84.6%)	24/36 (66.7%)
10^4 - $<10^6$	0/1	0/2	0/1	0/4
10^6 or higher	0	0	0/1	0/1
PA prior to treatment (ng/mL)				
0 - < 10	2/8 (25%)	8/8 (100%)	6/7 (85.7%)	16/23 (69.6%)
10 - < 50	0/4	1/1 (100%)	5/5 (100%)	6/10 (60%)
50 or higher	0/2	2/5 (40.0%)	0/3	2/10 (20%)

6.2.2.5 Conclusions

Study AP201 supports the efficacy of both 4 mg and 8 mg IV in monkeys. The 30-day survival rate was 79% for the 4 mg dose and 73% for the 8 mg dose compared to 14% for the placebo group. Lower survival was seen at higher PA and bacteremia levels. Time to treatment was 42.78 (SD 8.34) hours on average. An increased rate of brain lesions were seen in the treated animals that died compared to control.

6.2.3 AP204

6.2.3.1 Study Design and Endpoints

Primary Objective
The primary objective of this study was to evaluate the efficacy of single IV bolus doses of 4 or 16 mg/kg ETI-204, when administered therapeutically as compared with control material (normal saline), to protect cynomolgus macaques from lethality due to inhalational anthrax.

Secondary Objective
The secondary object was to perform expanded microscopic evaluations of brain and meninges for non-surviving and surviving NHPs as well as neurological examinations pre-study and at 28 and 56 days post challenge.

Study Design

This was a randomized, blinded, placebo-controlled study, conducted at (b) (4) in 2010.

Randomization for receiving challenge was performed in three steps. In the first step, NHPs were randomized (prior to challenge) by weight into one of the three groups of 16 animals (with each group containing 8 males and 8 females). In the second step, they were randomized to one of three aerosol challenge days (16 animals per day). In the third step, they were randomized to a challenge order per day. A staff member not associated with the conduct of this study randomly assigned viral identification to each of the three groups:

- Placebo: saline
- 4 mg/kg ETI-204
- 16 mg/kg ETI-204

The test product was manufactured at Baxter Bioscience.

Treatment vials were labeled as "Y", "X" and "Z" for saline, ETI-204 4 mg and ETI-204 16 mg. Because of this, this study is not considered a blinded study because those involved in the study had knowledge of masked treatment group assignment.

NHPs were challenged on Study Day 0 with a targeted 200 LD_{50} dose of *B. anthracis* (Ames strain) spores. Animals were monitored regularly after challenge and blood collected frequently post-treatment for assessment of bacteremia and serum PA levels.

A positive PA-ECL was used as a trigger for starting treatment. In the case of PA-ECL assay failure treatment was given when directed by the Study Director. If PA-ECL was negative at all time points including the 54-hour post-challenge time point, treatment would be started.

Complete gross necropsies and histopathology evaluations were conducted on all animals that were euthanized due to illness or found dead. At the end of the in-life portion of the study, either Day 28 or 56, all surviving animals were euthanized and the presence or absence of anthrax bacteria in samples was determined.

Primary Endpoints

The primary endpoint was survival. Since some surviving animals were euthanized at Day 28, survival out to Day 28 will be used in the primary efficacy analysis.

6.2.3.2 Statistical Methodologies

Sample Size Calculation
Assuming the probabilities of survival were 65% and 10% in the treated group and control group, respectively, the sample size of 16 animals per group provided 80.9% statistical power to detect a difference in survival rates between an ETI-204 treated group and the control group. This power calculation was for a one-sided, overall 0.025 level Fisher's exact test and included a Bonferroni adjustment for multiple comparisons. Each comparison should be assessed using a 0.0125 one-sided type I error.

Analysis Populations
There were three populations mentioned in the primary analysis:

1) Excluding animals that were not positive for bacteremia by culture prior to treatment and including animals that died prior to treatment as treatment failures. This population was for the primary analysis.
2) Including all challenged animals. This was for a secondary analysis.
3) Including only those animals that received treatment. However since all challenged animals survived to treatment, this population was the same as in 2).

Primary Analysis
The survival data from each treatment group was compared to the control group using a one-sided Fisher's exact test (at a level of 0.025).

This analysis was also performed using a Bonferroni adjustment for multiple comparisons.

Secondary Analyses
The primary analysis was repeated with all challenged animals. This secondary analysis was also adjusted for multiple comparisons using the Bonferroni method.

The time-to-death data were analyzed to determine if there were differences in protection for any of the groups based on a time-to-death model. The Kaplan-Meier estimators were plotted for each group and the log-rank test was conducted to determine if differences between groups were

statistically significant. If the overall log-rank was significant, then pairwise log-rank tests were computed to determine which pairs of groups were significantly different from each other.

6.2.3.3 Animal Disposition, Demographic and Baseline Characteristics

Demographic variables and baseline characteristics are listed in Table 26. Age, gender, body weight, and challenge dose were comparable among the three groups. It was noticed that in this study about 42% of animals received a challenge dose less than 200 LD50s. The bacteremia levels in the ETI-204 groups appear lower; however, there were no statistically significant differences among the three groups according to the p-value of 0.31 from an ANOVA. The mean PA level was slightly higher in the 4 mg/kg group.

Table 26. Study AP204: Demographic variables and baseline characteristics by treatment group

	Placebo (N=16)	**ETI-204 4 mg/kg IV (N=16)**	**ETI-204 16 mg/kg IV (N=16)**	**All (N=48)**
Age (years)				
Mean (SD)	3.1 (0.2)	3.0 (0.2)	3.1 (0.2)	3.0 (0.2)
Range	2.6, 3.3	2.7, 3.3	2.8, 3.3	2.6, 3.3
Gender [n (%)]				
Male	8 (50.0)	8 (50.0)	8 (50.0)	24 (50.0)
Female	8 (50.0)	8 (50.0)	8 (50.0)	24 (50.0)
Body weight (kg)				
Mean (SD)	2.8 (0.3)	2.8 (0.2)	2.8 (0.2)	2.8 (0.2)
Range	2.3, 3.5	2.5, 3.3	2.5, 3.3	2.3, 3.5
Challenge dose (LD_{50})				
Mean (SD)	220.1 (49.2)	207.4 (34.7)	209.2 (47.0)	212.2 (43.5)
Range	136.0, 327.0	155.0, 279.0	136.0, 325.0	136.0, 327.0
Challenge dose (LD_{50}), n(%)				
<200	6 (37.5)	7 (43.8)	7 (43.8)	20 (41.7)
200 or higher	10 (62.5)	9 (56.3)	9 (56.3)	28 (58.3)
Bacteremia enriched prior to treatment (n(%))	16 (100.0)	16 (100.0)	15 (93.8)	47 (97.9)
Bacteremia prior to treatment (cfu/mL)				
N	16	16	16*	48
Geometric mean	12287	14649	3139	9082
95% confidence interval	3344, 45140	4954, 43320	606, 16271	4276,19290
Mean (SD) of $\log_{10}$ bacteremia	4.09 (1.06)	4.17 (0.88)	3.50 (1.34)	3.92 (1.13)
PA-ECL at Trigger (n(%))	16 (100.0)	16 (100.0)	14 (87.5)	46 (95.8)
PA-ELISA Prior to Treatment				
Geometric mean	38.1	60.7	31.0	41.6
95% confidence interval	18.6, 78.2	36.5, 101	15.8, 60.9	29.3, 59.1
Mean (SD) of $\log_{10}$ PA-ELISA	1.58 (0.59)	1.78 (0.41)	1.49 (0.55)	1.62 (0.53)

*Only one animal had negative bacteremia. If this animal was excluded, the mean (SD) were 3.71 (1.07); range: 1.7, 5.63. Geometric mean was 5127 (95% confidence interval: [1307, 20109])

Time to treatment trigger and treatment

Table 27 shows the time between challenge, trigger, and treatment. These time variables were slightly higher in the treated groups. This may be due to the lower mean challenge doses in the two ETI-204 groups.

Table 27. Study AP204: Time between challenge, trigger, and treatment

	Placebo (N=16)	ETI-204 4 mg/kg IV (N=16)	ETI-204 16 mg/kg IV (N=16)	All (N=48)
Time to bacteremia (hours)				
Mean (SD)	29.89 (3.58)	31.7 (5.64)	33.18 (9.96)	31.56 (6.82)
Range	21.93, 34.8	23.62, 42.25	21.62, 58.73	21.62, 58.73
Time to trigger (hours)				
Mean (SD)	35.68 (5.32)	37.12 (6.24)	41.37 (8.97)	38.05 (7.29)
Range	25.10, 46.52	29.67, 48.10	27.13, 55.90	25.10, 55.90
Time to randomized treatment (hours)				
Mean (SD)	39.18 (4.96)	40.42 (5.97)	44.41 (8.70)	41.34 (6.96)
Range	28.47, 49.65	33.32, 51.22	30.18, 58.78	28.47, 58.78
Time from trigger to treatment (hours)				
Mean (SD)	3.50 (0.97)	3.31 (0.92)	3.05 (1.26)	3.28 (1.05)
Range	0.12, 4.22	0.03, 3.98	0.05, 4.20	0.03, 4.22

6.2.3.4 Results

Survival

Table 28 shows the survival status at Day 28 by treatment group. There was no difference between the ETI-204 4 mg/kg group and the placebo group. There was a difference between the ETI-204 16 mg/kg group and the placebo group. These findings were true in the two analysis populations. The applicant defined the bacteremic population as the primary analysis population. To be consistent with other monkey treatment studies, we also used an mITT population (randomized and received treatment) as an analysis population. Therefore the following analyses for this study will use the mITT population and bacteremic population. In this study, all randomized animals received treatment, so the mITT population includes all animals.

Table 28. Study AP204: Survival at Day 28 by treatment group

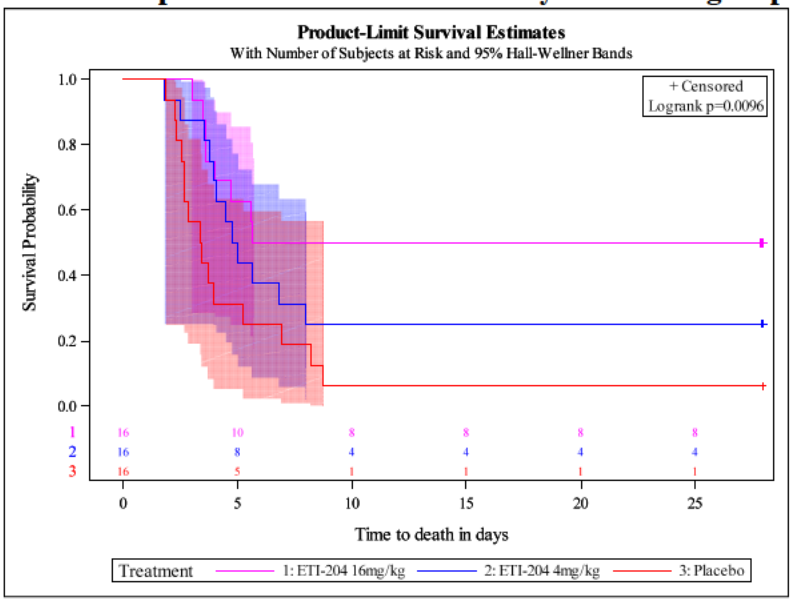
	Placebo (N=16)	ETI-204 4 mg/kg IV (N=16)	ETI-204 16 mg/kg IV (N=16)
Including all animals			
n (%)	1 (6.3)	4 (25.0)	8 (50.0)
Difference in survival proportion [exact 95% confidence interval] one-sided p-value compared with control		0.188 [-0.090, 0.473] 0.1077	0.438 [0.113, 0.703] 0.0036*
Adjusted exact 95% confidence interval		-0.135, 0.513	0.070, 0.733

	Placebo (N=16)	ETI-204 4 mg/kg IV (N=16)	ETI-204 16 mg/kg IV (N=16)
Excluding one animal without bacteremia prior to treatment (primary)			
n/N (%)	Same as above	Same as above	7/15 (0.467)
Difference in survival proportion [exact 95% confidence interval] one-sided p-value compared with control		Same as above	0.404 [0.089, 0.681] 0.0058*
Adjusted exact 95% confidence interval		Same as above	0.048, 0.712

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025/2=0.0125

Figure 12 shows the Kaplan-Meier survival curves by treatment group. The overall p-value from a log-rank test was statistically significant (p-value=0.0096). The p-value from a log-rank test for the comparison of the time to death in the ETI-204 4mg/kg group and the placebo was 0.0955, not statistically significant. The p-value for the comparison of the 16 mg/kg group and the placebo group was 0.0030, statistically significant at a two-sided significance level of 0.025, using the Bonferroni adjustment method for multiple comparisons.

Figure 12. Study AP204: Kaplan-Meier survival curve by treatment group



Including one surviving animal without bacteremia prior to treatment in the ETI-204 16mg/kg

As shown in Figure 13 and Figure 14, a lower bacteremia or PA-ELISA level prior to treatment was more likely to survive.

Figure 13. Study AP204: Time to death versus bacteremia prior to treatment by survival status at Day 28

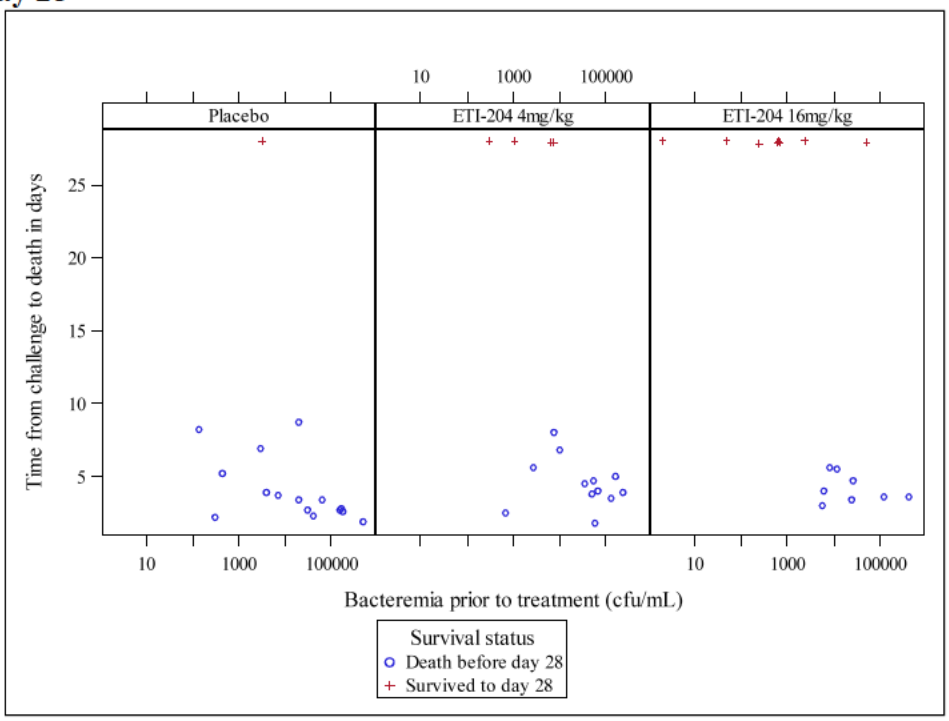


Figure 14. Study AP204: Time to death versus PA-ELISA prior to treatment by survival status at Day 28

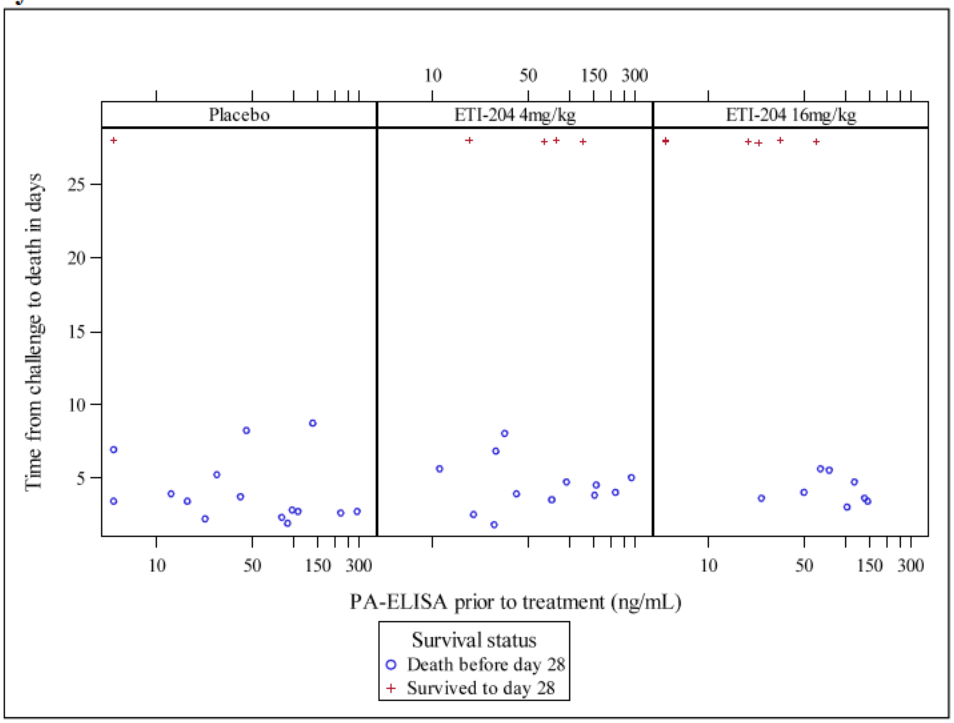
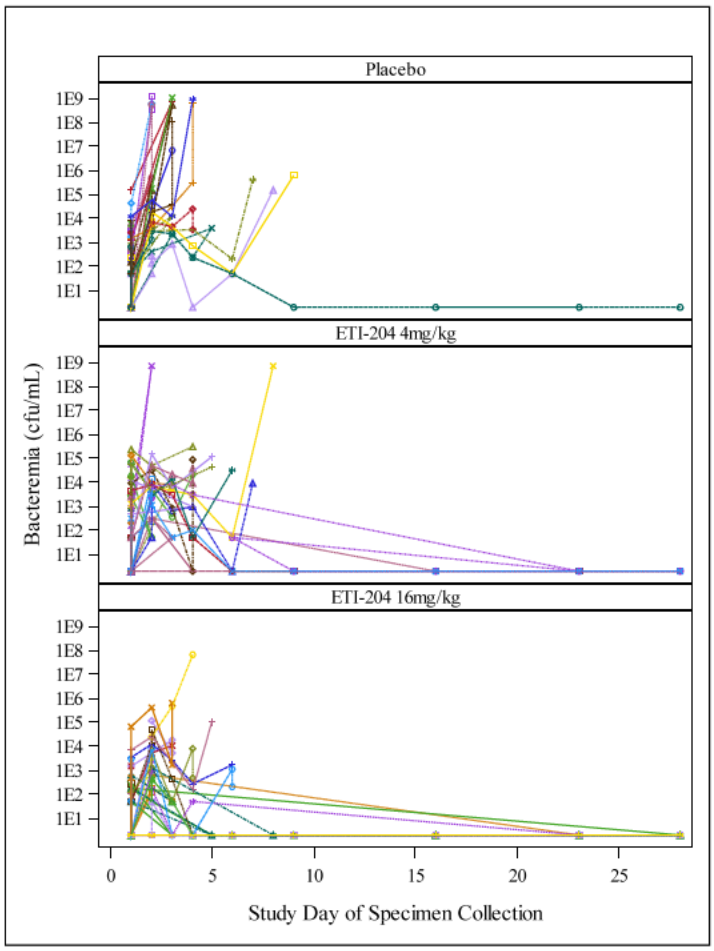


Figure 15. Study AP204 Bacteremia over time by animal



Bacteremia level over time

Figure 15 shows bacteremia over time by treatment arm and animal. In the two treatment groups, bacteremia levels in most animals were lower after receiving treatment, compared with the placebo group.

PA-ELISA level over time

Figure 16 shows PA-ELISA levels over time. After 6 hours of treatment or on Study Day 3, the two treatment groups demonstrated a significant decrease in PA-ELISA level.

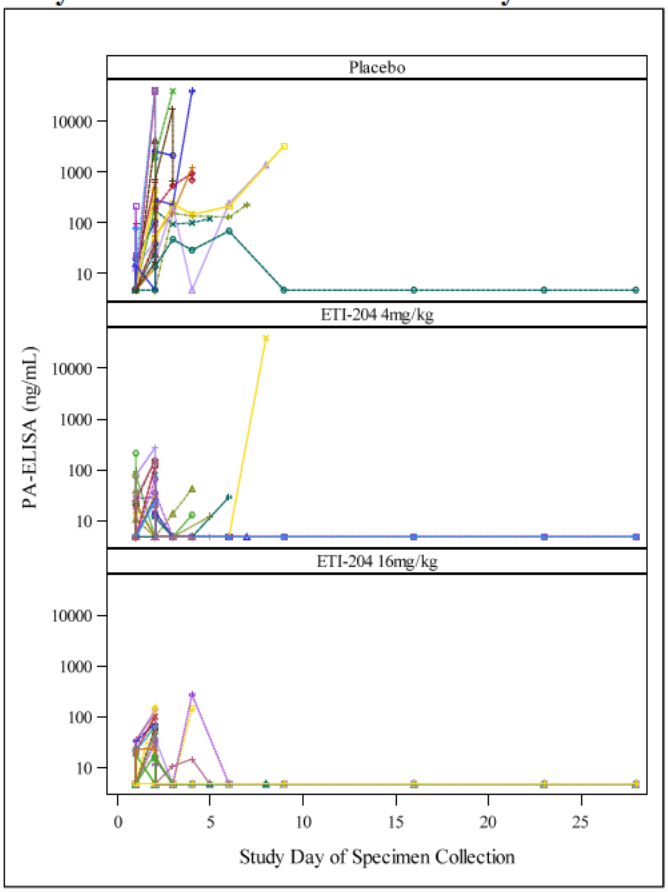
Tissue bacterial assessments and pathological findings in the brain

In the surviving animals, only 1 (100%), 2 (50%), and 6 (75%) animals in the placebo, 4 mg/kg IV, and 16 mg/kg IV groups had a bacterial load value of 0.5 or 1 (positive result) in the lung; no

bacterial load in the brain, liver, kidney, and spleen. Only one animal in the 16 mg/kg group had a 0.5 bacterial load in bronchial lymph node.

Among the animals that died, 14/15 (93.3%), 11/12 (91.7%), and 7/8 (87.5%) in the placebo, 4 mg/kg IV, and 16 mg/kg IV groups had a positive bacterial result in the brain.

Figure 16. Study AP204: PA-ELISA over time by animal and treatment



Among the animals that died, 1, 5, and 3 (6.7%, 41.7%, and 37.5%) animals in the placebo, 4 mg/kg IV, and 16 mg/kg IV groups had a positive microscopic pathological result (discoloration(s)) in the brain. No survivors had a positive pathological result in the brain. According to the study report, as compared to controls, non-survivors were "more likely to have extravascular bacteria (mostly in the meninges) and other morphologic abnormalities including meningitis (inflammation in the meninges), encephalitis (inflammation in the brain), vasculitis, and hemorrhage. These other abnormalities are consistent with the ETI-204-treated animals attempting to mount an immune response to the bacteria/bacterial products. The meninges were most commonly and typically the most severely affected area, indicating meningitis was the main morphologic finding associated with inhalation anthrax in ETI-204-treated animals. In the brain, the areas most affected tended to be those with the greatest surface area (cerebrum and cerebellum) and therefore with the most exposure to the meninges."

Regarding the secondary objective, the applicant reports "in all the animals that survived (regardless of group) until Days 28 or 56, there was no sign of anthrax infections, including a total lack of any visible bacteria in the blood stream."

Subgroup Analysis Results

Error! Not a valid bookmark self-reference. shows the survival status at Day 28 by gender, challenge dose, bacteremia, and PA prior to treatment. There was considerable variability in survival proportions by gender and challenge dose. It appears that a lower bacteremia level was associated with a higher survival proportion. A higher PA level in the 4 mg/kg group was associated with a higher survival proportion, compared with a lower PA level in the same treatment group. However the sample size was small in general, it was not possible to reach reliable conclusions from these subgroup analyses. One female with a challenge dose of 163 LD_{50}s and a bacteremia level of 3130 cfu/mL and a PA-level less than the LLOQ in the placebo group survived.

Table 29. Study AP204: Survival at Day 28 by gender, challenge dose, log_{10} bacteremia, and PA prior to treatment

	Placebo (N=16)	ETI-204 4 mg/kg IV (N=16)	ETI-204 16 mg/kg IV (N=16)	All (N=48)
Gender				
Female	1/8 (12.5%)	2/8 (25%)	3/8 (37.5%)	6/24 (25%)
Male	0/8	2/8 (25%)	5/8 (62.5%)	7/24 (29.2%)
Challenge dose (LD_{50}) (n(%))				
<250	1/13 (7.7%)	2/14 (14.3%)	8/15 (53.3%)	11/42 (26.2%)
250 or higher	0/3	2/2 (100%)	0/1	2/6 (33.3%)
<200	1/6 (16.7%)	1/7 (14.3%)	4/7 (57.1%)	6/20 (30%)
Bacteremia prior to treatment (cfu/mL)				
$<10^2$	0	0	2/2 (100%)	2/2 (100%)
10^2 - 10^4	1/7 (14.3%)	4/8 (50%)	5/8 (62.5%)	10/23 (43.5%)
10^4 - $<10^6$	0/9	0/8	1/6 (16.7%)	1/23 (4.3%)
PA prior to treatment (ng/mL)				
0 - < 10	1/3 (33.3%)	0	4/4 (100%)	5/7 (71.4%)
10 - < 50	0/6	1/7 (14.3%)	3/5 (60%)	4/18 (22.2%)
50 or higher	0/7	3/9 (33.3%)	1/7 (14.3%)	4/23 (17.4%)

6.2.3.5 Conclusion

Study AP204 was conducted after AP201 and repeated the 4 mg/kg dose and an intermediate dose of 8 mg/kg. However unlike in study AP201, the 4 mg/kg dose was not significantly better than placebo in terms of survival. The 8 mg/kg dose was significant in terms of survival compared to placebo. These differing results could be attributed to a higher challenge dose and more severe disease at the time of treatment. In all the animals that survived, PA and bacteremia levels became undetectable.

6.2.4 AP203

6.2.4.1 Study Design and Endpoints

Primary Objective

The primary objective was to evaluate the efficacy of ETI-204 when administrated therapeutically, IV, against lethality due to inhalation exposure to *B. anthracis* in cynomolgus macaques. The goal of this study was to evaluate the efficacy of a higher dose (32 mg/kg) than in previously conducted studies (AP201 and AP204).

Study Design

This was a randomized, blinded, placebo-controlled study, conducted by (b) (4) in 2012.

The study director, applicant, microbiologists, pathologist, neuropathologist, technicians performing the dosing, and all technicians assessing the animals were blinded to the contents of the dosing vials and animal group assignments. The paperwork that documented the treatment group and dosage information on each vial was maintained by the Quality Assurance (QA) Auditor.

There were three groups in the study:

- Group 1: placebo (saline)
- Group 2: ETI-204 8 mg/kg
- Group 3: ETI-204 32 mg/kg

The test product was manufactured at the Lonza facility.

The animals were randomized by weight into three groups of 16 animals (with each group containing ~50% male, ~50% female). Animals were stratified by sex to one of three challenge days and randomized to challenge order. ETI-204 or placebo vials were randomized to blocks of size 6 or 9. Treatment order and vial assignment was be determined by the following rules: 1) the chronologic order animals trigger for treatment, 2) in the case where animals trigger for treatment at the sample time point, the treatment order was determined by the challenge order, and 3) in the case where animals do not have a positive serum PA-ECL screening assay result by the 54 hours post challenge time point, the treatment order was determined by the challenge order. Those involved in the conduct of the trial were blinded to animal group assignments.

NHPs were aerosol challenged with a targeted 200 LD_{50} dose of *B. anthracis* Ames strain spores on Study Day 0. Animals were monitored regularly after challenge until Day 28 for clinical signs of disease, quantitative bacteremia, and levels of *B. anthracis* free PA.

Detection of PA via PA-ECL assay was used as a trigger for treatment. Treatment was planned to be administered within 3 hours of determining a positive serum PA-ECL.

Primary Endpoint

The primary endpoint was survival to 28 days post challenge.

6.2.4.2 Statistical Methodologies

Sample Size Calculation

Assuming the true probabilities of survival in the control group were 10% and 65%, respectively, and an overall two-sided type I error of 0.05, with 16 animals per group the statistical power was 80.9% to detect a difference in survival rates between each treated group and the control group.

Fisher's exact test was used with a Bonferroni adjustment to control for multiple comparisons, meaning that each test arm was compared to the control with a two-sided type I error of 0.025 (or one-sided 0.0125).

Analysis Populations

There were three study populations defined in the protocol:
Protocol-defined dataset was based on the treatment animals received, but would exclude animals that were not positive for bacteria by enriched culture prior to treatment. No animals were excluded from this population because all were bacteremic. Note that all animals received the randomized treatment.

ITT dataset included all challenged animals regardless of bacteremia status and would exclude animals that died prior to treatment.

mITT dataset included animals that were positive for bacteremia at any time point prior to treatment and would exclude animals that died prior to treatment.

Primary analysis
The survival proportion from each treatment group was compared to the control group using a one-sided Fisher's exact (0.025 level) using a Bonferroni-Holm adjustment for multiple comparisons, using the Protocol-defined dataset.

Secondary analyses
Secondary analyses were the same as primary analysis but using mITT and ITT populations. Since no animals were excluded from mITT and ITT population, the protocol-defined data set is the same as the ITT data set, and these secondary analyses were not needed.

If the primary analysis showed at least one statistically significant difference in survival rates, then a one-sided Fisher's exact (0.025 level) would have been used to test for a significant difference in survival rates between the two treated groups.

6.2.4.3 Animal Disposition, Demographic and Baseline Characteristics

All randomized animals received the planned treatment. Demographic variables and baseline characteristics of study AP203 are listed in Table 30. Age, gender, body weight, and challenge dose were comparable among three groups. The bacteremia level was slightly higher in the 8 mg/kg ETI-204 group. All animals were qualitatively bacteremic (enriched) prior to treatment.

Table 30. Study AP203: Demographic variables and baseline characteristics by treatment group

	Placebo (N=16)	**ETI-204 8 mg/kg IV (N=16)**	**ETI-204 32 mg/kg IV (N=16)**	**All (N=48)**
Age (years)				
Mean (SD)	4.4 (0.6)	4.3 (0.6)	4.4 (0.6)	4.4 (0.6)
Range	3.0, 5.0	3.0, 5.0	3.0, 5.0	3.0, 5.0
Gender [n (%)]				
Male	8 (50.0)	8 (50.0)	8 (50.0)	8 (50.0)
Female	8 (50.0)	8 (50.0)	8 (50.0)	8 (50.0)
Body weight (kg)				
Mean (SD)	3.88 (0.56)	3.83 (0.64)	3.99 (0.62)	3.90 (0.60)
Range	3.00, 4.70	2.90, 4.90	3.00, 4.80	2.90, 4.90
Challenge dose (LD_{50})				
Mean (SD)	294.6 (76.7)	279.4 (59.2)	291.8 (79.7)	288.6 (71.2)
Range	166.0, 462.0	160.0, 384.0	185.0, 430.0	160.0, 462.0
Bacteremia prior to treatment, n (%)	16 (100)	16 (100)	15 (93.8)	48 (100)
Bacteremia enriched prior to treatment, n (%)	16 (100)	16 (100)	16 (100)	48 (100)
Bacteremia prior to treatment (cfu/mL)				
Geometric mean	$5.90x10^4$	$1.19x10^5$	$2.48x10^4$*	$5.57x10^4$
95% confidence interval	$1.57x10^4$, $2.21x10^5$	$2.42x10^4$, $5.83x10^5$	$3.25x10^3$, $1.89x10^5$	$2.24x10^4$, $1.39x10^5$
Mean (SD) of log_{10} bacteremia	4.77 (1.08)	5.07 (1.30)	4.39 (1.66)*	4.84 (1.21)
PA-ECL at trigger, n(%)	16 (100)	16 (100)	16 (100)	48 (100.0)
Log_{10} PA-ELISA prior to Treatment				
Mean (SD)	1.89 (0.72)	2.12 (0.87)	1.96 (0.75)	1.99 (0.77)
Range	0.70, 3.26	0.70, 3.94	0.70, 3.3	0.70, 3.94
PA-ELISA prior to treatment				
Geometric mean	77.6	133.3	90.3	97.8
95% confidence interval	32.2, 186.8	46.1, 385.5	36, 226.6	58.4, 163.5
Mean (SD) of log_{10} PA	1.89 (0.72)	2.12 (0.87)	1.96 (0.75)	1.99 (0.77)

*Only one animal had negative bacteremia. If this animal was excluded, the mean (SD) were 4.67 (1.29), range: 2.18, 6.61. Geometric mean was 4.64 $x10^4$ [95% confidence interval: 8974, 239867].

Time to trigger, bacteremia and treatment

Table 31 shows the time between challenge, bacteremia, trigger to treatment, and treatment by treatment group. There was no statistically significant difference in these variables between each of the two treatment groups and the placebo group.

Table 31. Study AP203: Time between challenge, bacteremia, trigger, and treatment by treatment group

	Placebo (N=16)	**ETI-204 8 mg/kg IV (N=16)**	**ETI-204 32 mg/kg IV (N=16)**	**All (N=48)**
Time to quantitative bacteremia (hours)				
N	16	16	15*	47
Mean (SD)	29.98 (4.92)	28.34 (4.95)	29.87 (4.70)	29.39 (4.81)
Range	22.65, 39.22	22.2, 37.32	22.37, 37.83	22.2, 39.22
Time to trigger (hours)				
Mean (SD)	33.3 (4.7)	32.5 (5.5)	33.4 (4.2)	33.1 (4.7)
Range	27.9, 45.1	22.8, 45.5	28.5, 42.7	22.8, 45.5
Time to randomized treatment (hours)				
Mean (SD)	37.1 (4.2)	36.2 (5.2)	37.5 (4.0)	37 (4.4)
Range	32.4, 47.4	26.3, 47.5	32.6, 46.5	26.3, 47.5
Time from trigger to treatment (hours)				
Mean (SD)	3.8 (0.6)	3.8 (0.7)	4.1 (0.4)	3.9 (0.6)
Range	2.3, 4.7	1.9, 5	3.4, 4.8	1.9, 5

*****C40915 had no quantitative bacteremia count and was not included in the calculation. However enriched bacteremia prior to treatment was positive. In the study result, 16 animals were used because bacteremia was based on both quantitative and enriched (qualitative) bacteremia measurements.

6.2.4.4 Results

Survival

Table 32 shows survival proportion by treatment group. There was no statistically signficant difference between either ETI-204 group and the placebo group. An additonal analysis was conducted that excludes the one animal that was non-bacteremic using the quantitative method, that result was also non-significant.

Table 32. Study AP203: Survival at Day 28 by treatment group

	Placebo (N=16)	ETI-204 8 mg/kg IV (N=16)	ETI-204 32 mg/kg IV (N=16)
Including all animals			
n (%)	2 (12.50)	1 (6.25)	6 (37.50)
Difference in survival proportion [exact 95% confidence interval] one-sided p-value compared with control		-0.063 [-0.329,0.194] 0.887	0.25 [-0.065, 0.541] 0.11
Adjusted exact 95% confidence interval		-0.358, 0.238	-0.114, 0.577
Including only quantitatively bacteremic animals			
N (%)	Same as above	Same as above	5/15 (33.3)
Difference in survival proportion [exact 95% confidence interval] one-sided p-value compared with control		Same as above	0.208 [-0.104, 0.510] 0.104
Adjusted exact 95% confidence interval		Same as above	-0.148, 0.550

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer

There were no significant differences in survival (time to death) among or between groups, as shown in Figure 17 and Table 33. The p-value for the comparison of 32 mg/kg group and the placebo group was statistically significant at a two-sided significance level of 0.05 with no multiple comparison adjustment. However, per the protocol this analysis was only to be conducted if there was a significant effect between treatment and placebo.

Figure 17. Study AP203: Kaplan-Meier survival curve by treatment group

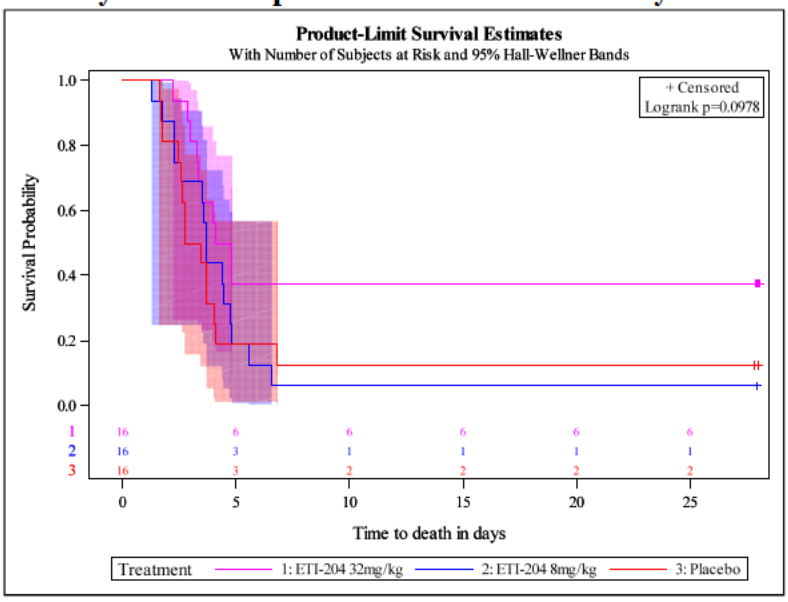
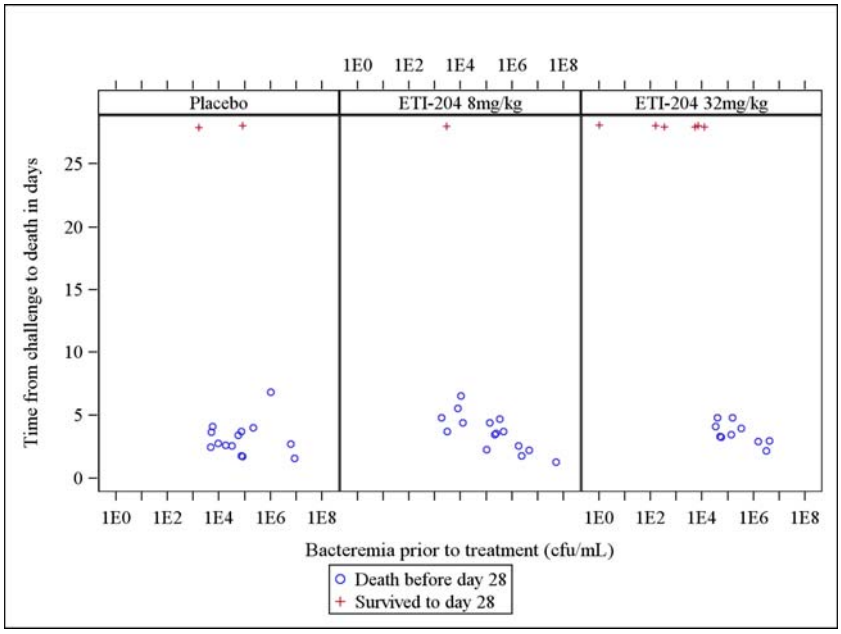


Table 33. Study AP203: two-sided p-values of pairwise log-rank tests comparing time from challenge to death between groups

	ETI-204 8 mg/kg IV (N=16)	ETI-204 32 mg/kg IV (N=16)
Placebo	0.817	0.044
ETI-204 8mg/kg		0.083

Figure 18 shows the time to death versus bacteria level prior to treatment. It was clear that animals with a lower bacteremia level were more likely to survive to Day 28.

Figure 18. Study AP203: Time to death versus bacteremia prior to treatment by survival status at Day 28



The following figure shows that animals with a lower PA-ELISA level were more likely to survive. The following table shows the odds ratio of survival at Day 28 associated with treatment and bacteremia or PA-ELISA prior to treatment. The only statistically significant effect was bacteremia in a model with treatment group, bacteremia, and PA-ELISA prior to treatment. So Model 1 only included treatment group and bacteremia prior to treatment. PA-ELISA was statistically significant in a model with treatment group and itself. The high correlation coefficient between $\log_{10}$ PA-ELISA and $\log_{10}$ bacteria (0.87) did not allow including both variables in the same model.

The analysis showed that a higher bacteremia level was associated with a lower survival probability.

Figure 19. Study AP203: Time to death versus PA-ELISA prior to treatment by survival status at Day 28

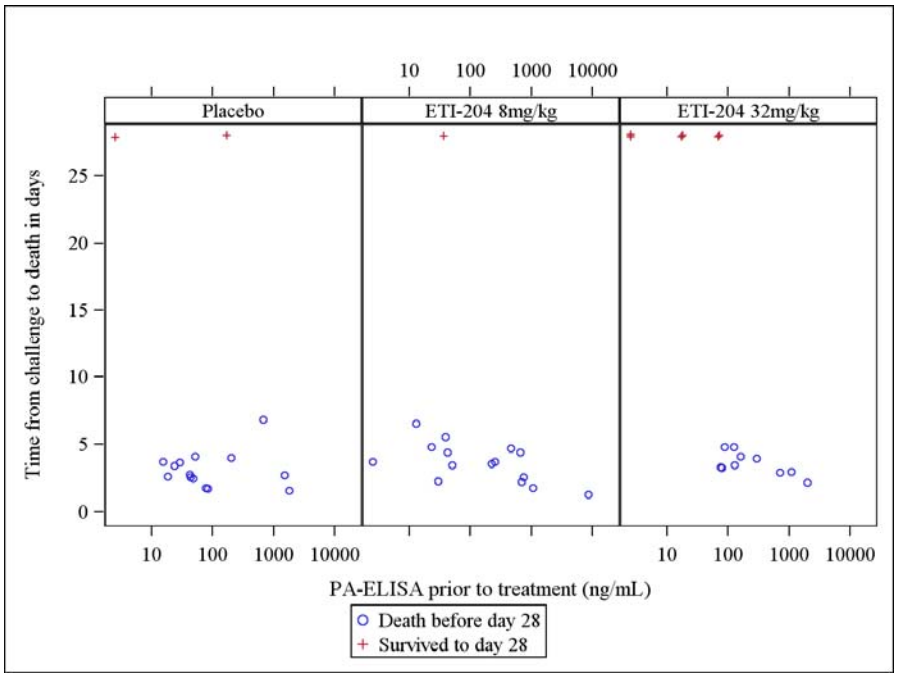


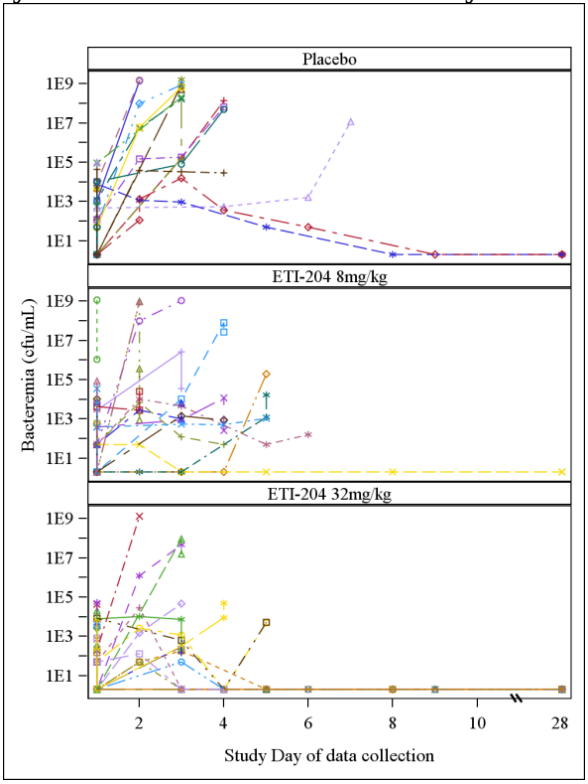
Table 34. Study AP203: Estimated odds ratio of survival at Day 28 associated with treatment and bacteremia or PA-ELISA prior to treatment from logistic regression on survival

Covariate	Odds ratio	95% confidence interval	p-value
Model 1			
ETI-204 8 mg/kg	0.323	0.02, 5.98	0.1360
ETI-204 32 mg/kg	8.276	0.51, 133.20	0.4478
Log_{10} bacteremia prior to treatment	0.055	0.005, 0.621	0.0189
Model 2			
ETI-204 8 mg/kg	0.436	0.025, 7.744	0.5717
ETI-204 32 mg/kg	8.642	0.876 85.229	0.0648
Log_{10} PA-ELISA prior to treatment	0.094	0.016, 0.571	0.0101

Bacteremia level over time

Figure 20 shows the bacteremia level for each animal in different groups. In the two ETI-204 groups, there was no dramatic decrease after receiving treatment. All surviving animals had a bacteremia below the LOD after Day 9.

Figure 20. Study AP203: Bacteremia over time by treatment and animal



PA-ELISA level over time

The following figure shows the PA-ELISA levels by treatment and animal. In the placebo group, the PA-ELISA level increased post-challenge. There were two surviving animals with a PA-ELISA level below the LLOQ after Day 9.

In summary, in this study both treatment regimens were not effective, although PA-ELISA level decreased after initiation of treatment, bacteremia did not reduce quickly enough to improve survival. Therefore, most animals died between 24 to 96 hours post-treatment, as in the placebo groups.

Subgroup Analysis Results

Table 35 shows the survival status by gender, challenge dose, bacteremia, and PA level. Because of small sample sizes, it was inconclusive about the effect of each grouping variable. The two surviving animals in the placebo groups were male, one with a challenge dose of 205 LD_{50}s, bacteremia of 81300 cfu/mL and PA-ELISA of 168 ng/mL, the other with a challenge dose of 315 LD_{50}s, bacteremia of 1640 cfu/mL and PA-ELISA of <LLOQ.

Figure 21. Study AP203: PA-ELISA level by treatment and animal

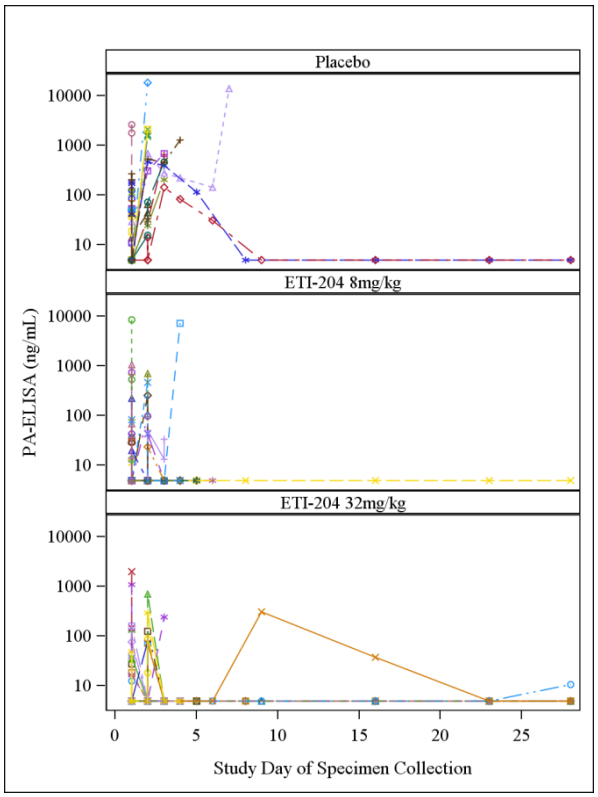


Table 35. Study AP203: Survival at Day 28 by gender, challenge dose, log_{10} bacteremia, PA prior to treatment

	Placebo (N=16)	**ETI-204 16 mg/kg IV (N=16)**	**ETI-204 32 mg/kg IV (N=16)**	**All (N=48)**
Gender				
Female	0/8	2/8 (25%)	1/8 (12.5%)	3/24 (12.5%)
Male	2/8 (25%)	4/8 (50%)	0/8	6/24 (25%)
Challenge dose (LD_{50})				
<250	1/5 (20%)	0/5	2/6 (33.3%)	3/16 (18.8%)
250 or higher	1/11 (9.1%)	1/11 (9.1%)	4/10 (40%)	6/32 (18.8%)
Log_{10} bacteremia prior to treatment				
$< 10^4$	1/5 (20%)	1/4 (25%)	5/5 (100%)	7/14 (50%)
10^4 - $<10^6$	1/9 (11.1%)	0/8	1/8 (12.5%)	2/25 (8%)
10^6 or higher	0/2	0/4	0/3	0/9
PA prior to treatment (ng/mL)				
0 - < 10	1/1 (100%)	0/1	2/2 (100%)	3/4 (75%)
10 - < 50	0/7 (0)	1/7 (14.3%)	2/2 (100%)	3/16 (18.8%)
50 or higher	1/8 (12.5%)	0/8	2/12 (16.7%)	3/28 (10.7%)

Tissue bacterial assessments and pathological findings in the brain

In the surviving animals, 0, 5 (83.3%), and 0 in the three groups had a bacterial load of 0.5 or 1 in the lung; 0, 1 (16.7%), and 0 in the bronchial lymph node. No surviving animals had bacterial load in the brain, kidney, liver, and spleen. Among non-survivors, all animals except for one in the placebo group (1/14, 7.1%) had a positive result in the brain.

Among dead animals, 3, 13, and 5 (21.4%, 86.7%, and 50.0%) from the placebo, 8 mg/kg, and 32 mg/kg groups had positive microscopic pathological results in the brain. Among survivors, only one survivor animal (16.7%) from the 32 mg/kg group had a positive result in the brain (deformity, 4 x 3 x 2mm depression; duramater fused to skull cap).

6.2.4.5 Conclusions

This study was conducted at higher doses of ETI-204 than in the previous study, AP204, where only the 16 mg/kg dose was found significantly different than placebo. In this study, neither the 8 mg/kg dose nor the 32 mg/kg dose was significantly difference from placebo. The applicant conducted analyses that show that both pre-treatment PA-ELISA and pre-treatment bacteremia level can affect survival rates. The large concern given these study results is that this was the first monkey treatment study using the Lonza product.

6.2.5 AP202

Comment: This study was conducted after studies AP201, AP203 and AP204 were conducted. As discussed above, AP201 and AP204 using the Baxter product were positive and AP203 using the Lonza product was not. Though there was a belief that the differences in survival rates were likely due to differences in the severity of anthrax as demonstrated by pre-treatment bacteremia and PA levels, there was an interest to conduct one study with both the Lonza and the Baxter product included.

6.2.5.1 Study Design and Endpoints

Primary Objective
To evaluate the efficacy of a single, intravenous dose of ETI-204 manufactured as Lonza on survival rate in cynomolgus monkeys infected with inhalational anthrax compared to placebo control.

Secondary Objective
To provide data from a treatment arm using ETI-204 manufactured at Baxter to compare to a treatment arm using ETI-204 manufactured at Lonza.

Study Design
This was a randomized, blinded, placebo-controlled study, conducted by (b) (4) in 2014.

Randomization was performed by four blocks of six (2:2:2 in each block) and three blocks of nine (3:3:3 in each block) vials, with the blocks being in a random order. Individual vials within each block were placed in a random order. Then the treatment dosing order and vial assignment were determined by the order in which animals triggered for treatment.

The treatment group and dose are as follows:

- Group 1 (placebo): 0 mg/kg IV
- Group 2 (ETI-204 Lonza) 16 mg/kg IV
- Group 3 (ETI-204 Baxter) 16 mg/kg IV

Animals were stratified by sex to one of three challenge days with each 17 non-human primates (NHPs) per day. NHPs were aerosol-challenged with a targeted 200 LD_{50} [(b) (4) *B. anthracis* spores] dose via a head-only inhalation exposure chamber.

Animals were monitored every 6 hours post challenge up to day 8. From study day 9 to 28 animals were monitored twice daily. Blood was sampled for bacteremia at pretreatment, 15 minutes (enriched bacteremia), 96 hours, 7 days and 28 days or at unscheduled termination post-treatment. PA was assessed at pre-treatment, 15 minutes post treatment, and at day 28 or at unscheduled termination. For details regarding animal care and microbiology methods see primary pharmacology-toxicology and microbiology reviews.

A positive serum PA result via the electronchemiluminescence (ECL) assay was the criteria for treatment trigger and used to determine treatment order and vial assignment. If animals did not have a positive serum PA-ECL by 54 hours post challenge, the treatment order was determined by the challenge order.

Regarding blinding, the dosing vial randomization scheme prepared by the viral randomization statistician was submitted to Quality Assurance Unit for audit. The applicant, study director, and staff who evaluated animals were blind. PA-ELISA analysis and ETI-204 concentration analysis were not conducted in a blinded fashion because samples were shipped for analysis after the study was unblinded.

Primary Endpoints

Survival to Day 28 post-challenge was the primary endpoint.

6.2.5.2 Statistical Methodologies

Sample Size Calculation

Sample size was calculated based on the following assumptions: the probability of survival was 55% and 10% in the ETI-204 treated group and placebo group, respectively; one-sided test with a 0.025 level using Boschloo's test. No adjustment was made for multiple comparisons because the primary analysis was the comparison of Lonza ETI-204 and placebo.

Study Population

Two analysis populations were defined in the protocol.

Intent to treat (ITT) population: All animals assigned to a treatment regardless of bacteria status prior to treatment.

Modified intent to treat (mITT) population: all animals assigned to a treatment excluding those animals that were not positive for bacteremia by enriched culture at any time point prior to placebo or ETI-204 dosing.

Since all animals were positive for bacteremia prior to dosing, the mITT and ITT population are the same for this study.

Statistical Methods

The survival proportion of animals in the Lonza ETI-204 treatment group was compared to that in the placebo group using a one-sided 0.025 level Boschloo's unconditional exact test with Berger-Boos correction (γ=0.001). The study was not powered for demonstrating Lonza ETI-204 was non-inferior to Baxter ETI-204.

The primary comparison was Lonza ETI-204 versus placebo. A comparison between Baxter and Lonza ETI-204 was a secondary analysis.

Missing Values

Missing values were planned not be included in the statistical analysis.

The limit of detection (LOD) for quantitative bacteremia was 3 cfu/mL. Quantitative bacteremia levels less than the LOD or reported as "0" were replaced with one half of the LOD rounded to the nearest integer (2 cfu/mL) for the statistical analysis.

The lower limit of quantification (LLOQ) for PA-ELISA was 5 ng/mL. PA-ELISA values reported as less than the LLOQ were replaced with one half of the LLOQ (2.5 ng/mL) for the statistical analysis.

6.2.5.3 Animal Disposition, Demographic and Baseline Characteristics

A total of 51 animals were challenged. One animal (C59383) died before treatment and was not randomized. The remaining 50 animals were randomized into 3 groups (placebo 17, Lonza ETI-204 16, and Baxter ETI-204 17).

As shown in the following table, age was reported as 2.7 to 5 years for all animals in the data set, although in the study report mean and SD for each group were included.

There were more males in the Lonza group and more females in the Baxter groups, although the differences were not statistically significant via a Chi-square test by the reviewer (p-value=0.29).

The mean challenge dose and standard deviation (SD) for all animals on study was 256 (±49) LD_{50}s, including the animal that died before receiving treatment. The challenge dose summary excluding this animal is summarized in the following table. The mean doses were comparable between groups.

All animals were bacteremic prior to treatment. The reviewer conducted an ANOVA of log_{10} bacteremia level prior to treatment and there were no differences between three groups.

All animals except for one had positive ECL results, most of which occurred between the 30 and 42 hour collection time points.

Log_{10} bacteremia levels were comparable between the Baxter ETI-204 group and the placebo group. The Lonza group had a higher mean log_{10} bacteremia level (not statistically significant), which may have an effect on survival in this group. This also was reflected by the geometric means.

There were no discernible differences in log_{10} PA-ELISA levels between the two treatment groups. The PA levels in the two treated groups were slightly higher than that in the control group.

Table 36. Study AP202: Demographic variables and baseline characteristics by treatment group

	Placebo (N=17)	**Lonza ETI-204 16 mg/kg IV (N=16)**	**Baxter ETI-204 16 mg/kg IV (N=17)**	**All (N=50)**
Age (years) estimated range	2.7-5	2.7-5	2.7-5	2.7-5
Gender [n (%)]				
Male	8 (47.1)	10 (62.5)	6 (35.3)	24 (48.0)
Female	9 (52.9)	6 (37.5)	11 (64.7)	26 (52.0)
Body weight (kg)				
Mean (SD)	2.91 (0.52)	2.88 (0.42)	2.85 (0.37)	2.88 (0.4)
Range	2.5, 4.6	2.2, 3.7	2.4, 3.9	2.2, 4.6
Challenge dose (LD_{50})				
Mean (SD)	247.6 (52.6)	270.2 (54.8)	254.4 (41.0)	257.1 (49.6)
Range	172.0, 318.0	166.0, 402.0	182.0, 323.0	166.0, 402.0
Challenge dose (LD_{50}) (n(%))				
<200	4 (23.5)	1 (6.3)	3 (16.7)	8 (16.0)
200 or higher	13 (76.5)	15 (93.8)	14 (82.4)	42 (84.0)
Challenge dose (x 10^7 cfu)				
Mean (SD)	1.53 (0.33)	1.67 (0.34)	1.57 (0.25)	1.59 (0.31)
Range	1.06, 1.97	1.02, 2.49	1.13, 2.00	1.02, 2.49
Bacteremia prior to treatment (n(%))	17 (100)	16 (100)	17 (100)	50 (100)
Bacteremia prior to treatment (cfu/mL)				
Geometric mean	89196	327589	120588	149853
95% confidence interval	23934, 332412	71210, 1507014	18063, 805039	62668, 358334
Mean (SD) of log_{10} bacteremia	4.95 (1.11)	5.52 (1.24)	5.08 (1.60)	5.18 (1.33)
PA-ECL positivity at trigger (n(%))	17 (100)	15 (93.8)*	17 (100)	49 (98.0)
Log_{10} PA-ELISA prior to treatment				
Mean (SD)	1.20 (0.92)	1.50 (0.94)	1.49 (1.20)	1.39 (1.02)
Range	0.40, 3.93	0.40, 3.71	0.40, 4.31	0.40, 4.31
PA-ELISA prior to treatment (ng/mL)				
Geometric mean	15.9	31.9	30.7	24.8 (10.4)
95% confidence interval	5.4, 46.9	10, 101.5	7.4, 127.2	12.8, 48.3
Mean (SD) of log_{10} PA	1.20 (0.92)	1.50 (0.94)	1.49 (1.20)	1.39 (1.02)

*C60822 was negative

Time between challenge, trigger, and treatment

In this study the treatment trigger was positive PA via ECL (PA-ECL). The time between challenge, bacteremia, trigger, and treatment by treatment group is shown in the following table. There were no differences in these variables between different treatment groups. Note there was only one bacteremia measurement between post-challenge and prior to treatment, so the time to

bacteremia was very close to the time from challenge to treatment, which may not reflect the actual time to bacteremia.

Table 37. Study AP202: Time between challenge, trigger, and treatment

	Placebo (N=17)	Lonza ETI-204 16 mg/kg IV (N=16)	Baxter ETI-204 16 mg/kg IV (N=17)	All (N=50)
Time to bacteremia (hours)				
Mean (SD)	38.8 (5.4)	39.2 (5.6)	39.2 (4.3)	39.1 (5)
Range	28.3, 51.9	31.0, 53.1	32.3, 46.0	28.3, 53.1
Time to trigger (hours)				
N	17	15*	17	49
Mean (SD)	34.5 (5.5)	34.1 (4.6)	35.1 (4.5)	34.6 (4.8)
Range	24.8, 48.5	27.1, 43.1	28.4, 42.8	24.8, 48.5
Time from trigger to treatment (hours)				
N	17	15*	17	49
Range	4.3 (0.8)	4.2 (0.8)	4.2 (0.7)	4.3 (0.7)
Mean (SD)	3.2, 6.2	3.2, 5.8	3.3, 5.8	3.2, 6.2

*Animal C60822 did not have a positive PA-ECL and was not included in the calculations. This animal was treated at 54 hours and was bacteremic and survived.

6.2.5.4 Results

Survival

The following table shows the survival proportion at Day 28 for each group. In the primary analysis, comparing with the placebo group, the Lonza group had a significantly higher survival proportion (31.3% versus 0%) with a difference of 0.312 (95% confidence interval [0.078, 0.587]), p-value=0.0085 from Boschloo's one-sided test. Since there was only one primary analysis, no multiple adjustment was needed for the primary efficacy.

As a secondary analysis, the Baxter group also had a significantly higher survival proportion than the placebo group. Even with a Bonferroni's adjustment for the two comparisons, the treatment effects were still statistically significant at a one-sided significance level of 0.0125 for each test.

The second objective of this study was to compare the efficacy of ETI-204 manufactured at Lonza and Baxter. As the applicant's analysis shows, there was no statistically significant difference between the Lonza and Baxter ETI-204 groups. The difference in survival proportions between the two products was -0.04 [95% confidence interval: [-0.365, 0.29]) (Lonza-Baxter). Considering the lower limit and width of the 95% confidence interval, this analysis is not conclusive about the non-inferiority of Lonza, given the small sample size in the two groups. It

was noticed that mean bacteremia and PA-ELISA levels were numerically higher in the Lonza group than in the Baxter group (not statistically significant). This would likely put the Lonza group at a disadvantage, leading to a conservative analysis.

Table 38. Study AP202: Survival at Day 28 in both ITT and mITT populations

	Placebo (N=17)	Lonza ETI-204 16 mg/kg IV (N=16)	Baxter ETI-204 16 mg/kg IV (N=17)
N (%)	0 (0)	5 (31.3)	6 (35.3)
Difference in survival proportion compared with placebo [exact 95% confidence interval] p-value		0.313 [0.078, 0.587] 0.0085*	0.353 [0.113, 0.617] 0.0046*

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Significant at a one-sided significance level of 0.025/2=0.0125

The following figure shows Kaplan-Meier curves and 95% confidence bands by treatment group. The p-value for the comparison of three groups was 0.0148. The p-value was 0.026 for the comparison of the Lonza ETI-204 and placebo group and 0.0073 for the comparison of the Baxter ETI-204 and placebo group (Table 39). These results demonstrated a statistically significant treatment effect, compared with the placebo group, without adjustment for multiple comparisons. Using Bonferroni's adjustment for the two comparisons with the placebo group, these differences were still statistically significant at a significance level of 0.05/2=0.025 (two-sided).

Figure 22. Study AP202: Kaplan-Meier curve and 95% confidence band by treatment group

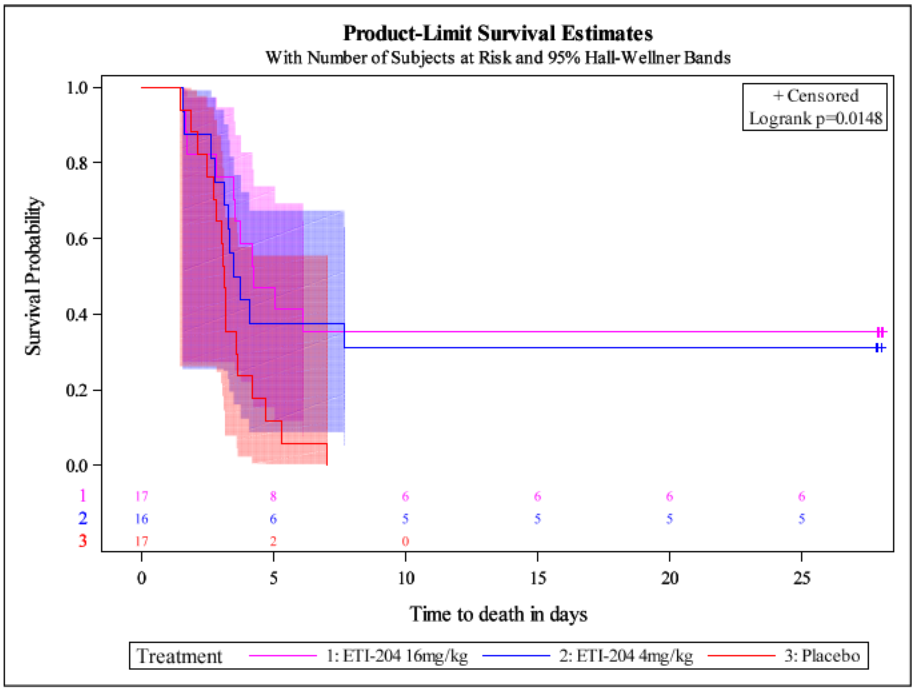


Table 39. Study AP202: two-sided p-values of pairwise log-rank tests comparing time from challenge to death among groups

	Lonza ETI-204 16 mg/kg IV (N=16)	Baxter ETI-204 16 mg/kg IV (N=17)
Placebo	0.026	0.0073*
Lonza ETI-204		0.6409

Source: Study Report Table 11.
*Significant at a two-sided significance level of 0.05/2=0.25

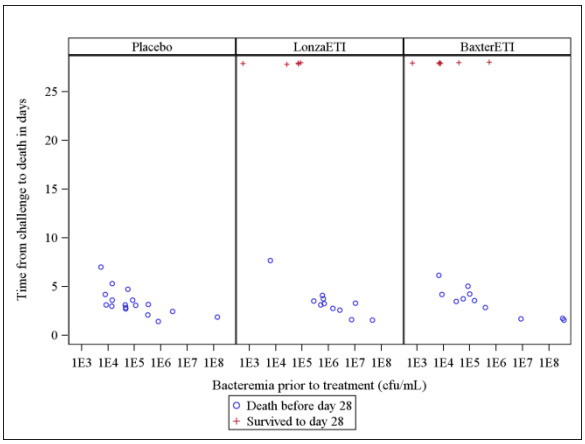
The following table shows the results for a proportional hazards model on time to death with treatment and $\log_{10}$ bacteremia prior to treatment. Compared with the placebo group, both treatment groups had a significantly reduced risk of death. Higher bacteremia prior to treatment was also significantly associated with an increased risk of death. No interaction terms between treatment and bacteremia were statistically significant. Log_{10} PA-ELISA and challenge dose were not statistically significantly associated with survival in a model with bacteremia. Bacteremia and PA-ELISA were highly correlated; therefore, this correlation likely explains no statistical association between PA-ELISA and survival.

Table 40. Study AP202: Log hazard ratio estimates from a proportional hazards regression model on time from challenge to death

Parameter	Parameter Estimate	Standard Error	Chi-Square	p-value
Lonza ETI-204	-1.58741	0.42812	13.7485	0.0002
Baxter ETI-204	-1.35765	0.41845	10.5264	0.0012
Log_{10} bacteremia prior to treatment	1.12175	0.18408	37.1346	<.0001

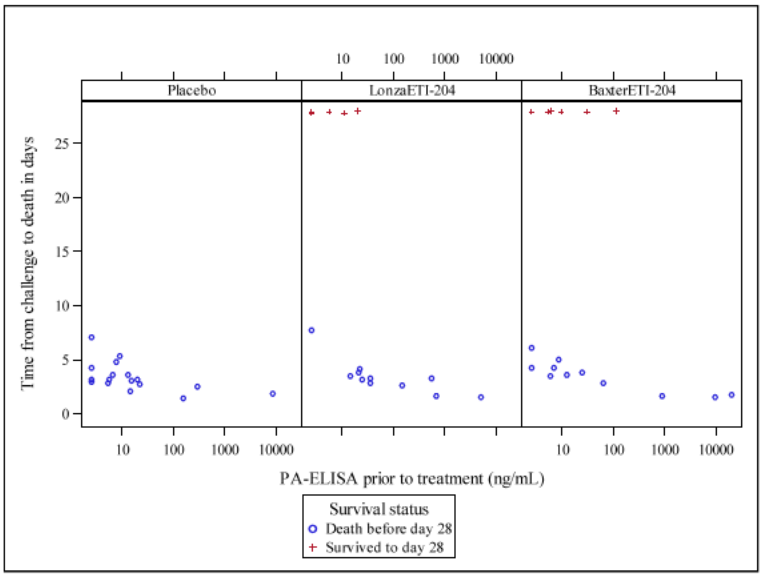
The following figure shows time to death versus bacteremia prior to treatment by treatment and survival status at day 28. It is evident that an animal with a higher level of bacteremia was more likely to die in the two treatment groups.

Figure 23. Study AP202: Time to death versus bacteremia prior to treatment by survival status at Day 28



The following figure shows PA-ELISA prior to treatment versus time to death by treatment. In the two treatment groups, animals with a lower PA-ELISA level were more likely to survive to Day 28. No animals with a PA-ELISA lever over 1000 ng/mL survived.

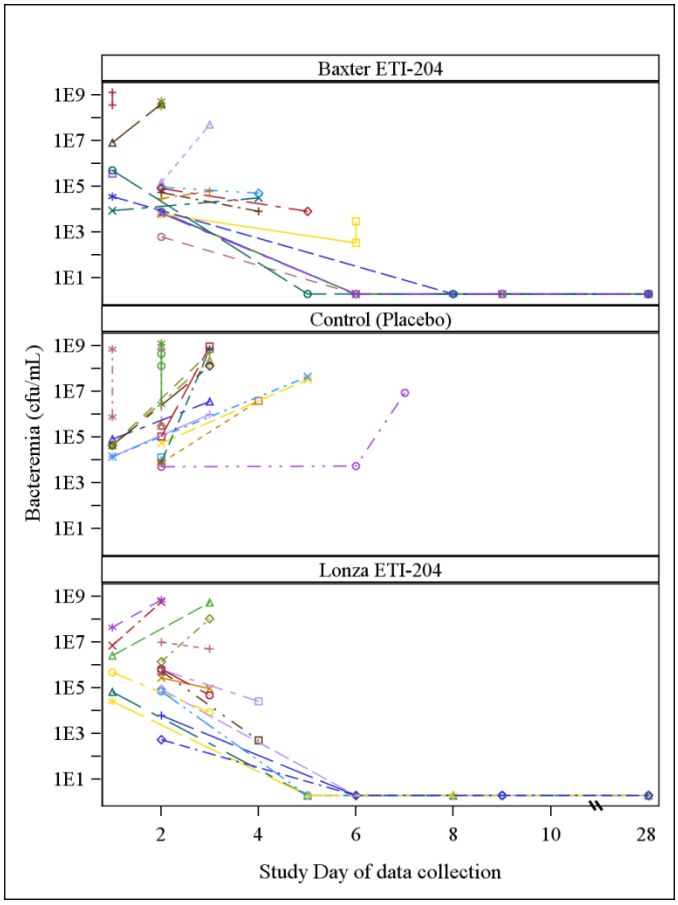
Figure 24. Study AP202: Time to death versus PA-ELISA prior to treatment by survival status at Day 28



Bacteremia level over time

The following figure shows bacteremia data by animal at all available time points, including terminal measurement at death. Most animals in the placebo group had an increased bacteremia level over time before death. To the contrary, most animals in the ETI-204 groups had decreased bacteremia over time after initiation of treatment (around 39 hours or 1.6 days of study).

Figure 25. Study AP202: Study 202: Bacteremia over time by animal

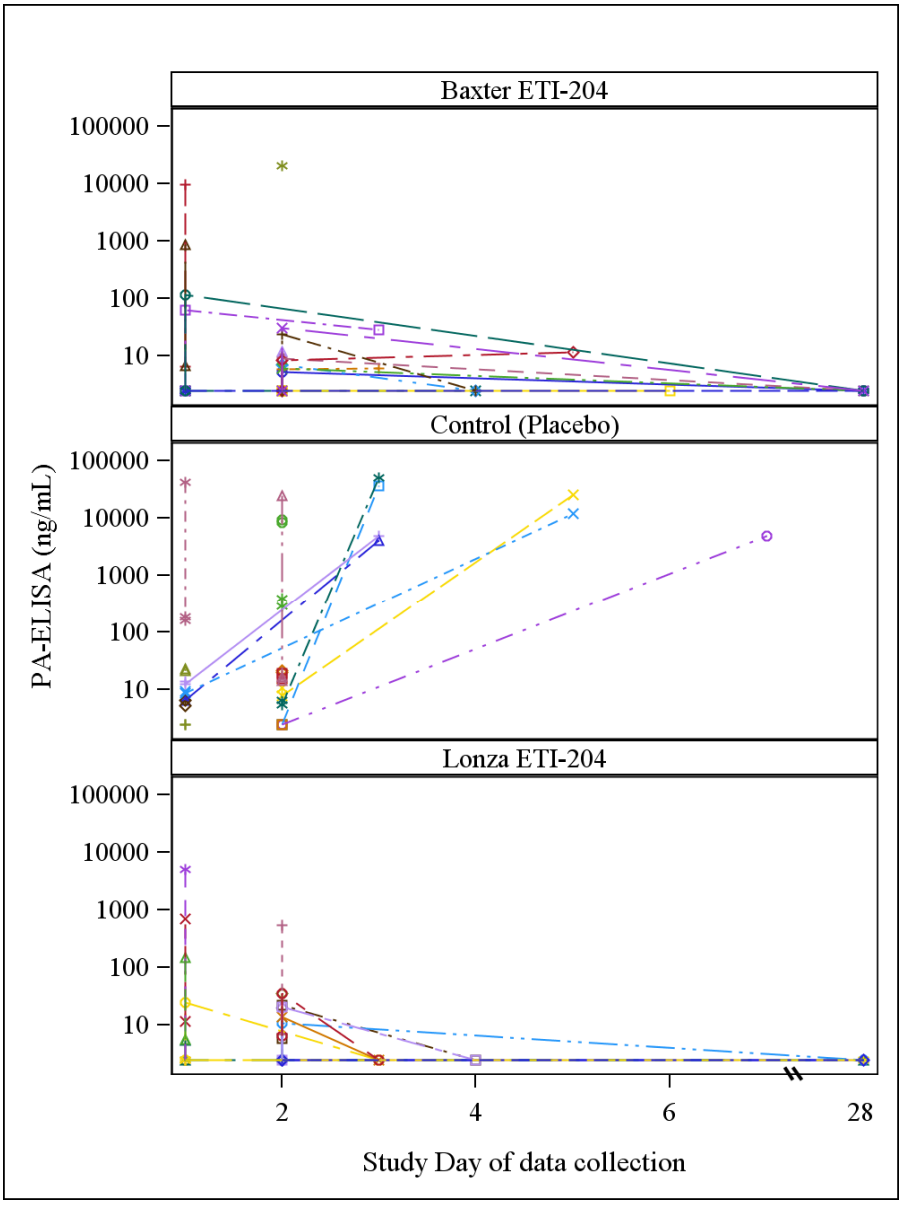


This graph includes all available bacteremia data including terminal bacteremia. For example, for control animal C59240, it was 8.83E+6. The previous graph only includes data at selected visits.

PA-ELISA over time

The following figure shows PA-ELISA over time starting from challenge by animal. The pattern was similar to that of bacteremia over time. At Day 2 from challenge, the levels in the treatment groups reduced.

Figure 26. Study AP202: PA-ELISA over time by animal and treatment



Subgroup Analysis Results

Table 41 shows survival status by gender, challenge dose, bacteremia, and PA prior to treatment. The survival proportions in females in the two treatment groups were much higher than in males. However, these differences were not statistically significant (two-sided p-values were 0.20 and 0.24 from the Boschloo's test in the Lonza and Baxter groups).

As expected, in the two treatment groups, a higher bacteremia level, and a higher PA level were associated with a lower survival proportion. A higher challenge dose was associated with a higher survival proportion.

Table 41. Study AP202: Survival at Day 28 by gender, challenge dose, bacteremia, and PA prior to treatment

	Placebo (N=17)	**Lonza ETI-204 16 mg/kg IV (N=16)**	**Baxter ETI-204 16 mg/kg IV (N=17)**	**All (N=50)**
Gender				
Female	0/9	3/6 (50%)	5/11 (45.5%)	8/26 (30.8%)
Male	0/8	2/10 (20%)	1/6 (16.7%)	3/24 (12.5%)
Challenge dose (LD_{50}) (n(%))				
<250	0/9	1/5 (20%)	2/7 (28.6%)	3/21 (14.3%)
250 or higher	0/8	4/11 (36.4%)	4/10 (58.8%)	8/29 (27.6%)
<200	0/4	0/1 (0)	0/3	0/8
Bacteremia prior to treatment (cfu/mL)				
$< 10^4$	0/3	1/2 (50%)	4/6 (66.7%)	5/11 (45.5%)
10^4 - $<10^6$	0/12	4/9 (44.4%)	2/8 (25.0%)	6/29 (20.7%)
10^6 or higher	0/17	0/5	0/3	0/10
PA-ELISA prior to treatment (ng/mL)				
0 - < 10	0/9	3/4 (75%)	4/9 (44.4%)	22 (44%)
10 - < 50	0/5	2/8 (25%)	1/3 (33.3%)	16 (32%)
50 or higher	0/3	0/4	1/5 (20%)	12 (24%)

Tissue bacterial assessments and pathological findings in the brain

At terminal sacrifice, all surviving animals had no bacteremia loads in the brain, kidney, liver, and spleen. The lung was positive for bacterial load. According to the study report, this is consistent with the results from previous studies which have shown that spores can be found in the lung up to 56 days after challenge in surviving NHPs. All non-survivors in the two treated groups had a negative bacterial result in the brain.

One (1), 3, and 3 dead animals (5.9%, 27.3%, and 27.3%) in the placebo and two ETI-204 groups had positive pathological findings in the brain.

6.2.5.5 Conclusions

Study AP202 was conducted after AP201, AP203 and AP204. AP 201 and AP204 used ETI-204 manufactured at Baxter while AP203 used ETI-204 manufactured at Lonza. The survival of the

ETI-204 product in study AP203 was much lower than expected. The applicant theorized that the severity of illness at baseline was the cause for the different survival rates across the studies; however, since the applicant wants to market the Lonza product, the Division believed that it was important to conduct an additional monkey treatment study in order to assess the effect of Lonza and to compare the two products in one study. Study AP202 was primarily designed to test the effect of the Lonza product versus placebo, but to also descriptively compare the Lonza product with the Baxter product.

The results of this study showed that 16 mg/kg IV of the Lonza product (31%) was statistically significantly higher than placebo (0) in terms of 28 day survival rate. Additionally, the study results suggested the products from two manufacturers were numerically comparable and the survival rate for the Baxter product was 35%. This study showed along with previous studies that severity of disease as measured by bacteremia and PA-ELISA affects the probability of surviving in the ETI-204 treatment arms.

6.2.6 NIAID1056

Study 1056-G607605: Efficacy of a Monoclonal Antibody Given in Combination with Ciprofloxacin in the Cynomolgus Macaques Therapeutic Model of Inhalational Anthrax
Conducted (b) (4) for NIAID

This study randomized animals to four arms: no treatment, ETI-204-alone given at when animal became PA positive, ciprofloxacin alone given 24 hours after PA positivity and a combination of ciprofloxacin plus ETI-204 given 24 hours after PA positivity. This is essentially two studies in one, with the comparison of the combination to ciprofloxacin can be considered to assess the added benefit of ETI-204 when given with an antibacterial and the comparison of ETI-204 alone compared to the untreated control. This review will focus only on the comparison of ETI-204 compared to the untreated control arm. Please see the statistical review by Ling Lan for a discussion of the contribution of ETI-204 when given with antibiotics.

6.2.6.1 Study Design and Endpoints

Primary Objective

The primary objective was to

1) To evaluate the efficacy of a monoclonal antibody in combination with ciprofloxacin when administered as a therapeutic treatment in a delayed fashion, following inhalation exposure to *B. anthracis* in cynomolgus macaques

2) To determine the efficacy of a monoclonal antibody when administered following detection of circulating PA by ECL

3) To evaluate the efficacy of ciprofloxacin when administered in a delayed fashion following inhalational exposure to B. anthracis in cynomolgus macaques

4) To fully evaluate all untreated controls until death or euthanasia to further develop a database of information pertaining to disease progression in *B. anthracis* aerosol challenged cynomolgus macaques

Comment: As stated above, the focus of this review will be the second primary objective that compared ETI-204 alone compared to untreated control.

Study Design

This was a randomized, controlled, open-label, parallel group, and factorial design study, conducted at (b) (4) in 2010.

The study included 4 groups as given in the table below. This review will focus on the comparison of the ETI-204 alone IV group versus the control group. Dose received was upon positive PA (ETI-204 only treatment) or 24±12 hours after detection of elevated PA (ETI 204 + ciprofloxacin; ciprofloxacin alone). The control group was not treated. The ETI-204 was manufactured at the Baxter facility.

Group	Dose (mg/kg)	Number of Animals Planned
Untreated control	0	8
ETI-204	8	8
Ciprofloxacin	10	16
ETI-204+Ciprofloxacin	8 + 10	16

Animals were randomized by body weight into two groups (ETI-204 and control) of eight animals each and two groups of sixteen animals each (50% male, and 50% female) to have a balanced sex and body weight distribution across groups. Then animals were randomized to one of three challenge days and a challenge order per day such that animals from each group were randomized to each of the three challenge days.

Animals were aerosol challenged with a targeted 200 LD_{50} dose of *B. anthracis* (Ames strain) spores. Animals in the ETI-204 group were treated within three hours of obtaining a positive PA-ECL result. Animals were monitored and blood collected regularly post challenge.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.2.6.2 Statistical Methodologies

Sample Size Calculation

There were several samples size calculations for 3 comparisons (combination versus ETI-204, antibiotic treatment versus control, and ETI-204 versus control). The calculations for sample sizes did not consider these multiple comparisons. With 8 animals in the antibody only group (group 1) and in the untreated control group (group 4) there was 81.2% power to detect a difference in survival between these two groups. This assumed that the probability of survival in the antibody only group was 80% and in the control group was less than 10%. Power calculations were for a one-sided, 0.05 level Fisher's exact test.

Analysis Populations

In the protocol there was no analysis population defined clearly. It states that each treated group was compared to the control group. Survival analysis would be repeated only including those animals that were positive for bacteremia by culture at some time point prior to treatment. Therefore, two analysis populations were used. But in the study report, the results from two

populations were included: 1) all randomized animals; and 2) only animals that received at least one treatment). The analyses did not follow the protocol closely. We will report the results from all randomized animals because no control animals had bacteremia data prior to treatment.

Statistical Methods

One-sided Fisher's exact tests were used to compare the survival rates between each treatment group and the control group. Type I error was not specified. In the report, unadjusted and Bonferroni-Holm adjusted p-value were reported, signifying a significance value using a significance level of 0.05.

Comment: This review will consider a one-sided significance level of 0.025 for this study.

6.2.6.3 Animal Disposition, Demographic and Baseline Characteristics

In this review, the focus of this study's results was the comparison of ETI-204 and the control group. The two groups were comparable in the most variables analyzed. However, the challenge dose was lower in the control group. Because the control group was untreated, many variables prior to treatment were not applicable. Therefore it was not possible to explore the effect of a lower challenge dose on pre-treatment bacteremia and PA level.

Table 42. Study NIAID 1056: Demographic variables and baseline characteristics by treatment group

	Control (N=8)	ETI-204 8 mg/kg IV (N=8)	Total (N=16)
Age (years)			
Mean (SD)	3.0 (0.5)	3.0 (0.0)	3.0 (0.4)
Range	2.0, 4.0	3.0, 3.0	2.0, 4.0
Gender [n (%)]			
Female	4 (50.0)	4 (50.0)	8 (50.0)
Male	4 (50.0)	4 (50.0)	8 (50.0)
Body weight (kg)			
Mean (SD)			
Range	3.0 (0.5)	3.0 (0.0)	3.0 (0.3)
Challenge dose (LD_{50})			
Mean (SD)	187.3 (28.0)	201.6 (84.4)	194.4 (61.2)
Range	146.0, 218.0	83.0, 360.0	83.0, 360.0
Challenge dose (LD_{50}) (n(%))			
<200	4 (50.0)	5 (62.5)	9 (56.3)
200 or higher	4 (50.0)	3 (37.5)	7 (43.8)
Challenge dose (x 10^7 cfu)			
Mean (SD)	1.16 (0.17)	1.25 (0.52)	1.20 (0.38)
Range	0.90, 1.35	0.51, 2.22	0.51, 2.22
Positive quantitative bacteremia prior to treatment (n(%))*	NA	8 (100.0)	8 (100.0)

	Control (N=8)	ETI-204 8 mg/kg IV (N=8)	Total (N=16)
Log_{10} bacteremia prior to challenge (cfu/mL)			
Mean (SD)	NA	4.51 (0.69)	4.51 (1.23)
Range		3.76, 5.82	3.76, 5.82
Bacteremia (cfm/mL)			
Geometric mean		32697.6	32697.6
95% confidence interval		8724.1, 122549	8724.1, 122549
Mean (SD) of log_{10} bacteremia	NA	4.51 (0.69)	4.51 (1.23)
PA-ECL positivity at trigger	NA	8 (100)	8 (100)
PA-ELISA (ng/mL) prior to treatment			
N		7	7
Geometric mean	NA	41.9	41.9
95% confidence interval		11.8, 148.4	11.8, 148.4
Mean (SD) of log_{10} PA	NA	1.62 (0.59)	1.62 (0.59)

*****The numbers were the same for qualitative bacteremia
NA: not applicable because of no treatment

Time to bacteremia, trigger, and treatment

The time to qualitative bacteremia was comparable between the two groups. Other variables for the control group were not applicable so no comparison could be made.

Table 43. Study NIAID 1056: Time between challenge, trigger, and treatment

	Control (N=8)	ETI-204 8 mg/kg IV (N=8)	Total (N=16)
Time to qualitative bacteremia (hours)			
Mean (SD)	33.2 (4.8)	31.2 (4.6)	32.2 (4.7)
Range	24.2, 39	24.7, 37.5	24.2, 39
Time to trigger (hours)			
Mean (SD)	NA	31.93 (5.0)	31.9 (5.0)
Range		24.7, 37.5	24.7, 37.5
Time to treatment (hours)			
Mean (SD)	NA	35.81 (5.04)	35.81 (5.04)
Range			
Time from trigger to treatment (hours)			
Range	NA	3.88 (0.39)	3.88 (0.39)
Mean (SD)		3.45, 4.48	3.45, 4.48

6.2.6.4 Results

Survival

The 8 mg/kg IV group demonstrated a statistically significant effect on survival proportions, compared with the control group.

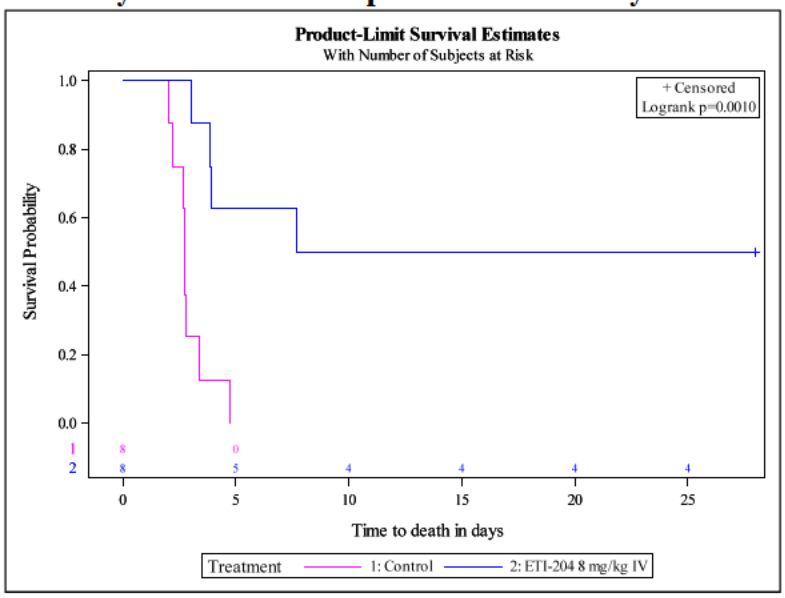
Table 44. Study NIAID 1056: Survival at Day 28 by treatment group

	Control (N=8)	ETI-204 8 mg/kg IV (N=8)	Total (N=16)
n (%)	0 (0)	4 (50)	4 (25)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.50 [0.058, 0.843] 0.014*	

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025

All control animals died before Day 4. Survival analysis of time to death in Table 84 shows that the ETI-204 group had a statistically significant improvement on survival compared with the placebo group, using a two-sided significance level of 0.05.

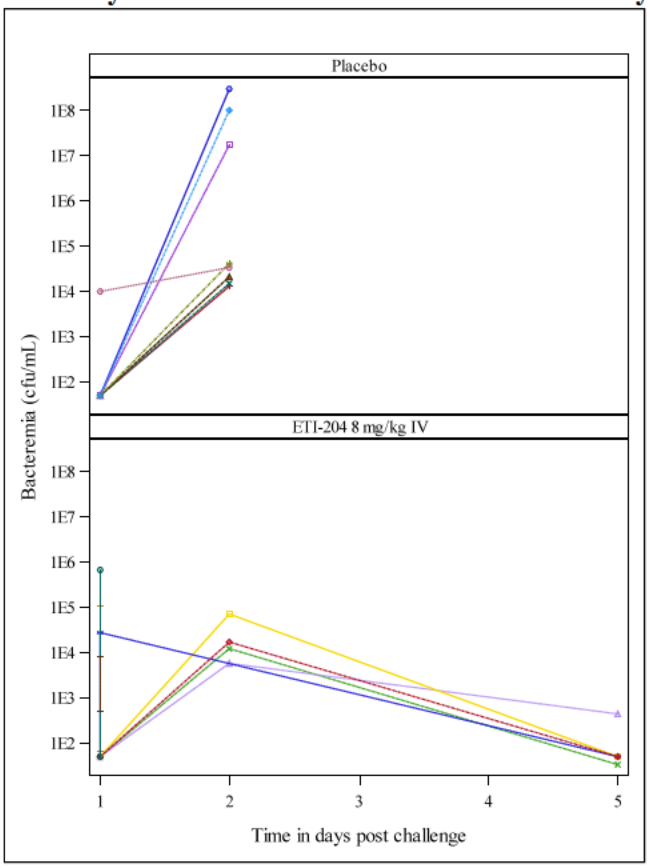
Figure 27. Study NIAID 1056: Kaplan-Meier curve by treatment group



Bacteremia over time

As shown in the following figure, 24 hours post-challenge the two groups were comparable in bacteremia. At 48 hours post-challenge, the bacteremia level in the control group increased dramatically. At 5 days post-challenge the ETI-204 group had lower bacteremia levels.

Figure 28. Study NIAID 1056: Bacteremia over time by animal



PA-ELISA over time

At 24 hours post-challenge the two groups were similar as shown in the following figure. The levels increased and reached a high level at 48 and 72 hours post-challenge in most animals. At or after 5 days post-challenge, the levels in the ETI-204 group dropped in most animals.

Subgroup Analyses

The following table shows the results of subgroup analyses. The sample sizes were too small to see a reliable trend by gender and challenge dose.

Pathological findings in the brain

Among dead animals in the two groups, only 1 (1/8) and 3 (3/4) from the untreated control and ETI-204 8 mg/kg group had positive pathological findings (discoloration(s)) in the brain. No positive results were recorded for survivors.

Figure 29. Study NIAID 1056: PA-ELISA over time by animal

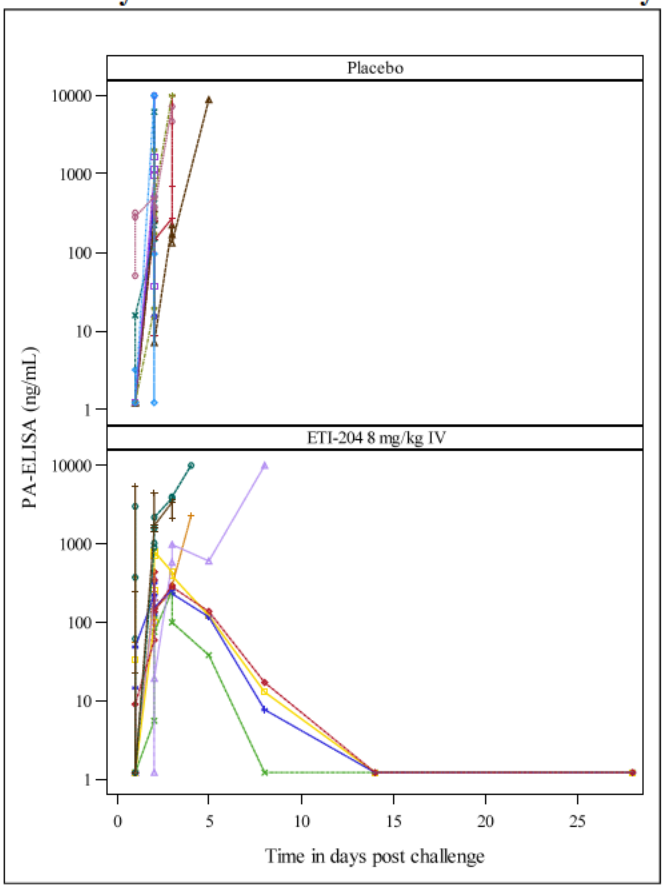


Table 45. Study NIAID1056: Survival at Day 28 by challenge dose, bacteremia, and PA-ELISA

	Placebo (N= 8)	ETI-204 8 mg/kg IV (N= 8)	Total (N= 16)
Gender			
Male	0/4	2/4 (50%)	2/8 (25%)
Female	0/4	2/4 (50%)	2/8 (25%)
Challenge dose (LD_{50})			
<250	0/8	3/6 (50%)	3/14 (21.4%)
250 or higher	0	1/2 (50%)	1/2 (50%)
<200	0/4	3/5 (60%)	3/9 (33.3%)
Bacteremia prior to treatment (cfu/mL)			
$<10^2$		0/2	0/2
10^2 - 10^4		4/6 (66.7%)	4/6 (66.7%)
PA prior to treatment (ng/mL)			
0 - < 10		1/2 (50.0%)	1/2 (50.0%)
10 - < 50		2/4 (50.0%)	2/4 (50.0%)

6.2.6.5 Conclusions

This study conducted by NIH was an open-label comparative trial. This trial compared the 8 mg/kg dose of ETI-204 to an untreated control. The ETI-204 treatment arm had a 50% survival rate compared to 0% on control. This difference was not quite significant due to the small sample size of 8 per arm. Compared to the other animal studies with an 8 mg/kg dose, this study had a lower response rate compared to AP201 (73%), but a higher response rate compared to AP203 (6%). The severity of disease at baseline based on pre-treatment bacteremia fell between these two studies, with the higher the disease severity at baseline the lower the survival rate.

6.3 IV Rabbit Treatment Studies

6.3.1 Summary of IV rabbit treatment studies

There were four rabbit studies that assessed the efficacy of ETI-204 IV as monotherapy, two were conducted by the applicant and two by NIH. All of the studies used the Baxter product. These studies varied the doses of ETI-204, typically based on the results of the previous studies. The survival results were very variable across the studies, which was possibly due to the severity of disease at the time of therapy.

Table 46. Survival Results in Rabbit Treatment IV studies testing mono-therapy

Study manufacturer year	Blinded	ETI-204 Dose (mg/kg)	Average challenge dose (LD_{50}) mean (SD)	Average time to treatment (hrs) mean (SD)	Pre-Bacter-emia (log_{10}) mean	Survival %	One-side p-value
AR021 Baxter 2008	Unclear	0 1 4 16	184.6 (71.8) 167.7 (41.3) 200.0 (51.8) 174.9 (61.2)	31.68 (7.28) 28.38 (4.97) 29.04 (3.87) 30.38 (4.88)	NA	0% (0/1) 33% (3/10) 76% (13/17) 94% (16/17)	 0.076 0.0005* <0.0001*
AR033 Baxter 2011	Yes	0 1 4 8 16	201.6 (33.8) 208.7 (27.8) 208.5 (45.4) 188.6 (38) 196.1 (30.2)	26.74 (5.21) 27.78 (3.79) 29.00 (5.64) 27.39 (4.94) 28.45 (5.44)	2.8 3.1 3.3 3.3 3.1	0% (0/14) 29% (4/14) 43% (6/14) 71% (10/14) 64% (9/14)	 0.0208 0.003* <0.001* 0.001*
1030 Baxter 2009	No	0 8	183.8 (20.1) 178.9 (68.9)	32.41 (7.01)	NA	0% (0/6) 75% (12/16)	 0.0008*
1045 Baxter 2014	No	0 8	202.3 (30.3) 194.4 (57.9)	73.18 (2.12)	NA	0 (0/6) 43% (7/16)	0.0296

*Significant at an overall one-sided significance level of 0.025 using Bonferroni adjustment for multiple comparisons if needed.

6.3.2 AR021

AR021: Evaluating the Efficacy of ETI-204 When Administered Therapeutically in the New Zealand White Rabbit Inhalational Anthrax Model
Conducted under (b) (4) Study 832-G924202 for Elusys Therapeutics, Inc.

6.3.2.1 Study Design and Endpoints

Study Objective
The objective of this study was to evaluate the efficacy of ETI-204 when administered therapeutically against lethality due to inhalation exposure to *B. anthracis* in NZW rabbits. The goal of this dose ranging study was to identify a target dose for ETI-204.

Study Design

This was a randomized, placebo-controlled study, conducted by (b) (4) in 2008.

There were 5 groups in this study:
- Placebo
- 1 mg/kg ETI-204 IV
- 4 mg/kg ETI-204 IV
- 16 mg/kg ETI-204 IV
- 50 mg levofloxacin (daily oral administration for 3 days)

The ETI-204 was manufactured at the Baxter facility.

Sixty four rabbits (32 male, 32 female) were randomized into five dose groups (based on weights collected during quarantine) with 17 animals per arm in the 4 and 16 mg/kg arms and 10 per arm in the other three arms. In addition, animals were also randomized to one of three challenge days and a challenge order per day.

The targeted inhaled dose of *B. anthracis* (Ames strain) was 200 median LD_{50}s. 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 (whichever came first). 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. Baseline body temperature was taken from study day -7 through the morning of study day 0. Standard deviations were calculated separately for each animal using all of the pre-challenge temperature. For calculation of time until significant increase in body temperature (SIBT), the last elevated temperature that caused the criteria to be met was selected as time that temperature was abnormal.

Beyond 48 hours post-challenge (until 72 hours post-challenge), only temperature would be used as a trigger for treatment. If an animal had not been treated by 72 hours, the animal will be

treated after its last hourly temperature. Animals were monitored for abnormal clinical signs for 28 days post-challenge and blood samples were taken regularly.

There were no quantitative bacteremia data and PA-ELISA data available in this study.

Primary Endpoints

The primary efficacy endpoint was survival to 28 days post challenge.

6.3.2.2 Statistical Methodologies

Sample Size Calculation

Assuming that the true probability of survival in the control group (group 1) was less than 5% and the true probability of survival in either of the two highest dose treatment groups (group 3 or 4) was greater than 55%, then 10 control animals and 17 treated animals provided 81.3% power to detect a difference in survival probabilities between these two groups. If the probability of survival in the levofloxacin treatment group (group 5) was assumed to be greater than 65%, then 10 control animals and 10 treated animals provided 86.1% power to detect a difference in survival probabilities between the levofloxacin treated group and the control group. These were for a one-sided, 0.05 level Fisher exact test.

Comment: This sample size calculation uses a one-sided level that is twice what would be expected and does not consider multiple comparisons.

Analysis Populations

In the protocol, there were no analysis populations defined. In the study report it is stated that the survival analysis was done four separate times. It was performed:

- with all animals included,
- with the animals that were inadvertently dosed with levofloxacin (Animal K99373 from the placebo group and Animal K99383 from the ETI-204 1mg/kg group) removed,
- with all animals that were not bacteremic at any study time point prior to and including treatment time removed, and
- with all animals that were not bacteremic through treatment and Animals K99373 and K99383 removed.

Statistical Methods

One-sided Fisher's exact tests were utilized to perform all pairwise comparison of survival rates between the groups. A Bonferroni-Holm adjustment was used to maintain an overall 0.05 significance level. However, it was not clear if this was a pre-specified analysis because it was stated in the statistical report but not in the protocol. Since our interest in this study is to compare ETI-204 to control, we will consider a basic Bonferroni adjustment which divides the overall one-sided p-value of 0.025 by 3.

The time-to-death data were analyzed to determine if there were differences in protection for the treatment groups based on a time-to-death model. When the log-rank test was significant, pairwise log-rank tests were computed to determine which groups were significantly different.

6.3.2.3 Animal Disposition, Demographic and Baseline Characteristics

Demographic variables and baseline characteristics are listed in Table 47. All animals were randomized and treated. Animal K99373 from the placebo group and animal K99383 from ETI-204 1 mg/kg group were inadvertently dosed with levofloxacin and are included in the randomized groups in this table. Fifty percent (31/62) of the animals were treated based on a positive PA-ECL, and 50% were treated based on SIBT. Note we could replicate the time to significant increase in body temperature for most of these animals treated on temperature trigger, with only a few animals having an about one hour longer or shorter time than the above criterion indicated. Therefore, the applicant defined time to SIBT was used.

It was noted that PA-ECL positivity was slightly lower in the ETI-204 4 mg and 16 mg groups. Other variables were comparable across different treatment groups.

Table 47. Study AR021: Demographic variables and baseline characteristics by treatment group

	Placebo (N=10*)	**ETI-204 1 mg/kg IV (N=10*)**	**ETI-204 4 mg/kg IV (N=17)**	**ETI-204 16 mg/kg IV (N=17)**	**Levofloxacin 50 mg/kg orally (N=10)**	**Total (N=64)**
Age (month) Range	7, 8	7, 8	7, 8	7, 8	7, 8	7, 8
Gender [n (%)] Female Male	5 (50) 5 (50)	5 (50) 5 (50.)	9 (52.9) 8 (47.1)	8 (47.1) 9 (52.9)	5 (50.0) 5 (50.0)	32 (50) 32 (50)
Body weight (kg) Mean (SD) Range	3.2 (0.1) 3.0, 3.3	3.2 (0.2) 3.0, 3.3	3.2 (0.1) 2.9, 3.4	3.2 (0.2) 2.9, 3.5	3.2 (0.2) 2.9, 3.5	3.2 (0.2) 2.9, 3.5
Challenge dose (LD_{50}) Mean (SD) Range	184.6 (71.8) 85.0, 343.0	167.7 (41.3) 99.0, 217.0	200.0 (51.8) 89.0, 309.0	174.9 (61.2) 86.0, 300.0	164.8 (48.2) 79.0, 221.0	180.4 (55.9) 79.0, 343.0
Challenge dose (cfu x 10^7) Mean (SD) Range	1.937 (0.754) 0.891, 3.600	1.764 (0.435) 1.040, 2.280	2.102 (0.544) 0.936, 3.250	1.837 (0.643) 0.907, 3.150	1.729 (0.506) 0.834, 2.320	1.895 (0.587) 0.834, 3.600
Challenge dose (LD_{50}) (n(%)) <200 200 or higher	7 (70) 3 (30)	8 (80) 2 (20)	9 (52.9) 8 (47.1)	10 (58.8) 7 (41.2)	8 (80) 2 (20)	40 (64.5) 22 (35.5)
Enriched bacteremia prior to treatment [n (%)]	10 (100)	9 (90)	15 (88.2)	14 (82.4)	9 (90)	57 (89.1)

	Placebo (N=10*)	ETI-204 1 mg/kg IV (N=10*)	ETI-204 4 mg/kg IV (N=17)	ETI-204 16 mg/kg IV (N=17)	Levofloxacin 50 mg/kg orally (N=10)	Total (N=64)
PA-ECL positivity at trigger (n(%))	5 (50)	6 (60)	6 (35.3)	8 (47.1)	6 (60)	31 (48.4)

*Animal K99373 from the placebo group and animal K99383 from ETI-204 1 mg/kg group were inadvertently dosed with levofloxacin and were included in the randomized groups in this table.

Time to bacteremia, trigger, and treatment

Table 48 includes the time between challenge, trigger, and treatment. The time to qualitative bacteremia was longer in the first three groups, compared with that in the ETI-204 16 mg/kg group and the levofloxacin group. As described previously, the time to SIBT in the data set from most animals was the same as the time derived from the temperature data by the reviewer, with only a few animals with a shorter time in the data set, possibly due to rounding in defining critical temperature. Therefore, in this table, the trigger time in the data set was used.

Table 48. Study AR021: Time between challenge, trigger, and treatment

	Placebo (N=10)	ETI-204 1 mg/kg IV (N=10)	ETI-204 4 mg/kg IV (N=17)	ETI-204 16 mg/kg IV (N=17)	Levofloxacin 50 mg/kg orally (N=10)	Total (N=64)
Time to qualitative bacteremia (hours)						
N	9*	9*	15	14	7	54
Mean(SD)	37.7 (21.8)	43.3 (25.5)	38.2 (15.2)	27.5 (3.7)	25.0 (2.3)	34.5 (16.7)
Range	23.8, 94.1	23.7, 104.3	23.6, 60.7	23.8, 35.7	23.7, 30.1	23.6, 104.3
Time to trigger (hours)						
Mean (SD)	29.95 (7.61)	26.49 (4.70)	27.65 (4.13)	28.77 (5.25)	24.85 (3.38)	27.69 (5.20)
Range	20.88, 43.82	21.80, 35.57	22.20, 35.58	21.62, 40.30	18.48, 30.43	18.48, 43.82
Time to significant increase in body temperature (hours)						
N	5	4	11	9	4	33
Mean (SD)	32.3 (10.4)	25.9 (5.1)	28.5 (4.5)	30.2 (5.8)	24.4 (4.9)	28.7 (6.2)
Range	20.9, 43.8	21.8, 32.9	22.2, 35.6	21.6, 40.3	18.5, 30.4	18.5, 43.8
Time from trigger to treatment (hours)						
Mean (SD)	1.73 (1.19)	1.73 (1.19)	1.40 (1.21)	1.61 (1.34)	2.09 (1.38)	1.69 (1.27)
Range	0.27, 3.45	0.27, 3.45	0.23, 3.45	0.23, 3.50	0.20, 3.88	0.18, 3.88

*K99373 and K99383 were negative for *B. anthracis* in the LB data set

6.3.2.4 Results

Survival

Table 49 includes survival status at Day 28 by treatment group. The first panel includes all randomized animals, including two animals, one in the placebo group and one in the 1 mg/kg group, which were inadvertently treated with levofloxacin and survived to Day 28. Because the two animals survived, comparison of the 4 mg/kg and 16 mg/kg arm to the placebo control group was a conservative analysis. These analyses showed a statistically significant difference between the 4 mg/kg and 16 mg/kg groups and the placebo group. The comparison between the 1 mg/kg ETI-204 group was not significantly different from placebo even including the ETI-204 1 mg/kg animal that received levofloxacin and survived. The levofloxacin group had the similar survival proportion as the ETI-204 16 mg/kg group. The next two analyses remove the 2 animals that were treated with levofloxacin inadvertently and the results were consistent with the mITT analysis.

Table 49. Study AR021: Survival at Day 28 by treatment group

	Placebo (N=10)	**ETI-204 1 mg/kg (N=10)**	**ETI-204 4 mg/kg (N=17)**	**ETI-204 16 mg/kg (N=17)**	**Levofloxacin 50 mg/kg (N=10)**
n (%)	1 (10)	4 (40)	13 (76.5)	16 (94.1)	9 (90.0)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.3 [-0.107, 0.659] 0.0755	0.665 [0.249, 0.878] 0.0005	0.841 [0.443, 0.978] <0.0001	0.80 [0.366, 0.975] 0.0002
Adjusted exact 95% confidence interval		-0.219, 0.732	0.155, 0.918	0.352, 0.989	0.244, 0.988
Calculations only including animals that were bacteremic at some time prior to treatment					
n/N (%)	1/10 (10)	4/9 (44.4)	11/15 (73.3)	13/14 (92.9)	8/9 (88.9)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.344 [-0.078, 0.709] 0.059	0.633 [0.232, 0.878] 0.0011*	0.829 [0.431, 0.976] <0.0001*	0.789 [0.335, 0.972] 0.0004*
Adjusted exact 95% confidence interval		-0.192, 0.779	0.120, 0.905	0.326, 0.989	0.209, 0.987
Calculations not including animal K99373 and K99383 in the first two groups					
n/N (%)	0/9 (0)	3/9 (33.3)	13/17 (76.5)	16/17 (94.1)	9/10 (90.0)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.333 [0.071, 0.701] 0.0488	0.765 [0.400, 0.932] <0.0001*	0.941 [0.619, 0.999] <0.0001*	0.900 [0.477, 0.998] <0.0001*
Adjusted exact 95% confidence interval		-0.1952, 0.7714	0.219, 0.955	0.426, 1.000	0.354, 0.999
Calculation only includes animals that were bacteremic at some time prior to treatment (enriched bacteremia), excluding animal K99373 and K99383 in the first two groups					
n/N (%)	0/9 (0)	3/8 (37.5)	11/15 (73.3)	13/14 (92.9)	8/9 (89)

	Placebo (N=10)	ETI-204 1 mg/kg (N=10)	ETI-204 4 mg/kg (N=17)	ETI-204 16 mg/kg (N=17)	Levofloxacin 50 mg/kg (N=10)
Difference in survival proportion compared with placebo [exact 95% confidence] one-sided p-value		0.375 [-0.022, 0.755] 0.032	0.733 [0.298, 0.9251] 0.0002*	0.929 [0.593, 0.998] <0.0001*	0.889 [0.454, 0.997] <0.0001*
Adjusted exact 95% confidence interval		-0.142, 0.822	0.208, 0.955	0.413, 1.000	0.326, 0.999

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025/4=0.00625

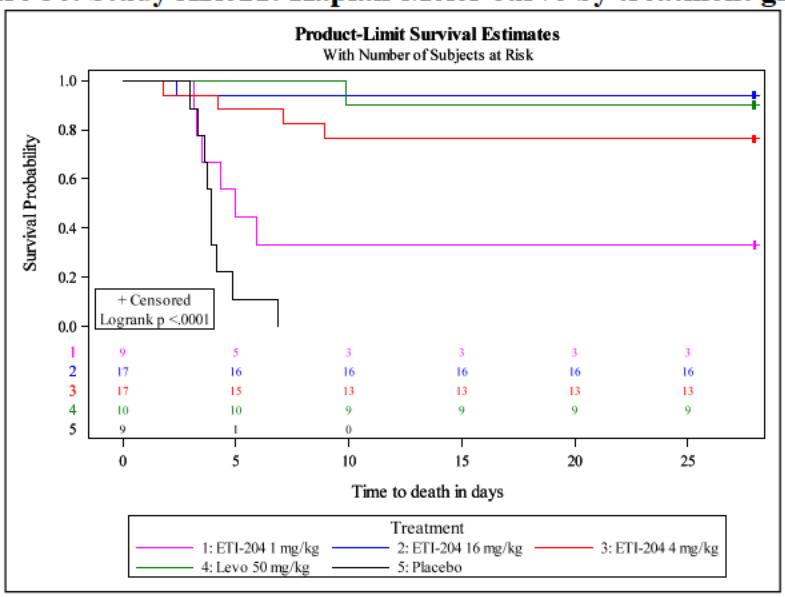
Figure 30and Figure 30 demonstrated that there was a significant difference between the 4 mg/kg dose and 16 mg/kg dose of ETI-204 groups and the placebo group, using a two-sided significance level of 0.05/3=0.0167. The levofloxacin group was also significantly different from placebo.

Table 50. Study AR021: two-sided p-values of pairwise log-rank tests comparing time from challenge to death among groups

	ETI-204 1 mg/kg IV	ETI-204 4 mg/kg IV	ETI-204 16 mg/kg IV	Levofloxacin 50 mg/kg IV
Placebo	0.0878	<0.0001*	<0.0001*	<0.0001*

Not including animals inadvertently doses with levofloxacin (K99373 and K99383). Including these two surviving animals in the intended groups only changed the first p-value from 0.0878 to 0.147.
*Statistically significant at a two-sided significance level of 0.05/3=0.0167

Figure 30. Study AR021: Kaplan-Meier curve by treatment group



Not including animals inadvertently doses with levofloxacin (K99373 and K99383). Including these two surviving animals in the intended groups did not change the overall p-value indicated in the figure.

Subgroup Analysis Results

The following table shows the results of subgroup analyses. It appears that the survival proportions were higher in the female group than in the male group. An exact logistic regression including treatment group and gender demonstrated that gender was statistically significant. The reason for this significant effect was not clear, because there was no bacteremia and PA measured prior to treatment and the mean challenge dose and the proportion of qualitative bacteremia were comparable between males and females. The sample sizes for challenge dose were too small to make a conclusion on the effect of challenge dose.

Table 51. Study AR021: Survival at Day 28 by gender and challenge dose

	Placebo (N= 10)	**ETI-204 1 mg/kg IV (N= 10)**	**ETI-204 4 mg/kg IV (N= 17)**	**ETI-204 16 mg/kg IV (N= 17)**	**Levofloxacin 50 mg/kg Orally (N= 10)**	**Total (N= 64)**
Gender						
Female	1/5 (20%)	3/5 (60%)	9/9 (100%)	8/8 (100%)	5/5 (100%)	26/32 (81.3%)
Male	0/5	1/5 (20%)	4/8 (50%)	8/9 (88.9%)	4/5 (80%)	17/32 (53.1%)
Challenge dose (LD_{50}) (n(%))						
<250	1/9 (11.1%)	4/10 (40%)	11/14 (78.6%)	15/16 (93.8%)	9/10 (90.0%)	40/59 (67.8%)
250 or higher	0/1		2/3 (66.7%)	1/1 (100%)		3/5 (60%)

Tissue bacterial assessment and pathological findings in the brain

Among the dead animals, 9, 5, 4, 1 from the placebo, 1, 4, and 16 mg/kg groups had a positive result in the spleen, and 9, 5, 3, 1 in bronchial lymph node. There were no positive bacterial loads in these two issues among the survivors. No results from the brain were included in the data set.

Among non-survivors, 9 (100%), 5 (83.3%), 2 (50%) animals from the placebo, 1 mg/kg, and 4 mg/kg groups had positive pathological findings in the brain. No survivors had positive pathological findings in the brain.

6.3.2.5 Conclusion

In this study in New Zealand White rabbits, the 16 mg/kg dose of ETI-204 was statistically superior to placebo in rate of survival at day 28. This study also supports the efficacy of the 4 mg/kg dose. This study used the Baxter product.

6.3.3 AR033

AR033: Evaluating the Efficacy of ETI-204 When Administered Therapeutically in New Zealand White Rabbits

Conducted under (b) (4) Study 1185-100003006 for Elusys Therapeutics, Inc.

6.3.3.1 Study Design and Endpoints

Primary Objective

The objective of this study was to further explore a range of therapeutic doses of ETI-204 in *B. anthracis* challenged rabbits and to collect data for pharmacokinetic (ETI-204 serum levels) and pharmacodynamic (quantitative free PA, quantitative bacteremia) analysis to support selection of the human clinical dose.

Study Design

This was a randomized, blinded, placebo-controlled, parallel group, trigger-to-treat (dosing upon positive PA-ECL or SIBT), dose ranging study in anthrax challenged animals, conducted at (b) (4) in 2011.

Seventy (70) NZW rabbits (35 males and 35 females) were planned and randomized to the following five groups of 14 animals each and analyzed.

- Placebo
- ETI-204 1 mg/kg IV
- ETI-204 4 mg/kg IV
- ETI-204 8 mg/kg IV
- ETI-204 16 mg/kg IV

The test product was manufactured at the Baxter facility.

All animals were aerosol challenged with a targeted 200 LD_{50} inhaled dose of *Bacillus anthracis* spores on Study Day 0. Animals were monitored for a positive PA-ECL result or a significant increase in body temperature (SIBT). After one of these occurred, animals were treated. Between 42 hours post-challenge and 54 hours post-challenge, only temperature was used as a trigger for treatment. If an animal was not treated within 54 hours post-challenge, then the animal was treated after its last hourly temperature.

Except for Study Coordinator and QA Auditor, all other personnel were blind to the treatment assignment.

Treatment was started when they had exhibited SIBT. 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.

Clinical signs were monitored every 6 hours between 18 hours and 168 hours post median challenge time for a challenge cohort and once daily on all other study days.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.3.3.2 Statistical Methodologies

Sample Size Calculation
With an assumption that the true probabilities of survival were 5% and 70% in the control group and a treated group, respectively, 14 animals per group would provide 83.4% statistical power, using a two-sided, 0.05 level, Fisher's exact taking into account a Bonferroni adjustment to control for multiple comparisons across four tests.

Comment: Using a two-sided type I error of 0.0125 replicated this sample size calculation.

Analysis Populations

The primary analysis excluded animals that were not positive for bacteremia by culture (qualitative, quantitative, or enriched) at some time point prior to treatment, but included animals that died prior to treatment as treatment failures regardless if they were ever positive for bacteremia.

A secondary analysis would include all challenged animals regardless of bacteremia status and include those animals that received treatment.

Statistical Methods

The survival data from each treatment group were compared to the control group using a two-sided Fisher's exact test, using a Bonferroni adjustment for multiple comparisons.

6.3.3.3 Animal Disposition, Demographic and Baseline Characteristics

All animals survived to treatment and were included in the analyses. Demographic variables and baseline characteristics are listed in the following table. These variables were comparable across different groups except for challenge dose, which was lower in the ETI-204 8 mg/kg group. Because other variables, such as the proportion of bacteremia and level of bacteremia were comparable, it was expected that this low challenge dose would not significantly affect the efficacy results. Twenty-four percent (17/70) and 75% (53/70) of the animals were treated based on a positive PA-ECL result and SIBT, respectively.

Table 52. Study AR033: Demographic variables and baseline characteristics by treatment group

	Placebo (N=14)	ETI-204 1 mg/kg (N=14)	ETI-204 4 mg/kg (N=14)	ETI-204 8 mg/kg (N=14)	ETI-204 16 mg/kg (N=14)	Total (N=70)
Age (month)						
Mean (SD)	8.9 (1.2)	9.3 (2.0)	9.8 (2.2)	8.9 (2.2)	9.9 (3.5)	9.4 (2.3)
Range	7.0, 12.0	7.0, 12.0	7.0, 15.0	7.0, 13.0	7.0, 19.0	7.0, 19.0
Gender [n (%)]						
Female	7 (50.0)	7 (50.0)	7 (50.0)	7 (50.0)	7 (50.0)	35 (50.0)
Male	7 (50.0)	7 (50.0)	7 (50.0)	7 (50.0)	7 (50.0)	35 (50.0)
Body weight (kg)						
Mean (SD)	3.6 (0.2)	3.5 (0.2)	3.6 (0.2)	3.5 (0.1)	3.6 (0.2)	3.5 (0.2)
Range	3.3, 3.8	3.2, 3.9	3.2, 3.8	3.2, 3.7	3.2, 4.0	3.2, 4.0
Challenge dose (LD_{50})						
Mean (SD)	201.6 (33.8)	208.7 (27.8)	208.5 (45.4)	188.6 (38.0)	196.1 (30.2)	200.7 (35.4)
Range	132.0, 263.0	155.0, 255.0	102.0, 278.0	137.0, 290.0	129.0, 238.0	102.0, 290.0
Qualitative direct bacteremia prior to treatment [n (%)]	13 (92.9)	12 (85.7)	11 (78.6)	13 (92.9)	13 (92.9)	62 (88.6)
Bacteremia prior to treatment (cfu/mL)						
Geometric mean	705.9	1310.1	1937.1	2050.0	1362.2	1379.9
95% confidence interval	81.7, 6098.6	131.2, 13085	248.4, 15108.3	280.8, 14966.8	193.7, 9581.3	593.2, 3209.9
Mean (SD) log_{10} bacteremia	2.8 (1.6)	3.1 (1.7)	3.3 (1.5)	3.3 (1.5)	3.1 (1.5)	3.1 (1.5)
PA-ECL positivity at trigger (n(%))	2 (14.3)	3 (21.4)	5 (35.7)	5 (35.7)	2 (14.3)	17 (24.3)
PA-ELISA prior to treatment (ng/mL)						
Geometric mean	5.3	5.5	5.7	5.7	5.8	5.6
95% confidence interval	4.3, 6.6	4.2, 7.2	4, 8.2	4.5, 7.3	3.9, 8.8	5, 6.4
Mean (SD) of log_{10} PA	0.7 (0.2)	0.7 (0.2)	0.8 (0.3)	0.8 (0.2)	0.8 (0.3)	0.7 (0.2)

Time between, challenge, trigger, and treatment

As the following table shows, these variables were comparable between different groups. As in Study AR021, the time to SIBT in the data set from most animals was the same as the time derived from the temperature data by the reviewer, with only a few animals with a difference of within one half hour in the data set, possibly due to rounding in defining a critical temperature. Therefore, in this table, the trigger time in the data set was used for the time to trigger.

Table 53. Study AR033: Time between challenge, trigger, and treatment

	Placebo (N=14)	ETI-204 1 mg/kg (N=14)	ETI-204 4 mg/kg (N=14)	ETI-204 8 mg/kg (N=14)	ETI-204 16 mg/kg (N=14)	Total (N=70)
Time to bacteremia (hours)						
N	14	12	13	14	12	65
Mean (SD)	36.7 (20.8)	31.3 (13.6)	28.2 (6.5)	30.1 (12.2)	34.8 (18.9)	32.2 (15.2)
Range	22.1, 103.7	22.9, 73.7	23, 44.8	22.4, 69	23.7, 92.6	22.1, 103.7
Time to trigger (hours)						
Mean (SD)	25.78 (5.30)	26.83 (3.61)	27.40 (5.87)	25.94 (4.75)	27.73 (5.34)	26.74 (4.95)
Range	18.42, 36.92	20.43, 33.35	19.78, 42.82	19.88, 36.32	17.83, 37.07	17.83, 42.82
Time from trigger to treatment (hours)						
Range	0.95 (1.23)	0.95 (1.05)	1.60(1.35)	1.45 (1.55)	0.71 (0.75)	1.13 (1.23)
Mean (SD)	0.30, 4.48	0.28, 3.22	0.37, 4.25	0.23, 4.22	0.27, 2.82	0.23, 4.48

6.3.3.4 Results

Survival

Table 54. Study AR033: Survival at Day 28 by treatment group

	Placebo (N=14)	ETI-204 1 mg/kg (N=14)	ETI-204 4 mg/kg (N=14)	ETI-204 8 mg/kg (N=14)	ETI-204 16 mg/kg (N=14)
n (%)	0	4 (28.6)	6 (42.9)	10 (71.4)	9 (64.3)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one sided p-value		0.286 [0.012, 0.581] 0.02081	0.429 [0.135, 0.711] 0.003*	0.714 [0.406,0.916] <0.001*	0.643 [0.334, 0.872] 0.001*
Exact 95% confidence interval		-0.077, 0.649	0.044, 0.769	0.312, 0.944	0.237, 0.909
Including only qualitatively bacteremic animals					
n/N (%)	0/13 (0)	2/12 (16.7)	3/11 (27.3)§	9/13 (69.2)	8/13 (61.5)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.167 [-0.098, 0.484] 0.118	0.273 [-0.031, 0.610] 0.036	0.692 [0.367, 0.909] <0.001*	0.615 [0.290, 0.861] <0.001*
Exact 95% confidence interval		-0.208, 0.563	-0.138, 0.683	0.268, 0.939	0.189, 0.901

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer

*Statistically significant at a one-sided significance level of 0.025/4=0.00625
§Animal L48722 was qualitatively negative, but positive in quantitative bacteremia, which was included in the applicant's analysis, but was excluded here. If this animal was included, the survival proportion was 0.333 (4/12), which would be less conservative in comparing with the placebo group.

The survival proportions are shown in Table 54. With a Bonferroni adjustment (one-sided significance level of 0.00625), 4, 8, and 16 mg/kg groups were statistically significantly different from the placebo group in the analysis including all animals. In the qualitatively bacteremic animals, only the 8 and 16 mg/kg treatment groups had a significant treatment effect.

Figure 31 and Table 55 show the results from survival analysis of the time to death. With a Bonferroni adjustment (two-sided significance level of 0.0125), the differences between all treatment groups and the placebo group were statistically significant.

Figure 31. Study AR033: Kaplan-Meier curve by treatment group

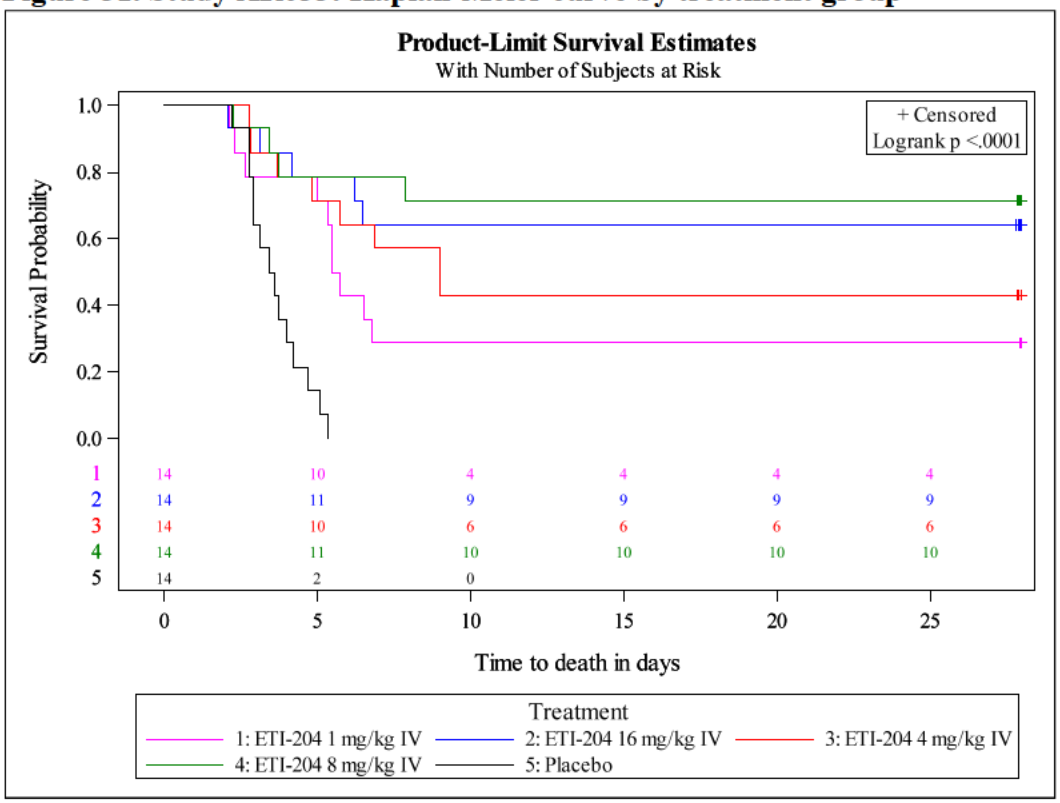


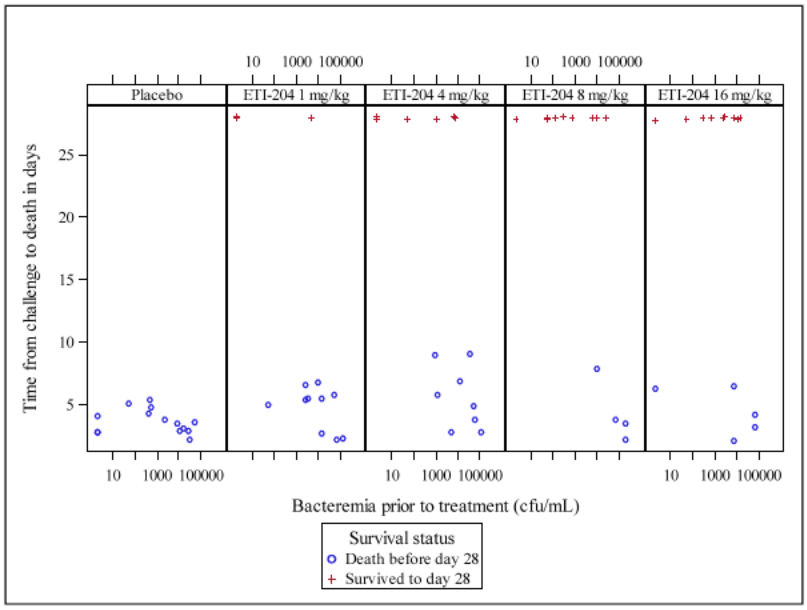
Table 55. Study AR033: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death among groups

	ETI-204 1 mg/kg (N=14)	ETI-204 4 mg/kg (N=14)	ETI-204 8 mg/kg (N=14)	ETI-204 16 mg/kg (N=14)
Placebo	0.0003*	0.0001*	<0.0001*	<0.0001*

*Statistically significant at a two-sided significance level of 0.05/4=0.0125

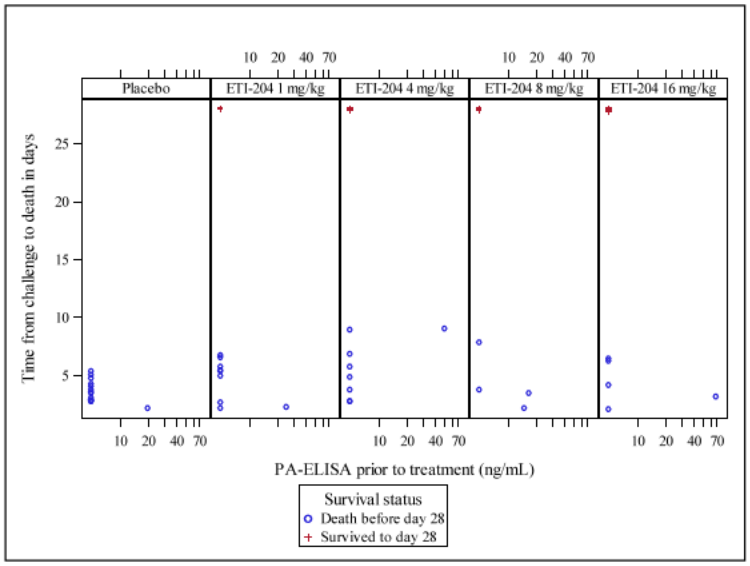
Figure 32 shows the time to death versus bacteremia prior to treatment. In this study, deaths occurred across all bacteremia levels. Therefore, it was not possible to find a cut-off point of bacteremia to separate surviving and non-surviving animals. However, there does appear to be a trend with animals with lower bacteremia being more likely to survive.

Figure 32. Study AR033: Time to death versus bacteremia prior to treatment by survival status at Day 28



All animals in the control group died regardless of the PA levels. Animals in the treatment groups with a PA less than the LLOQ were more likely to survive (Figure 33).

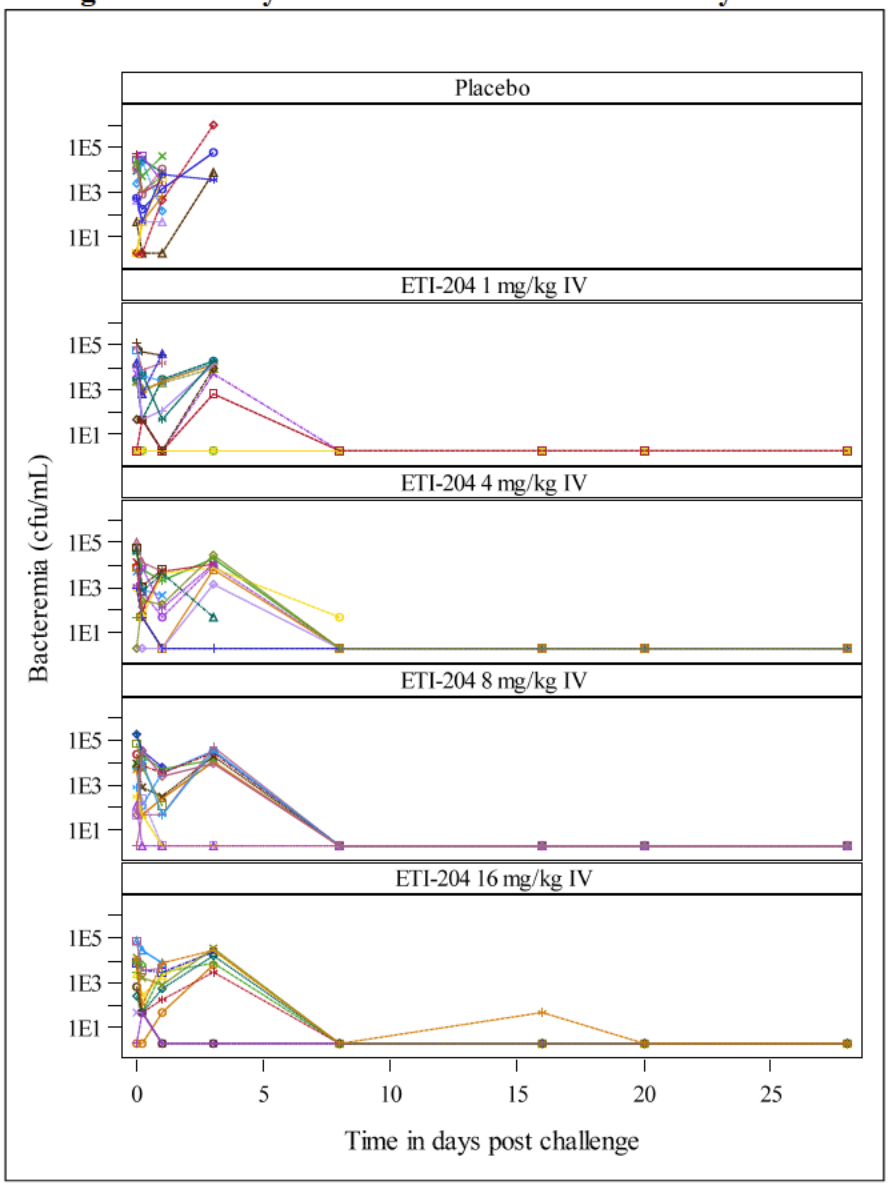
Figure 33. Study AR033: Time to death versus PA-ELISA prior to treatment by survival status at Day 28



Bacteremia over time

Figure 34 shows 8 or 9 days post-challenge, the bacteremia levels decreased to a very low level in the treatment groups. The control animals had an elevated mean level of bacteremia before death.

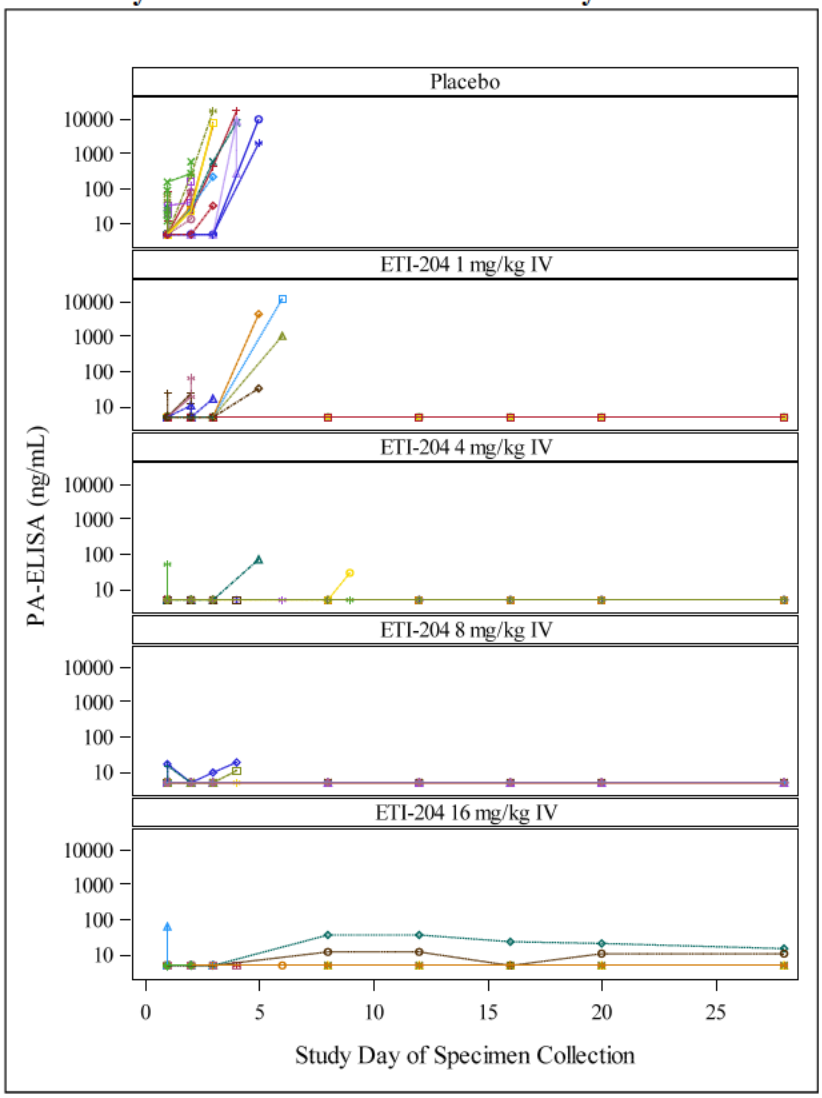
Figure 34. Study AR033: Bacteremia over time by animal



PA-ELISA over time

Figure 35 shows PA-ELISA levels over time. In the placebo group, PA-ELISA increased until death, in other groups, except for the 1 mg/kg group, the PA-ELISA levels did not increase so clearly, indicating a treatment effect. In the 16 mg/kg group, 3 surviving animals had a relatively high PA-ELISA level.

Figure 35. Study AR033: PA-ELISA over time by animal and treatment



Subgroup Analyses

The following table shows the results of subgroup analyses. The sample sizes were too small to see a reliable trend by each grouping variable.

Table 56. Study AR033: Survival at Day 28 by gender, challenge dose, log_{10} bacteremia, PA prior to treatment

	Placebo (N= 14)	ETI-204 1 mg/kg IV (N= 14)	ETI-204 4 mg/kg IV (N= 14)	ETI-204 8 mg/kg IV (N= 14)	ETI-204 16 mg/kg IV (N= 14)	Total (N= 70)
Gender						
Female	0/7	1/7 (14.3%)	4/7 (57.1%)	5/7 (71.4%)	5/7 (71.4%)	15/35 (42.9%)
Male	0/7	3/7 (42.9%)	2/7 (28.6%)	5/7 (71.4%)	4/7 (57.1%)	14/35 (40%)
Challenge dose (LD_{50})						
<250	0/1	1/1 (100%)	2/2 (100%)	0/1	0	3/5 (60%)
250 or higher	0/13	4/12 (33.3%)	6/13 (46.2%)	10/12 (83.3%)	9/13 (69.2%)	29/63 (46.0%)
PA prior to treatment (ng/mL)						
0 - < 10	0/13	4/12 (33.3%)	6/13 (46.2%)	10/12 (83.3%)	9/13 (69.2%)	29/63 (46.0%)
10 - < 50	0/1	0/1	0/1	0/2	0	0/5
50 or higher	0	0	0	0	0/1	0/1
Bacteremia prior to treatment (cfu/mL)						
$<10^2$	0/4	3/4 (75%)	3/3 (100%)	3/3 (100%)	2/3 (66.7%)	11/17 (64.7%)
10^2 - 10^4	0/5	1/5 (20%)	3/6 (50%)	6/7 (85.7%)	5/7 (71.4%)	15/30 (50%)
10^4 - $<10^6$	0/5	0/5	0/5	1/4 (25%)	2/4 (50%)	3/23 (13.0%)

Tissue bacterial assessments and pathological findings in the brain

Almost all dead animals had a positive bacterial load in the tissues tested (bronchial lymph node, brain, liver and spleen). Only one animal out of 8 surviving animals (11.1%) in the 16 mg/kg group had positive bacterial load in bronchial lymph node in all tissues tested (brain, kidney, lung, liver and spleen).

Among non-survivors, only 2 (14.3%), 1 (10%), 1 (25%), and 2 (40%) animals had brain discoloration(s) in the 0, 1, 8, 16 mg/kg groups, respectively. There were no positive pathological findings in the brain from survivors.

6.3.3.5 Conclusions

As in study AR021, the 16 mg/kg dose of ETI-204 was statistically superior to placebo in rate of survival at day 28. However, the survival rates in this study for both the 4 mg/kg dose and the 16 mg/kg dose were lower than what was seen in study AR021. In this study the 8 mg/kg dose was statistically superior to placebo for all analyses, while the 4 mg/kg dose was only significant in the analysis of all randomized animals. It is not clear why the survival rates were lower in this study compared to AR021, other than the challenge dose did seem to be higher in this study. This study used the Baxter product.

6.3.4 NIAID1030

Determining the Therapeutic Efficacy of a Novel Anti-PA Antibody Administered Alone or in Combination with Levofloxacin to New Zealand White Rabbits Following a *Bacillus* anthracis Inhalation Challenge

Conducted under (b) (4) Study No. 1030-G607604 for DMID/NIAID

This study randomized animals to four arms: no treatment, ETI-204-alone given when animal had an increase in body temperature, levofloxacin alone given 96 hours after challenge and a combination of levofloxacin plus ETI-204 given 96 hours after challenge. This is essentially two studies in one, with the comparison of the combination to levofloxacin can be considered to assess the added benefit of ETI-204 when given with an antibacterial and the comparison of ETI-204 alone compared to the untreated control. This review will focus only on the comparison of ETI-204 compared to the untreated control arm. Please see the statistical review by Ling Lan for a discussion of the contribution of ETI-204 when given with antibacterials.

6.3.4.1 Study Design and Endpoints

Primary Objective

The primary objective was to assess the efficacy of ETI-204 when administered following a SIBT and to assess the efficacy of delayed treatment (96 hours after exposure) with levofloxacin or anti-PA monoclonal antibody in combination of levofloxacin to New Zealand White (NZW) rabbits following aerosol exposure to *Bacillus anthracis*.

Study Design

This was a randomized, controlled, open-label, parallel-group study conducted at (b) (4) in 2009.

Animals were randomized to the following groups:

- ETI-204 8 mg/kg, started on SIBT
- Levofloxacin 50 mg/kg orally once daily for three days, started at 96 hours post median challenge ± 1 hour
- ETI-204 8 mg/kg IV (once) + Levofloxacin 50 mg/kg orally once daily for three days, started at 96 hours post median challenge ± 1 hour
- Non-treated Control

The test product was manufactured at the Baxter facility.

Comment: As discussed above, the focus of this review is the effect of ETI-204 monotherapy compared to untreated control.

Prior to the start of study, rabbits were randomized into three groups of 16 (50% male, 50% female) and one group of 6 rabbits for the control (50% male, 50% female). The rabbits were then randomized to two days of challenge (Challenge Day A and Challenge Day B) such that

50% of the animals from each group were challenged per day. Finally, the animals were randomized for challenge order for each day of challenge.

On Study Day 0, rabbits were challenged with a targeted dose of 200 LD_{50} *B. anthracis* (Ames strain) spores. Animals were monitored and blood samples were taken regularly. Treatment in the ETI-204 8 mg/kg arm was started after a significant increase in body temperature (SIBT) was observed. SIBT was defined as an animal had three consecutive measurements greater than or equal to a threshold of the animal's average pre-challenge temperature plus two standard deviations.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.3.4.2 Statistical Methodologies

Sample Size Calculation

The sample sizes of 16, 16, 16 and 6 in the four groups, respectively were considered sufficient in the protocol for showing treatment efficacy between the ETI-204 alone group or the combination group and the control group. The statistical power was 98% to detect a significant difference in survival rates between the ETI-204 group and the control group, assuming the probability of survival in the ETI-204 group was 75% and the probability of survival in the control group was 1%. Power calculations for all tests were for a one-sided, 0.05 level Fisher's exact test.

Comment: There are two independent comparisons of interest, the ETI-204 group versus untreated controls and the ETI-204 plus levofloxacin group compared to levofloxacin. Since there are separate control groups for the two comparisons, we do not believe that multiplicity adjustments need to be considered. We will consider the type I error of 0.025 one-sided.

Analysis Populations

1) All randomized animals.
2) The animals in each group that received treatment for a secondary analysis.

Statistical Methods

Fisher's exact tests were used to compare the survival rates between each treatment group and the control group.

6.3.4.3 Animal Disposition, Demographic and Baseline Characteristics

In the review, the focus of study was the comparison of ETI-204 and the control group. The proportion of challenge dose less than 200 LD_{50}s in the treated group was slightly lower, which was not a concern. The two groups were comparable in the other variables analyzed.

Table 57. Study NIAID1030: Demographic variables and baseline characteristics by treatment group

	Control (N=6)	ETI-204 8 mg/kg IV (N=16)	Total (N=22)
Age (months)			
Mean (SD)	4.0	4.0	4.0
Gender [n (%)]			
Female	3 (50.0)	8 (50.0)	11 (50.0)
Male	3 (50.0)	8 (50.0)	11 (50.0)
Body weight (kg)			
Mean (SD)	2.5 (0.1)	2.5 (0.1)	2.5 (0.1)
Range	2.4, 2.7	2.4, 2.7	2.4, 2.7
Challenge dose (LD_{50})			
Mean (SD)	183.8 (20.1)	178.9 (68.9)	180.3 (59.1)
Range	157.0, 209.0	87.0, 362.0	87.0, 362.0
Challenge dose (LD_{50}) (n(%))			
<200	5 (83.3)	11 (68.8)	16 (72.7)
200 or higher	1 (16.7)	5 (31.2)	6 (27.3)
Positive qualitative bacteremia prior to treatment (n(%))	NA	12 (75)	
PA-ECL positivity prior to treatment (n(%))		13 (81.3)	
Log_{10} PA-ELISA prior to treatment (ng/mL)	NA		
Mean (SD)		0.48 (0.55)	
Range		0.00, 1.51	
PA-ELISA prior to treatment (ng/mL)	NA		
Geometric mean		3.04	
95% confidence interval		1.5, 6	
Mean (SD) of log_{10} PA		0.48 (0.55)	

Time between challenge, trigger, and treatment

The time to qualitative bacteremia was longer in the control group (Table 58). This could be due to the lack of a bacteria measurement prior to treatment (less frequent measurements) in the control group. The time to trigger was based on the value provided in the data. We were not able to exactly replicate the time to SIBT calculated based on mean and SD of baseline temperature, but the values were close.

Table 58. Study NIAID 1030: Time between challenge, trigger, and treatment

	Control (N=6)	ETI-204 8 mg/kg IV (N=16)	Total (N=22)
Time to qualitative bacteremia (hours) N Mean (SD) Range	 6 44.00 (23.60) 24, 72	 11 34.91 (12.53) 24, 48	 17 38.12 (17.09) 24, 72
Time to trigger (hours) Mean (SD) Range		16 31.19 (7.02)	
Time from trigger to treatment (hours) Range Mean (SD)		 1.22 (2.02) 0.15, 8.12	

6.3.4.4 Results

Survival

As stated above a one-sided type I error or 0.025 was used. There was a statistically significant difference between the ETI-204 group and the control group in survival to day 28 (Table 59).

Survival analysis of time to death

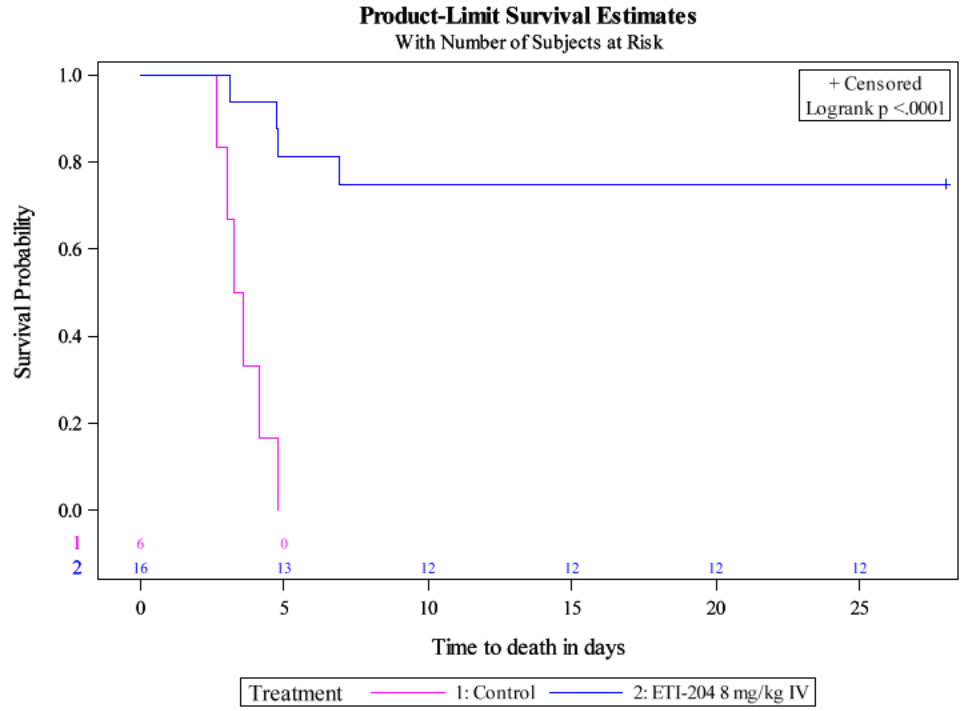
Figure 36
Figure 36 shows that the ETI-204 group had a statistically significant improvement in survival compared with the control group, using a two-sided significance level of 0.05.

Table 59. Study NIAID 1030: Survival at Day 28 by treatment group

	Control (N=6)	ETI-204 8 mg/kg IV (N=16)
All animals		
n (%)	0 (0)	12 (75)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.75 0.221, 0.927 0.0008*
Qualitatively bacteremic animals		
n/N (%)		8/12 (66.7)

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025

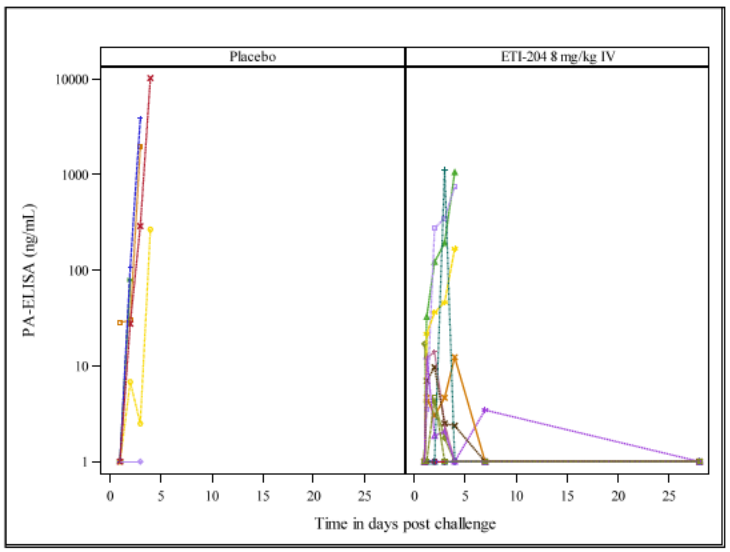
Figure 36. Study NIAID 1030: Kaplan-Meier curve by treatment group



PA-ELISA over time

The PA levels by treatment and animal are reported in the following figure. The animals in the ETI-204 group had very low levels by Day 7 and decreased to below LLOQ on Day 28.

Figure 37. Study NIAID 1030: PA-ELISA by treatment animals



Subgroup Analysis Results

The following table shows the results of subgroup analyses. The sample sizes were too small to see a reliable trend by gender and challenge dose. Animals with a lower PA-level were more likely to survive to the end of the study.

Table 60. Study NIAID1030: Survival at Day 28 by challenge dose and PA-ELISA

	Control (N= 6)	**ETI-204 8 mg/kg IV (N= 16)**	**Total (N= 22)**
Gender			
Female	0/3	7/8 (87.5%)	7/11 (63.6%)
Male	0/3	5/8 (62.5%)	5/11 (45.5%)
Challenge dose (LD_{50})			
<250	0/6	10/14 (71.4%)	10/20 (50%)
250 or higher	0	2/2 (100%)	2/2 (100%)
PA prior to treatment (ng/mL)			
0 - < 10		10/12 (83.3%)	10/12 (83.3%)
10 - < 50		2/4 (50%)	2/4 (50%)

Pathological finding in the brain

Among all dead animals, only 1 animal from each of the control group and 8 mg/kg group had a positive pathological result in the brain. No positive results were reported for survivors.

6.3.4.5 Conclusions

In this study 8 mg/kg of ETI-204 was statistically significantly superior to placebo in terms of 28 day survival. The survival rate at 8 mg/kg was 75%.

6.3.5 NIAID1045

Determining the Therapeutic Efficacy of a Novel Anti -toxin Administered Alone or in Combination with Levofloxacin to New Zealand White Rabbits Following *a Bacillus anthracis* Inhalation Challenge

Conducted under (b) (4) Study No. 1045-G607604 for DMID/NIAID

This study randomized animals to four arms: no treatment, ETI-204-alone, levofloxacin alone and a combination of levofloxacin plus ETI-204. All treatment was given at a fixed time point of 72 hours. As the no treatment arm never received treatment, it is an appropriate control for the ETI-204 alone arm. This review will focus only on the comparison of ETI-204 compared to the untreated control arm. Please see the statistical review by Ling Lan for a discussion of the contribution of ETI-204 when given with antibacterials.

Note that this study is a delayed treatment study. Treatment was delayed past the point when an animal would have developed symptoms.

6.3.5.1 Study Design and Endpoints

Primary Objective

The primary objective was to determine the efficacy of treatment with ETI-204, levofloxacin or ETI-204 in combination with levofloxacin to NZW rabbits 72 hours following exposure to *Bacillus anthracis.*

Study Design

This was a randomized open label study with treatments administered as a fixed time, conducted at (b) (4) in 2010. Control group was not treated.

Animals were randomized to one of the following groups:

Group	ETI-204 Dose (mg/animal)	Levofloxacin Dose (mg/kg/day), once daily for 3 days	Number of animals planned	Description
1	0	50	16	Levofloxacin
2	8	50	16	ETI-204 + levofloxacin
3	8	0	16	ETI-204
4	0	0	6	Untreated control

The test product was manufactured at the Baxter facility.

Prior to start of study, rabbits were randomized into three groups of 16 (50% male, 50% female) and one group of 6 rabbits for the control (50% male, 50% female). The rabbits were then randomized to two days of challenge (Challenge Day A and Challenge Day B) such that 50% of

the animals from each group were challenged per day. Finally, the animals were randomized for challenge order for each day of challenge.

Treatment was initiated 72 hours ± 1 hour post-median challenge. Blood samples were collected at 24, 48, 72, 96 hours, Days 7, 14, and 28. Clinical observations were made at least twice daily during study (every 6 hours between 24 and 96 hours post-median challenge). Animals that succumbed to challenge, or were found moribund and euthanized or surviving to Day 20 underwent a complete gross necropsy.

This review will only cover the ETI-201 alone and un-treated control group.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.3.5.2 Statistical Methodologies

Sample Size Calculation

There was 57% power to detect a significant difference in overall survival rates between the antitoxin only group (groups 3) and the control group (group 4) assuming that the probability of survival in group 3 was 50% and the probability of survival in group 3 was 1%. Power calculations were for a one-sided, 0.05 level Fisher's exact test.

Analysis Populations

In the protocol there were two analysis populations mentioned:

- All randomized animals for the primary efficacy analysis
- All animals receiving treatment for a secondary analysis

Statistical Methods

The primary efficacy analysis compared the survival rates in the combination treatment group (group 2) to the antibiotic only treatment (group 1). However, interest in this review is the comparison of the ETI-204 alone group to untreated controls. We will consider a 0.025 one-sided Fisher's exact test to compare the survival rates between these two groups.

Animal Disposition, Demographic and Baseline Characteristics

Baseline variables were comparable between these two groups. The age for all animals was 5 months. Sample sizes were too small to make meaningful comparisons in bacteremia and PA levels.

Table 61. Study NIAID1045: Demographic variables and baseline characteristics by treatment group including all randomized animals

	Control (N=6)	ETI-204 8 mg/kg IV (N=16)	Total (N=22)
Gender [n (%)]			
Female	3 (50.0)	8 (50.0)	11 (50.0)
Male	3 (50.0)	8 (50.0)	11 (50.0)
Challenge dose (LD_{50})			
Mean (SD)	202.3 (30.3)	194.4 (57.9)	196.5 (51.2)
Range	164.0, 247.0	108.0, 289.0	108.0, 289.0
Challenge dose (x 10^7 cfu)			
Mean (SD)	2.13 (0.31)	2.04 (0.61)	2.06 (0.54)
Range	1.73, 2.59	1.14, 3.04	1.14, 3.04
Challenge dose (LD_{50}) (n(%))			
<200	4 (66.7)	8 (50.0)	12 (54.5)
200 or higher	2 (33.3)	8 (50.0)	10 (45.5)
Positive qualitative bacteremia prior to treatment (n(%))	3 (50.0)	3 (18.8)	6 (27.3)
PA-ECL positivity 24 hours post challenge (n(%))	3 (50.0)	4 (25.0)	7 (31.8)
Log_{10} PA-ELISA 24 hours post-challenge (ng/mL)			
Mean (SD)	0 (0)	0.19 (0.54)	0.14 (0.46)
Range		0.0, 1.8	0.0, 1.8
PA-ELISA 24 hours post-challenge (ng/mL)			
Geometric mean	1	1.56	1.38
95% confidence interval		0.8, 3	0.9, 2.2

Time to bacteremia

The following table shows the time to qualitative bacteremia in all randomized subjects that was bacteremic during the study. The time was comparable between the two groups.

Table 62. Study NIAID 1045: Time to qualitative bacteremia

	Control (N=6)	ETI-204 8 mg/kg IV (N=16)	Total (N=22)
Time to qualitative bacteremia (hours)			
N	6	13	19
Mean (SD)	55.36 (54.50)	48.76 (15.87)	50.84 (31.67)
Range	23.1, 164	25.7, 72.9	23.1, 164

6.3.5.3 Results

Survival

Only 69% (11/16) of the animals randomized to ETI-204 alone survived to the protocol specified treatment time of 72 hours post-challenge. In all randomized animals, there was no statistically significant treatment effect. In all animals that were randomized and received treatment, there was a statistically significant difference in survival proportions between the two groups, using a one-sided 0.025 level test. In animals that received treatment and were bacteremic prior to treatment, 5/9 (56%) in the ETI-204 survived through 28 days post-challenge, compared with 0/5 in the control group. In bacteremic animals, there was no statistically significant difference in survival proportions.

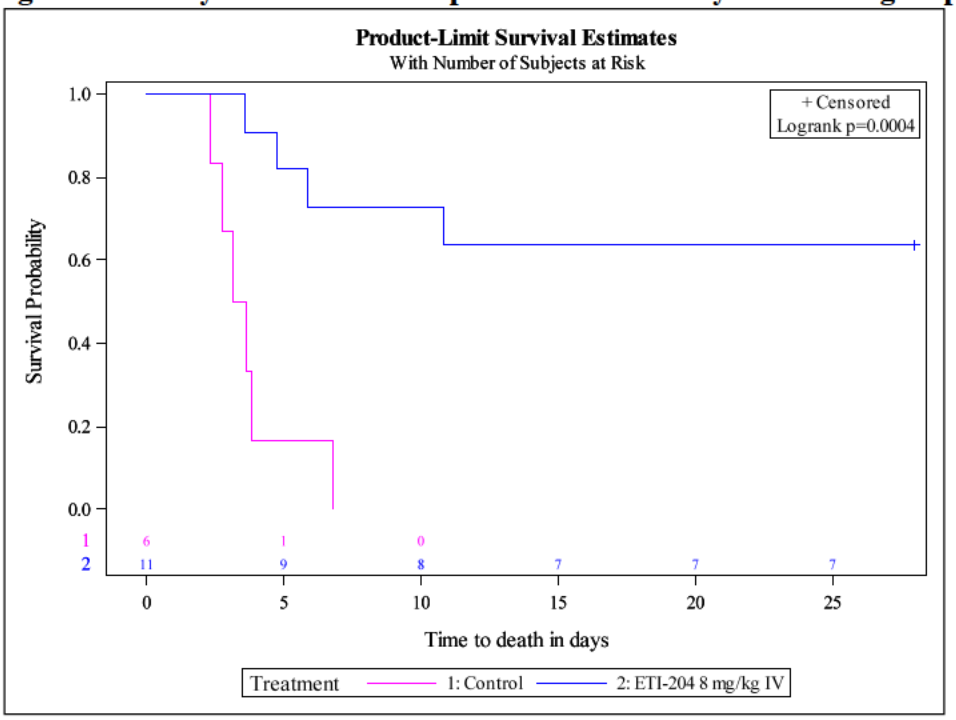
Table 63. Study NIAID 1045: Survival at Day 28 by treatment group

	Control (N=6)	**ETI-204 8 mg/kg IV (N=16)**
All randomized animals		
n (%)	0 (0)	7 (43.8)
Difference in survival proportion compared with control [exact 95% confidence interval] one-sided p-value		0.438 [-0.054, 0.701] 0.0296
Animals that received treatment at 72 hours post-challenge		
n/N (%)	0/5 (0)	7/11 (63.6)
Difference in survival proportion compared with control [exact 95% confidence interval] one-sided p-value		0.636 [0.078, 0.891] 0.0052*
Animals qualitatively bacteremic at or prior to 72 hours post challenge		
n/N (%)	0/3 (0)	5/9 (55.6)
Difference in survival proportion compared with control [exact 95% confidence interval] one-sided p-value		0.556 -0.162, 0.863 0.070

Two-sided 95% confidence interval and one-sided p-values were calculated by the reviewer
*Statistically significant at a two-sided significance level of 0.05

Survival (time-to-death) analysis shows that there was a statistically significant difference between the two groups in animals that received treatment 72 hours post challenge.

Figure 38. Study NIAID 1045: Kaplan-Meier curve by treatment group



PA level over time

As the following graph show, the PA level in the control group increased from below the LLOQ to a high level at terminal time point. The PA level for the treatment group also increased, but peaked at 96 hours post-challenge (about 1 day after starting treatment) for several animals. After Day 7 the levels decreased among these surviving animals.

Subgroup Analyses

The following table shows the results of subgroup analyses. In the ETI-204 group, males or lower challenge dose were associated with a higher survival proportions. The reason was not clear, but it could be due to the lower mean challenge dose. In the ETI-204 group, the mean challenge dose for males was 189 LD_{50}s, lower than 221 LD_{50}s for females.

Figure 39. Study NIAID 1045: PA-ELISA by treatment and animal

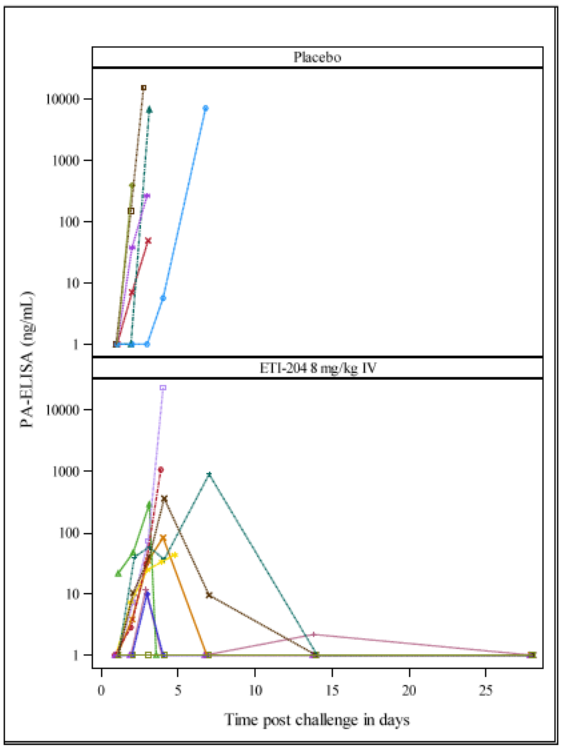


Table 64. Study NIAID1045: Survival at Day 28 by challenge dose, bacteremia, and PA-ELISA

	Control (N=6)	ETI-204 8 mg/kg IV (N=11)	Total (N=17)
Gender			
Female	0/3	2/5 (40%)	2/8 (25%)
Male	0/3	5/6 (83.3%)	5/9 (55.6%)
Challenge dose (LD_{50})			
<250	0/6	6/8 (75%)	6/14 (42.9%)
250 or higher	0	1/3 (33.3%)	1/3 (33.3%)
PA prior to treatment (ng/mL)			
0 - < 10	0/6	7/10 (70%)	7/16 (43.8%)
10-< 50		0/1 (0)	0/1 (0)

Pathological findings in the brain

Among all dead animals from the two groups, only 2 from the ETI-204 8 mg/kg IV group had positive pathological findings in the brain (discoloration(s), etc).

6.3.5.4 Conclusions

This study demonstrated that ETI-204 administered 72 hours post-challenge improved the survival proportion in the animals receiving treatment. There were no statistically significant differences in survival proportions between the ETI-204 and the control group in all randomized animals and in bacteremic animals prior to treatment, because 5 animals in the ETI-204 group died before receiving treatment and the number of bacteremic animals prior to treatment in the two groups were small.

6.4 Monkey Post-Exposure Prophylaxis Studies

6.4.1 Summary of monkey post-exposure prophylaxis studies

There were three monkey post-exposure prophylaxis studies to assess the efficacy of ETI-204 in the post-exposure prophylaxis. All of the studies were conducted by the applicant. AP107 used the Baxter product and AP301 and AP307 used the Lonza product. There were 2 IV treatment groups (2 and 8 mg/kg) and 2 IM groups (4 and 8 mg/kg) in AP107 and all groups in the other 2 studies only contained IM groups. AP107 did not demonstrate any significant treatment effects. The last two studies demonstrated significant treatment effects (≥83% in survival difference) when ETI-204 was administered by 24 hours post challenge with a dose of 8 or 16 mg/kg.

6.4.2 AP107

Post-Exposure Prophylaxis Dose Ranging Study in Cynomolgus Macaques Exposed to Bacillus Anthracis Spores followed by Treatment Intravenously or Intramuscularly with ETI-204

Conducted under (b) (4) 766-G924201 for NIAID

6.4.2.1 Study Design and Endpoints

Primary Objective

The objective was to evaluate the efficacy of ETI-204 in protecting non-human primates from death when given intravenously or intramuscularly 24 hours post-exposure to *B. anthracis* spores.

Study Design

This was a randomized, open-label, placebo-controlled, parallel group, IV and IM ETI-204 dose-ranging study (dosing at 24 hours following *B. anthracis* spore exposure), conducted at (b) (4) in 2008.

Monkeys were planned to be randomized into four treatment groups and one control group:

- Placebo (saline)
- ETI-204 2 mg/kg, IV
- ETI-204 4 mg/kg, IM
- ETI-204 8 mg/kg, IV
- ETI-204 8 mg/kg, IM

In the data set, there was one monkey that was randomized to the 8 mg/kg IV group but only received 6 mg/kg IV and 2 mg/kg subcutaneously. It was included in the 8 mg/kg IV group in the analysis.

The test product was manufactured at the Baxter facility.

All monkeys were challenged with a targeted dose of 200 LD_{50} *B. anthracis* (Ames strain) spores. The test article or control material was administered IV or IM at 24 hours ± 30 minutes post-challenge for each animal relative to the end of their challenge. Clinical observations were made twice daily during normal business hours. Blood samples were collected at 24, 32, 40, and 48 hours and 14 days and terminal time point.

Primary Endpoint

The primary endpoint was survival to 30 days post anthrax spore challenge.

6.4.2.2 Statistical Methodologies

Sample Size Calculation

The protocol states that the sample sizes of 9 animals per treatment group and 6 animals in the control group were sufficient to test treatment efficacy in comparison to untreated controls with 83% power, when the probability of survival in the treated group was 85% and the probability of survival in the control group was 15%. This was based on a one-sided, Fisher's exact test.

Comment: Using a one-sided 0.05 type I error could replicate this calculation. However, using a two-sided type I error of 0.05 only provides a 76.9% statistical power.

Analysis Population

There was no analysis population defined in the protocol, but the analysis included all randomized animals.

Statistical Methods

Fisher's exact tests were used to establish efficacy of individual treatments relative to the control group. A procedure was used to maintain an overall 0.05 significance level using the Bonferroni-Holm adjustment. However, it is not clear if this procedure was pre-specified because it only mentioned in the statistical analysis report, but not in the protocol. In addition, the overall one-sided type I error should be 0.025. Therefore, we will use the Bonferroni method for multiple comparison adjustment.

A time-to-death analysis may also be performed on these data to determine where there were differences in protection for the different groups.

6.4.2.3 Animal Disposition, Demographic and Baseline Characteristics

A total of 41 monkeys were randomized. Table 65 shows the demographic variables and baseline characteristics by treatment group. It is noticed that in the 4 mg/kg IM group the mean challenge dose was higher than in other groups but the proportion of qualitative bacteremia was lower than the average. At 24 hours post challenge the proportions of qualitative positive bacteremia were less than 23% in all groups, while the differences in these proportions among different groups were large, due to the small sample sizes.

Table 65. Study AP107: Demographic variables and baseline characteristics by treatment group

	Placebo (N=6)	ETI-204 2 mg/kg IV 24 hrs PC (N=9)	ETI-204 4 mg/kg IM 24 hrs PC (N=8)	ETI-204 8 mg/kg IM 24 hrs PC (N=9)	ETI-204 8 mg/kg IV 24 hrs PC (N=9)	Total (N=41)
Age (years)						
Range	2-5	2-5	2-5	2-5	2-5	2-5
Gender [n (%)]						
Female	3 (50.0)	5 (55.6)	4 (50.0)	4 (44.4)	5 (55.6)	21 (51.2)
Male	3 (50.0)	4 (44.4)	4 (50.0)	5 (55.6)	4 (44.4)	20 (48.8)
Body weight (kg)						
Mean (SD)	2.4 (0.2)	2.4 (0.2)	2.4 (0.2)	2.5 (0.3)	2.6 (0.4)	2.5 (0.3)
Range	2.2, 2.6	2.2, 2.7	2.1, 2.6	2.1, 3.1	2.1, 3.5	2.1, 3.5
Challenge dose (LD_{50})						
Mean	324.2	315.6	366.0	289.0	288.7 (49.1)	314.9 (78.3)
(SD)	(70.6)	(83.4)	(113.6)	(51.8)		
Range	254.0, 458.0	213.0, 451.0	198.0, 551.0	222.0, 351.0	225.0, 370.0	198.0, 551.0
Challenge dose (LD_{50}) (n(%))						
<200	0	0	1 (12.5)	0	0	1 (2.4)
200 or higher	6 (100)	9 (100)	7 (87.5)	9 (100)	9 (100)	40 (97.6)
Positive qualitative bacteremia 24 hours after challenge (n(%))	1 (16.7)	2 (22.2)	1 (12.5)	2 (22.2)	1 (11.1)	7 (17.1)

Time to quantitative bacteremia

As the following table shows, the time to quantitative bacteremia was comparable across different groups.

Table 66. Study AP107: Time to quantitative bacteremia

	Placebo	ETI-204 2 mg/kg IV 24 hrs PC	ETI-204 4 mg/kg IM 24 hrs PC	ETI-204 8 mg/kg IM 24 hrs PC	ETI-204 8 mg/kg IV 24 hrs PC	Total
Time to quantitative bacteremia (hours)						
N	6	9	7	7	7	36
Mean (SD)	33.3 (7.9)	30.2 (3.5)	33.2 (5.6)	29.7 (3.9)	32.0 (4.6)	31.6 (5.1)
Range	23.9, 48.1	24.0, 32.1	24.1, 40.2	24, 32	24.1, 40.1	23.9, 48.1

6.4.2.4 Results

Survival

There were no differences in survival proportions between any treatment groups and the placebo group if using a Bonferroni adjustment method (a one-sided significance level of 0.025/4=0.0063), as shown in the following table.

Table 67. Study AP107 Survival at Day 28 by treatment group

	Placebo (N=6)	ETI-204 2 mg/kg IV (N=9)	ETI-204 4 mg/kg IM (N=8)	ETI-204 8 mg/kg IM (N=9)	ETI-204 8 mg/kg IV (N=8)
n (%)	1 (16.7)	4 (44.4)	6 (75.0)	5 (55.6)	6 (75.0)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.278 [-0.295, 0.641] 0.210	0.583 [0.018, 0.902] 0.020	0.389 [-0.158, 0.777] 0.087	0.583 [0.018, 0.902] 0.020
Adjusted exact 95% confidence interval		-0.391, 0.765	-0.130, 0.941	-0.292, 0.835	-0.130 0.941

Two-sided 95% confidence interval and one-sided p-values were calculated by the reviewer

Also in the time-to-death survival analysis, there were no statistically significant differences between any treatment group and the placebo group, using a Bonferroni method for multiple comparison adjustment (0.05/4=0.0125), as shown in the following figure and table,

Figure 40. Study AP107: Kaplan-Meier curve by treatment group

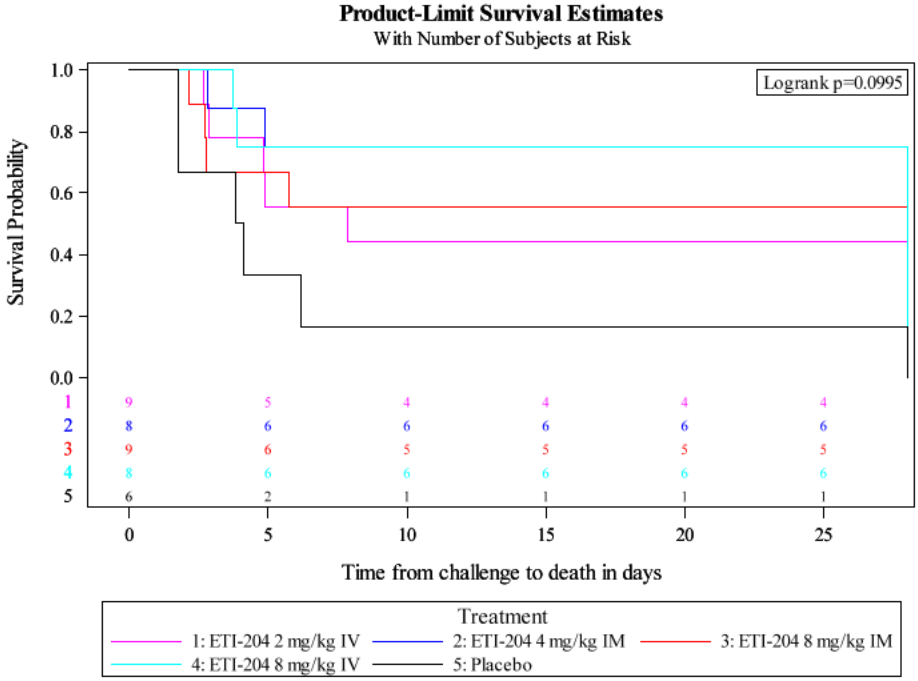


Table 68. Study AP107: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

ETI-204 2 mg/kg IV (N=9)	ETI-204 4 mg/kg IM (N=8)	ETI-204 8 mg/kg IM (N=9)	ETI-204 8 mg/kg IV (N=8)
0.1662	0.0278	0.1695	0.0380

Pathological findings in the brain

No tissue bacterial load data were available in the data sets. Microscopic findings showed that among dead animals, only 2 control animals (40%) had brain bacteria, hemorrhage, and/or meningitis in the brain.

6.4.2.5 Subgroup Analysis Results

The following table shows the results of subgroup analyses. The sample sizes were too small to observe a reliable trend by each grouping variable.

Table 69. Study AP107: Survival status by gender and challenge dose

	Placebo (N= 6)	ETI-204 2 mg/kg IV 24 hrs PC (N= 9)	ETI-204 4 mg/kg IM 24 hrs PC (N= 8)	ETI-204 8 mg/kg IM 24 hrs PC (N= 9)	ETI-204 8 mg/kg IV 24 hrs PC (N= 9)	Total (N= 41)
Gender						
Female	0/3	2/5 (40%)	4/4 (100%)	3/4 (75%)	4/5 (80%)	13/21(61.9%)
Male	1/3 (33.3%)	2/4 (50%)	2/4 (50%)	2/5 (40%)	2/4 (50%)	9/20 (45%)
Challenge dose (LD_{50})						
<250	0	1/2 (50%)	0/1	2/3 (66.7%)	1/3 (33.3%)	4/9 (44.4%)
250 or higher	1/6 (16.7%)	3/7 (42.9%)	6/7 (85.7%)	3/6 (50.0%)	5/6 (83.3%)	18/32 (56.3%)

6.4.2.6 Conclusions

After Bonferroni adjustment for multiple comparisons, no significant treatment effects were observed. The survival proportions in the 4 mg/kg IM and 8 mg/kg IV groups showed promising treatment effects (6/8 or 75%). However, after multiple-comparison adjustment, the effects were no longer statistically significant.

6.4.3 AP301

Study to Evaluate the Pharmacokinetics of ETI -204 Administered via Intramuscular (IM) Route in a Time of Treatment Post-Exposure Prophylaxis Model of Cynomolgus Monkey Anthrax Infection

Conducted under (b) (4) Study Number 2720 -100014200 for NIAID

6.4.3.1 Study Design and Endpoints

Primary Objective

The primary objective was to examine the PK of ETI-204 when administered IM to cynomolgus monkeys at increasing times following exposure to *Bacillus anthracis* spores.

Secondary Objective

The secondary objective was to evaluate the impact of the time of treatment on the PK of ETI-204 administered IM.

Study Design

This was a randomized, blinded, placebo-controlled IM ETI-204 dose-ranging study in monkeys challenged with inhalational anthrax (dosing at 18, 24, and 36 hours following *B. anthracis* spore exposure), conducted at (b) (4) in 2013.

Animals were randomized into the following 7 groups:

- Control/vehicle 18 hrs post challenge
- ETI-204 8 mg/kg 18 hrs post challenge
- ETI-204 8 mg/kg 24 hrs post challenge
- ETI-204 8 mg/kg 36 hrs post challenge
- ETI-204 16 mg/kg 18 hrs post challenge
- ETI-204 16 mg/kg 24 hrs post challenge
- ETI-204 16 mg/kg 36 hrs post challenge

The test product was manufactured at the Lonza facility.

Randomization was performed in three steps: stratified by weight to three weight strata for males and three strata for females, each stratus with 7 animals to be randomized to 7 groups; randomized to three challenge days; assigned to a random challenge order.

Assignment was only known to the statistician preforming the randomizations, product preparation technicians, (b) (4) Quality Assurance Unit, and the study subject matter expert.

Animals were exposed to aerosolized *B. anthracis* (Ames) spores (targeted 200 LD_{50}s).

The last day of observation was Day 28 for the placebo and 8 mg/kg groups, and Day 56 for the 16 mg/kg groups.

Monkeys were observed twice daily (at least 6 hours apart) for clinical signs.

Primary Endpoint

Survival was not the primary endpoint, but we considered survival at Day 28 was an efficacy endpoint.

6.4.3.2 Statistical Methodologies

Sample Size Calculation

In the protocol it was stated that the number of animals (6 in each group) used in this study was expected to be sufficient and to generate the necessary PK results while demonstrating survival trends between treatment and control groups. This was a PK study and no formal sample size calculation conducted for efficacy comparisons.

Analysis Populations

All animals that survived to treatment were included in the study population, regardless of bacteremia status.

Statistical Methods

For treatment group comparison, the survival data from each treatment group was compared to the control group using a one-sided, 0.025 level Fisher's exact test with and without adjustment for multiple comparisons. Although statistical comparisons were made between all group pairs, it should be noted that this study was not powered to determine statistical differences between groups. No specific adjustment methods were mentioned for multiple comparisons. We will use Bonferroni method in the following analyses.

6.4.3.3 Animal Disposition, Demographic and Baseline Characteristics

Demographic variables were comparable across different groups (Table 70). Bacteremia prior to treatment was measured at different time for different treatment groups. It is expected that as time increased from 18 to 36 hours post-challenge, the bacteremia levels and the proportions of quantitative bacteremia increased. The observed bacteremia data were consistent with this expectation.

Table 70. Study AP301: Demographic variables and baseline characteristics by treatment group

	Placebo 18 hrs PC (N=6)	ETI-204 8 mg/kg 18 hrs PC (N=6)	ETI-204 8 mg/kg 24 hrs PC (N=6)	ETI-204 8 mg/kg 36 hrs PC (N=6)	ETI-204 16 mg/kg 18 hrs PC (N=6)	ETI-204 16 mg/kg 24 hrs PC (N=6)	ETI-204 16 mg/kg 36 hrs PC (N=6)	Total (N=42)
Age (years)								
Mean(SD)	2.9 (0.5)	2.8 (0.1)	2.8 (0.2)	2.8 (0.1)	3.0 (0.6)	3.1 (0.7)	2.8 (0.1)	2.9 (0.4)
Range	2.6, 4.0	2.6, 2.9	2.7, 3.1	2.7, 3.0	2.6, 4.2	2.6, 4.6	2.7, 2.9	2.6, 4.6
Gender [n (%)]								
Female	3 (50)	3 (50)	3 (50)	3 (50)	3 (50)	3 (50)	3 (50)	21 (50)
Male	3 (50)	3 (50)	3 (50)	3 (50)	3 (50)	3 (50)	3 (50)	21 (50)
Body weight (kg)								
Mean (SD)	2.77 (0.21)	2.68 (0.18)	2.78 (0.15)	2.75 (0.22)	2.78 (0.26)	2.88 (0.19)	2.78 (0.16)	2.78 (0.19)
Range	2.50, 3.10	2.50, 2.90	2.60, 3.00	2.50, 3.10	2.60, 3.30	2.60, 3.10	2.60, 3.00	2.50, 3.30
Challenge dose (LD_{50})								
Mean	395.67	461.67	385.50	409.50	422.83	305.17	431.83	401.74
(SD)	(166.85)	(151.57)	(133.39)	(131.11)	(157.82)	(130.22)	(215.41)	(152.87)
Range	257, 725	278, 673	250, 602	266, 584	290, 700	152, 501	216, 810	152, 810
Positive quantitative bacteremia prior to treatment (n(%))	0	0	2(33.3)	6 (100)	0	2(33.3)	6 (100)	16 (38.1)
Bacteremia prior to treatment (cfu/mL)								
Geometric mean	2.0	2.0	12.8	60287.8	2.0	13.4	32327.4	59.6
95% confidence interval	NA	NA	0.4, 378.6	19996, 181766	NA	0.4, 442.5	255.4, 4091563	13, 273.3
Mean (SD) of log10 bacteremia	0.30 (0)	0.30 (0)	1.11 (1.40)	4.78 (0.46)	0.30 (0.00)	1.13 (1.45)	4.51 (2.00)	1.77 (2.12)

NA: Not available for only one value.

Time to bacteremia

The following table shows the time to quantitative bacteremia. It appears that in the treatment groups, for the same dose, animals developed quantitative bacteremia earlier as treatment further delayed.

Table 71. Study AP301: Time to quantitative bacteremia

	Placebo (N=6)	ETI-204 8 mg/kg 18 hrs (N=6) PC	ETI-204 8 mg/kg 24 hrs PC (N=6)	ETI-204 8 mg/kg 36 hrs PC (N=6)	ETI-204 16 mg/kg 18 hrs PC (N=6)	ETI-204 16 mg/kg 24 hrs PC (N=6)	ETI-204 16 mg/kg 36 hrs PC (N=6)	Total (N=42)
Time to quantitative bacteremia (hours)								
N	6	5	6	6	3	3	6	35
Mean (SD)	41.7 (1.0)	51.0 (21.6)	40.5 (12.9)	28.9 (8.2)	42.3 (1.3)	31.8 (13.8)	30.2 (7.1)	37.9 (12.9)
Range	40.8, 43.2	40.5, 89.6	23.1, 49.7	17.7, 36.8	41.3, 43.7	23, 47.7	23.4, 36.8	17.7, 89.6

6.4.3.4 Results

Survival

As the following table shows, there were statistically significant differences between the 8 mg/kg and 16 mg/kg groups and the placebo group if treatment was initiated 18 or 24 hours post challenge, using a one-sided significance level of 0.025/6=0.00417 (Bonferroni adjustment for multiple comparisons). There was a trend that longer treatment delay was associated with a lower survival proportion.

Survival (time-to-death) analyses (Figure 41 and Table 73) indicated that there were statistically significant differences between the 8 mg/kg and 16 mg/kg groups and the placebo group if treatment was initiated 18 hours post challenge, using a two-sided significance level of 0.05/6=0.00833 (Bonferroni adjustment for multiple comparisons). There was no treatment effect observed with any treatment started 36 hours post challenge. There was a trend that a longer treatment delay with the same dose was associated with a lower survival proportion.

Table 72. Study AP301: Survival at Day 28 by treatment group

	Placebo (N=6)	ETI-204 8 mg/kg 18 hrs PC (N=6)	ETI-204 8 mg/kg 24 hrs PC (N=6)	ETI-204 8 mg/kg 36 hrs PC (N=6)	ETI-204 16 mg/kg 18 hrs PC (N=6)	ETI-204 16 mg/kg 24 hrs PC (N=6)	ETI-204 16 mg/kg 36 hrs PC (N=6)
n (%)	0	6 (100)	5 (83.3)	0	6 (100)	5 (83.3)	3 (50.0)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		1 (0.47,1) 0.0002*	0.833 (0.230, 0.996) 0.0032*	0 (-0.493, 0.493) 0.5	1 (0.47,1) 0.0002*	0.833 (0.230, 0.996) 0.0032*	0.5 (-0.037, 0.882) 0.034
Adjusted exact 95% confidence interval		0.438, 1	0.196, 0.998	-0.483, 0.483	0.438, 1	0.196, 0.998	-0.069, 0.893

Two-sided 95% confidence interval and one-sided p-values were calculated by the reviewer
*Significant at a one-sided significance level of 0.025/6

Figure 41. Study AP301: Kaplan-Meier curve by treatment group

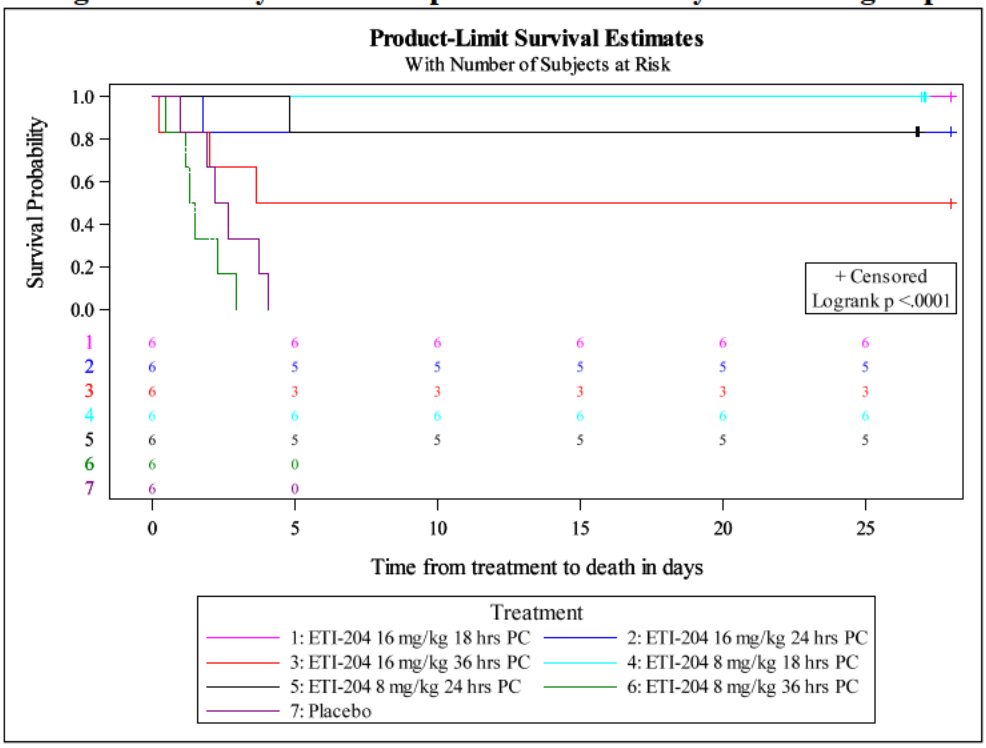


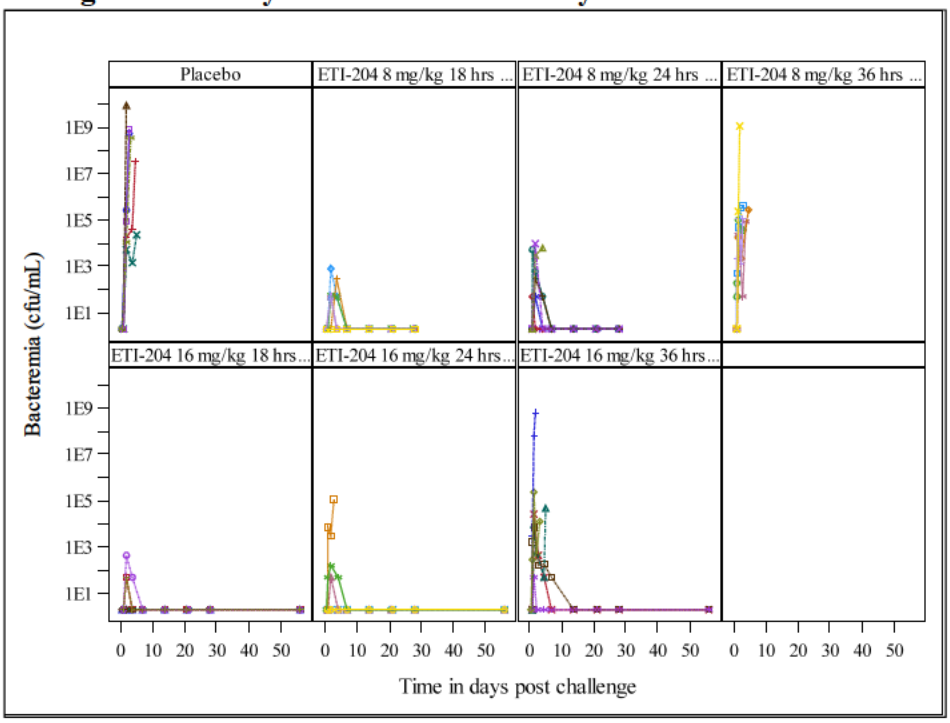
Table 73. Study AP301: Two-sided p-values of pairwise log-rank tests comparing time from treatment to death between a treatment group and the placebo group

ETI-204 8 mg/kg 18 hrs PC	ETI-204 8 mg/kg 24 hrs PC	ETI-204 8 mg/kg 36 hrs PC	ETI-204 16 mg/kg 18 hrs PC	ETI-204 16 mg/kg 24 hrs PC	ETI-204 16 mg/kg 36 hrs PC
0.0005*	0.0005*	0.162	0.005*	0.009	0.151

*Statistically significant at a two-sided significance level of 0.05/6=0.0083

The following figure shows bacteremia levels changes over time. The bacteremia levels prior to treatment reflected the timing of measurement. If treatment was initiated earlier the peaks for bacteremia levels were lower and after Day 14 all surviving animals had a bacteremia level of below the LOD.

Figure 42. Study AP301: Bacteremia by treatment and animal



Tissue bacterial assessments and pathological findings in the brain

Two and three surviving animals (or 33% and 60%) in the 8 mg/kg group 18 hour and 24 hours post-challenge group had a positive bacterial load in bronchial lymph node in all tissues tested (brain, liver and spleen). All dead animals had a positive result in the brain.

Among animals that died, only 2 out of 6 and 1 out of 3 (33.3%) in the 8 mg/kg 36 hour post challenge and 16 mg/kg 36 hours post challenge groups had positive microscopic pathological findings in the brain (discoloration(s)). No survivors had a positive result.

Subgroup Analysis Results

The following table shows the results of subgroup analyses. The sample sizes were too small to see a reliable trend by each grouping variable.

Table 74. Study AP301: Survival at Day 28 by challenge dose, log_{10} bacteremia

	Placebo (N= 6)	**ETI-204 8 mg/kg 18 hrs PC (N= 6)**	**ETI-204 8 mg/kg 24 hrs PC (N= 6)**	**ETI-204 8 mg/kg 36 hrs PC (N= 6)**	**1 ETI-204 6 mg/kg 18 hrs PC (N= 6)**	**ETI-204 16 mg/kg 24 hrs PC (N= 6)**	**ETI-204 16 mg/kg 36 hrs PC (N= 6)**	**Total (N= 42)**
Gender								
Female	0/3	3/3 (100%)	2/3 (66.7%)	0/3	3/3 (100%)	3/3 (100%)	1/3 (33.3%)	12/21 (57.1%)
Male	0/3	3/3 (100%)	3/3 (100%)	0/3	3/3 (100%)	2/3 (66.7%)	2/3 (66.7%)	13/21 (61.9%)
Challenge dose (LD_{50})								
<250	0	0	0	0	0	3/3 (100%)	1/2 (50%)	4/5 (80%)
250 or higher	0/6	6/6 (100%)	5/6 (83.3%)	0/6	6/6 (100%)	2/3 (66.7%)	2/4 (50%)	21/37 (56.8%)
Bacteremia prior to treatment (cfu/mL)								
$<10^2$	0/6	6/6 (100%)	4/5 (80%)	0	6/6 (100%)	5/5 (100%)	1/1 (100%)	22/29 (75.9%)
10^2 - 10^4	0	0	1/1 (100%)	0	0	0/1	1/2 (50%)	2/4 (50%)
10^4 - $<10^6$	0	0	0	0/6	0	0	1/2 (50%)	1/8 (12.5%)
10^6 or higher	0	0	0	0	0	0	0/1	0/1

6.4.3.5 Conclusions

This PK study was not designed to have efficacy as the primary objective. However, it demonstrated that 8 mg/kg or 16 mg/kg IM ETI-204 given either at 18 hours or 24 hours post challenge significantly improved survival in the treated animals.

6.4.4 AP307

Study to Evaluate the Post-Exposure Efficacy of ETI-204 via Intramuscular (IM) Administration in the Cynomolgus Macaque Inhalation Anthrax Model
Conducted under (b) (4) Study Number 2597-100011517

6.4.4.1 Study Design and Endpoints

Primary Objective

The primary objective was to determine the protective efficacy of ETI-204 when administered IM to cynomolgus macaques at increasing times following exposure to *B. anthracis* spores.

Secondary Objective

The secondary objective was to determine pharmacokinetics of ETI-204 via the IM route; to evaluate the impact of the time of ETI-204 administration on PA levels, and to evaluate the numbers of *B. anthracis* in the blood.

Study Design

This was a randomized, open-label, placebo-controlled, IM ETI-204 study (dosing at 24, 36, and 48 hours following *B. anthracis* spore exposure), conducted at (b) (4) in 2012.

A total of 54 animals (27 males and 27 females) were planned and randomized into one group of the following groups.

- Placebo, IM, 24 hrs post mean challenge
- 16 mg ETI-204, IM, 24 hrs post mean challenge
- 16 mg ETI-204, IM, 36 hrs post mean challenge
- 16 mg ETI-204, IM, 48 hrs post mean challenge

Randomization was performed in three steps to have balanced weight and sex distributions in each group. Animals were randomized by weight and sex in 10:14:14:16 to the four groups (first step). Once assigned groups, animals were randomized to one of the four challenge days (second step) and a challenge order with each day (third step). All animals were challenged with a targeted 200 LD_{50} dose of *B. anthracis* spores.

Although this was an open-label study, pathologist was blind to the treatment assignment.

Monkeys were observed twice daily for clinical signs. Blood samples were collected at planned time points.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.4.4.2 Statistical Methodologies

Sample Size Calculation

Assuming that the true probabilities of survival in control and treatment groups were 10% and 65% respectively, there was 80% power to detect a difference in survival rates between each treated group (n=14) and the control group (n=10). Power calculation was for a one-sided, 0.05 level, Fisher's exact test with no adjustment for multiple comparisons across the three tests.

Comment: An overall one-sided type I error of 0.025 should be used.

Analysis Populations

Two analysis populations were defined as follows:
1) Animals that survived to treatment, regardless of the bacteremia status. This was defined in the study protocol.
2) All-inclusive population that included all challenged animals based on assigned group. This only appeared in the study report.

Statistical Methods

The survival data from each treatment group were compared to the control group using a one-sided, 0.025 level Fisher's exact test with and without adjustment from multiple comparisons.

The study report states that for each of these tests, only control animals that survived to the matching time of treatment for the treated group in the comparison were included in the test.

6.4.4.3 Animal Disposition, Demographic and Baseline Characteristics

Two animals (C49209 and C51315; Group 4) did not survive to their group-specified treatment time of 48 hours post mean challenge and were not included in the following table, to be consistent with applicant's pre-treatment summary statistics table. These variables were well balanced across different treatment groups.

Table 75. Study AP307: Demographic variables and baseline characteristics by treatment group

	Placebo (N=10)	ETI-204 16 mg/kg 24 hrs PC IM (N=14)	ETI-204 16 mg/kg 36 hrs PC IM (N=14)	ETI-204 16 mg/kg 48 hrs PC IM (N=14)	Total (N=52)
Age (years)					
Mean(SD)	3.8 (0.4)	3.7 (0.5)	3.8 (0.4)	4.0 (0.0)	3.8 (0.4)
Range	3.0, 4.0	3.0, 4.0	3.0, 4.0	3.0, 4.0	3.0, 4.0

	Placebo (N=10)	ETI-204 16 mg/kg 24 hrs PC IM (N=14)	ETI-204 16 mg/kg 36 hrs PC IM (N=14)	ETI-204 16 mg/kg 48 hrs PC IM (N=14)	Total (N=52)
Gender [n (%)]					
Female	5 (50.0)	7 (50.0)	7 (50.0)	8 (57.1)	28 (51.9)
Male	5 (50.0)	7 (50.0)	7 (50.0)	6 (42.9)	26 (48.1)
Body weight (kg)					
Mean (SD)	3.21 (0.31)	3.16 (0.35)	3.12 (0.24)	3.35 (0.78)	3.21 (0.47)
Range	2.70, 3.80	2.60, 3.90	2.90, 3.60	2.60, 5.60	2.60, 5.60
Challenge dose (LD_{50})					
Mean	200.70	209.00	197.64	211.57	204.50
(SD)	(45.98)	(56.83)	(92.43)	(70.14)	(67.62)
Range	131, 265	112, 310	84, 346	131, 329	84, 346
Positive quantitative bacteremia prior to treatment (n(%))	5 (50.0)	1 (7.1)	12 (85.7)	14 (100.0)	32 (59.3)
Log_{10} bacteremia prior to treatment (cfu/mL)					
Mean (SD)	1.14 (0.93)	0.48 (0.66)	3.73 (2.21)	4.79 (1.75)	2.64 (2.37)
Range	0.30, 2.57	0.30, 2.78	0.30, 6.86	2.26, 7.94	0.30, 7.94
Bacteremia prior to treatment (cfu/mL)					
Geometric mean	13.8	3.0	5380.0	61537.9	438.4
95% confidence interval	3, 63.5	1.2, 7.2	286.8, 100921.9	6036.6, 627322.5	96.2, 1998
Mean (SD) of log_{10} bacteremia	1.14 (0.93)	0.48 (0.66)	3.73 (2.21)	4.79 (1.75)	2.64 (2.37)
PA-ELISA Positivity prior to treatment	0	0	7 (50)	14 (100)	23 (42.6)
PA-ELISA prior to treatment (ng/mL)					
Geometric mean	5.0	5.0	19.8	228.5	20.3
95% confidence interval	NA	NA	7.1, 55.5	72.5, 720.3	11.3, 36.2
Mean (SD) of log_{10} PA	0.70 (0.00)	0.70 (0.00)	1.30 (0.77)	2.36 (0.86)	1.31 (0.91)

Time to bacteremia

The following table shows the time between challenge and bacteremia. The 16 mg/kg administered 24 hours post challenge group had a longest time to bacteremia and only 50% (7/14) had positive quantitative bacteremia.

Table 76. Study AP307: Time between challenge and bacteremia

	Placebo (N=10)	ETI-204 16 mg/kg 24 hrs PC IM (N=14)	ETI-204 16 mg/kg 36 hrs PC IM (N=14)	ETI-204 16 mg/kg 48 hrs PC IM (N=14)	Total (N=52)
Time to quantitative bacteremia (hours)					
N	10	7	12	14	43
Mean (SD)	39.8 (22.7)	50.1 (20.8)	31.2 (5.3)	36.6 (7.5)	38.0 (15.4)
Range	22.2, 95.9	25.2, 93.5	21.9, 36.3	21.9, 49.8	21.9, 95.9

6.4.4.4 Results

Survival

Using a one-sided significance level of 0.025/3=0.0083 for multiple comparisons, only the 16 mg/kg administered 24 hours post challenge was statistically significant from the placebo group in survival proportions, as shown in the following table.

Table 77. Study AP307 Survival at Day 28 by treatment group

	Placebo	ETI-204 16 mg/kg 24 hrs PC IM	ETI-204 16 mg/kg 36 hrs PC IM	ETI-204 16 mg/kg 48 hrs PC IM
All randomized animals receiving treatment				
n/N (%)	1/10 (10.0)	13/14 (92.9)	6/14 (42.9)	4/14 (28.6)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.829 [0.431,0.976] <0.0001*	0.329 [-0.068, 0.643] 0.053	0.175** [-0.234, 0.504] 0.203
Adjusted exact 95% confidence interval		0.347, 0.987	-0.155, 0.699	-0.320, 0.570
All randomized animals				
n/N (%)	Same as above	Same as above	Same as above	4/16 (25%)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		Same as above	Same as above	0.15 [-0.214, 0.454] 0.219
Adjusted exact 95% confidence interval				-0.319, 0.516

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025/3=0.0083
**The corresponding control group size was 9 because 1 animal did not survive to hours 48 and the comparison was based on 1/9 survival in the control group.

Survival (time-to-death) analyses only demonstrated that the 16 mg/kg group administered 24 hours post-challenge was statistically significant from the placebo group (at a significance level of 0.05/3=0.0167 to adjust for multiple comparisons), as shown in the following figure and table.

Figure 43. Study AP307: Kaplan-Meier curves by treatment group

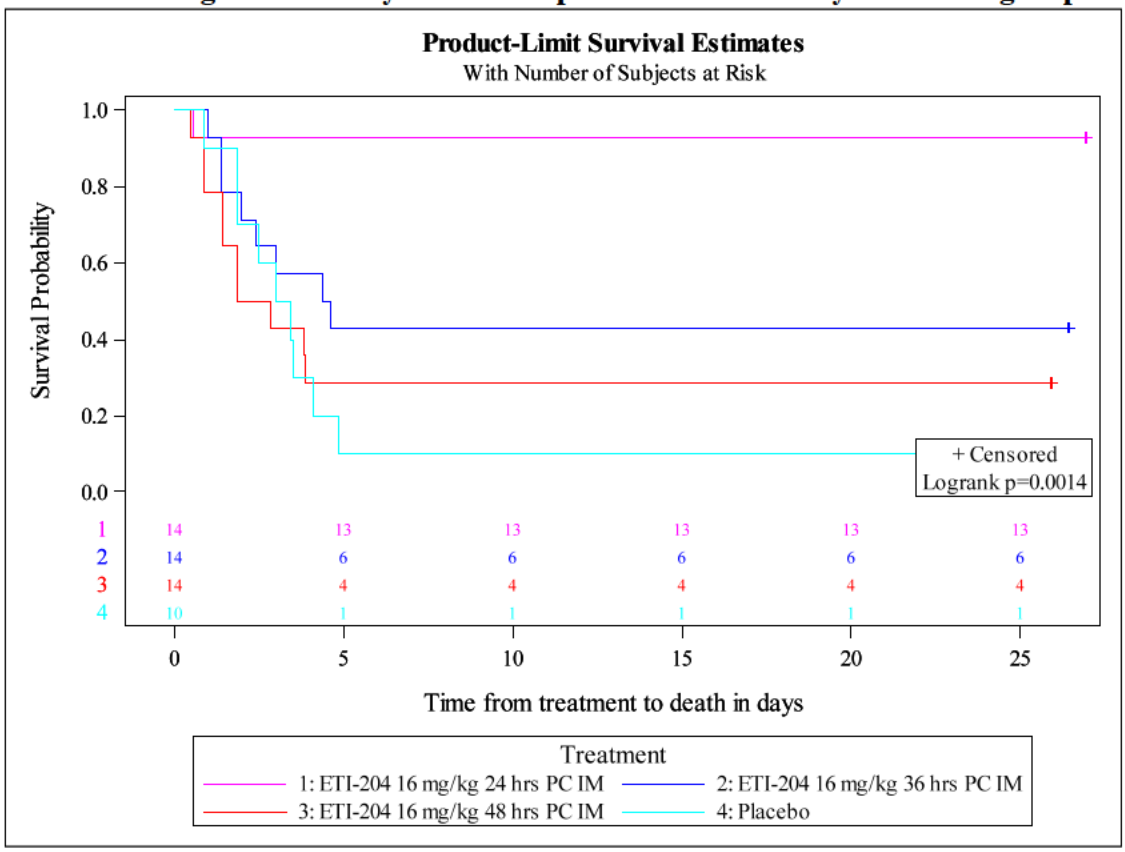


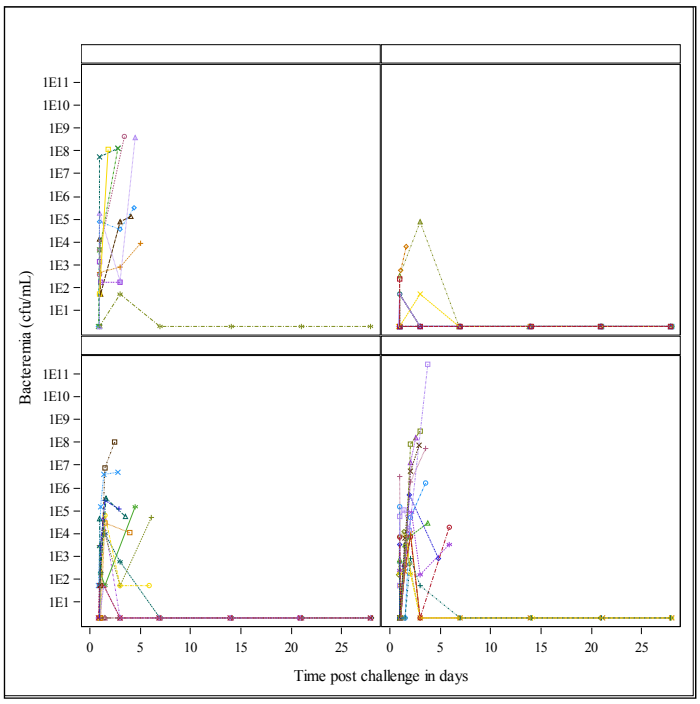
Table 78. Study AP307: Two-sided p-values of pairwise log-rank tests comparing time from treatment to death between a treatment group and the placebo group

ETI-204 16 mg/kg 24 hrs PC IM (N= 14)	ETI-204 16 mg/kg 36 hrs PC IM (N= 14)	ETI-204 16 mg/kg 48 hrs PC IM (N= 14)
<0.0001*	0.149	0.836

*Statistically significant at a two-sided significance level of 0.05/3=0.0167

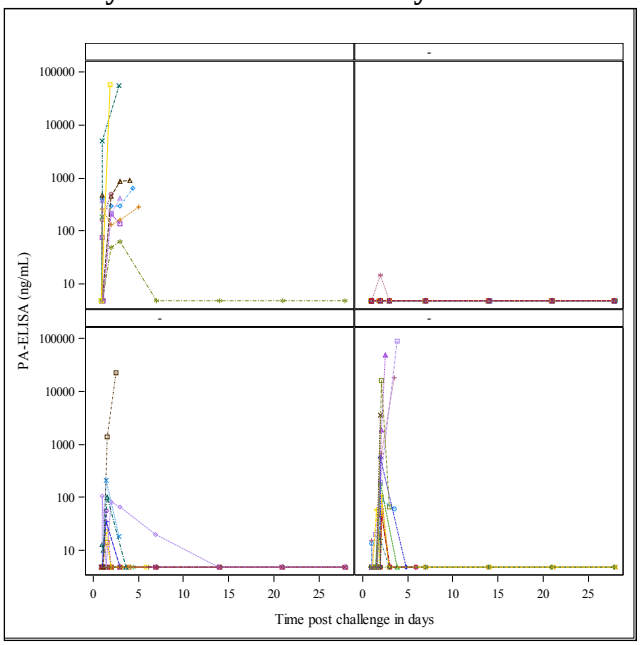
As the following graph shows, the two groups with 36 and 48 hour post-challenge treatment had higher bacteremia levels. From 7 days post-challenge, the bacteremia levels in surviving animals decreased to the level of the LOD.

Figure 44. Study AP307: Bacteremia by treatment and animals



As the following graphs show, prior to treatment the placebo and treatment groups administered 24 hours post challenge did not have any PA level above the LLOQ. At 36 and 48 hours post challenge, the PA levels increased. After administration of ETI-204, the PA levels decreased clearly.

Figure 45. Study AP307: PA-ELISA by treatment and animals



Tissue bacterial assessments and pathological findings in the brain

Tissue bacterial loads are shown in the following table. The values of 0.5 and 1 were considered as positive results. A small proportion of animals had some positive results in some issues in the ETI-204 groups.

Table 79. Study AP307: Bacterial load results by tissue

	Placebo (N=1)	ETI-204 16 mg/kg 24 hrs PC IM (N=13)	ETI-204 16 mg/kg 36 hrs PC IM (N=6)	ETI-204 16 mg/kg 48 hrs PC IM (N=4)
Lymph Node, n(%)				
0	1 (100.0)	8 (61.5)	4 (66.7)	3 (75.0)
0.5	0	4 (30.8)	1 (16.7)	1 (25.0)
1	0	1 (7.7)	1 (16.7)	0
Brain, n(%)				
0	1 (100.0)	10 (76.9)	5 (83.3)	4 (100.0)
0.5	0	3 (23.1)	1 (16.7)	0
Liver, n(%)				
0	1 (100)	13 (100)	5 (83.3)	4 (100)
0.5	0	0	1 (16.7)	0
Spleen, n(%)				
0	1 (100)	12 (92.3)	6 (100)	4 (100)
0.5	0	1 (7.7)	0	0

0.5 and 1 were considered as positive

Among animals that died, 4, 5, and 2 from the placebo group, 16 mg/kg 36 and 48 hours post challenge groups (44.4%, 62.5%, and 20.0%) had microscopic pathological findings (discoloration(s)) in the brain. No survivors had positive results.

Subgroup Analysis Results

The following table shows the results of subgroup analyses. The sample sizes were too small to see a reliable trend by each grouping variable.

Table 80. Study AP307: Survival at Day 28 by gender, challenge dose, log_{10} bacteremia, PA prior to treatment

	Placebo (N= 10)	ETI-204 16 mg/kg 24 hrs PC IM (N= 14)	ETI-204 16 mg/kg 36 hrs PC IM (N= 14)	ETI-204 16 mg/kg 48 hrs PC IM (N= 14)	Total (N= 54)
Gender	0/5	6/7 (85.7%)	2/7 (28.6%)	2/8 (25.0%)	10/28 (35.7%)
Female	1/5 (20%)	7/7 (100%)	4/7 (57.1%)	2/6 (33.3%)	14/26 (53.8%)
Male	0/5	6/7 (85.7%)	2/7 (28.6%)	2/8 (25%)	10/28 (35.7%)
Challenge dose (LD_{50})					
<250	0/8	10/11 (90.9%)	2/9 (22.2%)	3/10 (30%)	15/40 (37.5%)
250 or higher	1/2 (50%)	3/3 (100%)	4/5 (80%)	1/4 (25%)	9/14 (64.3%)

	Placebo (N= 10)	ETI-204 16 mg/kg 24 hrs PC IM (N= 14)	ETI-204 16 mg/kg 36 hrs PC IM (N= 14)	ETI-204 16 mg/kg 48 hrs PC IM (N= 14)	Total (N= 54)
Bacteremia prior to treatment (cfu/mL)					
$<10^2$	1/8 (12.5%)	13/13 (100%)	4/5 (80%)	0	18/26 (69.2%)
10^2 - 10^4	0/2	0/1	1/1 (100%)	3/5 (60%)	4/9 (44.4%)
10^4 - $<10^6$	0	0	1/6 (16.7%)	1/5 (20%)	2/11 (18.2%)
10^6 or higher	0	0	0/2	0/4	0/6
PA prior to treatment (ng/mL)					
0 - < 10	1/10 (10%)	13/14 (92.9%)	5/7 (71.4%)	0	19/31 (61.3%)
10 - < 50	0	0	0/3	2/3 (66.7%)	2/6 (33.3%)
50 or higher	0	0	1/4 (25.0%)	2/11 (18.2%)	3/15 (20.0%)

6.4.4.5 Conclusions

This study only supports the dose of 16 mg/kg IM administered 24 hours post-challenge. The same dose administered 36 or 48 hours post-expose failed to demonstrate any statistically significant treatment effects.

6.5 Rabbit Post-Exposure Prophylaxis Studies

6.5.1 Summary of rabbit post-exposure prophylaxis studies

Seven studies in rabbits (AR004, AR007, AR012, AR034 (phase 1), AR035, AR037, and AR0315) were also conducted to assess the efficacy of ETI-204 in the post-exposure prophylaxis. AR034 was included in this section because ETI-204 was administered 30 hours post-challenge in Phase I and was considered as a post-exposure prophylaxis study by the reviewer. The products Baxter, Elusys, (b) (4), and Lonza were used in 2, 2, 1, and 3 studies, respectively. Each study included IM, IV groups, or both. The study results varied across different studies.

6.5.2 AR004

Time Response Therapeutic Efficacy on the (b) (4) Monoclonal Anti-PA Antibody against Aerosolized Anthrax when Administered post-challenge in the Rabbit Model Against Experimental Anthrax in the Rabbit Model
Conducted under (b) (4) Study Number 380-G004907

6.5.2.1 Study Design and Endpoints

Primary Objective
The primary objective was to examine the efficacy of the (b) (4) anti-PA monoclonal antibody (ETI-204) in delaying or preventing death in rabbits from anthrax when administered as a therapeutic treatment at various time points following an inhalational exposure to *Bacillus anthracis.*

Study Design
This was a randomized, placebo-controlled, parallel group study with treatment administered at a fixed dose and at varying times post-challenge. It was conducted at (b) (4) in 2004.

- ETI-204 10 mg/animal IV, 24 hrs post challenge
- ETI-204 10 mg/animal IV, 36 hrs post challenge
- ETI-204 10 mg/animal IV, 48 hrs post challenge
- Placebo PBS IV, 48 hrs post challenge

Note that some animals received treatment after the point at which symptoms would have developed this study falls between a prophylaxis study and a treatment study. However, since treatment was not started based signs or symptoms, we have included it as a post-exposure prophylaxis trial.

This fixed dose of 10 mg/animal corresponds to approximately 4 mg/kg.

The product was manufactured at the Elusys facility.

Animals were randomized by sex and weight to a treatment group and then randomized into two challenge days and then a challenge order in a challenge day. Animals were challenged with a targeted dose of approximately 200 *B. anthracis* LD_{50}s (Ames).

Clinical observations were preformed twice daily during the study.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.5.2.2 Statistical Methodologies

Sample Size Calculation

Sample sizes of 10 control and 10 treated animals per group were considered in the protocol sufficient to provide greater than 80% power to detect a difference when the survival probabilities were 10% in the control group and 70% in the treated group, using a one-sided Fisher's exact test.

Comment: Type I error was not specified. Using a one-sided and two-sided level of 0.05, the statistical power would have been 82.4%, and 66.7%.

Analysis Population

The analysis population was not defined in the protocol. In the statistical analysis report the analysis population included randomized animals that survived to treatment. Three animals in Group 3 (ETI-204 48 hours post challenge) and one animal in Group 4 (placebo) died prior to the treatment time point. These animals were not included in the statistical analysis.

Note that because animals were treated at different time, all animals that died prior to treatment could bias the results against the regimens that were treated earlier. This should be kept in mind while considering the results of the study.

Statistical Methods

One-sided Fisher's exact tests, at a 0.05 level, were used to compare the survival rates between each individual antibody group and the control group by the applicant. The analysis in this review will consider a one-sided 0.025 level.

6.5.2.3 Animal Disposition, Demographic and Baseline Characteristics

One animal from the placebo group and 3 animals from the group starting treatment at 48 hour post-challenge (PC) died prior to the post-challenge treatment time and are not included in the analysis. Note that this will potentially bias the results in favor of the 48 hour treatment group

because 3 animals that were most likely the weakest animal were removed from the analysis population. Despite this possible bias, the results in the 48 hour group were quite poor. Demographic variables and baseline characteristics are shown in the following table. These variables were well balanced. Notice that 58% of animals received a challenge dose less than 200 LD_{50}s and the 24- and 48-hour groups had a higher proportion (~70%) of less than 200 LD_{50}s. The higher mortality rate in the 48-hour group suggested that the lower challenge dose should not be a problem for evaluating the efficacy in the 24-hour group. No animals were qualitatively bacteremic at 24 hours post-challenge.

Table 81. Study AR004: Demographic variables and baseline characteristics by treatment group

	Placebo (N=9)	**ETI-204 10 mg IV 24 hrs PC (N=10)**	**ETI-204 10 mg IV 36 hrs PC (N=10)**	**ETI-204 10 mg IV 48 hrs PC (N=7)**	**Total (N=36)**
Age (weeks) Range	13-17	13-17	13-17	13-17	13-17
Gender [n (%)] Female Male	4 (44.4) 5 (55.6)	5 (50.0) 5 (50.0)	5 (50.0) 5 (50.0)	3 (42.9) 4 (57.1)	17 (47.2) 19 (52.8)
Challenge dose (LD_{50}) Mean (SD) Range	193.1 (80.4) 86, 352.8	177.3 (62.4) 103.2, 266.9	195. 0 (58.679) 90.700, 262.600	159.914 (50.152) 62.200, 214.000	182.786 (63.058) 62.200, 352.800

6.5.2.4 Results

Survival

The applicant derived p-values for the three comparisons using a one-sided Fisher's exact test were 0.0006, 0.0217, and 0.0625 and concluded that the 24- and 36-hour antibody treatment groups demonstrated a significant increase in survival proportions. Our analysis showed that only the 24-hour treatment group was statistically significantly different from the placebo group, using a one-sided significance level of 0.025/3=0.0083 to adjust for multiple comparisons.

Figure 46 shows that overall there was a statistically significant difference in survival. The p-values from the pairwise log-rank tests in the following table also demonstrated that only the 24-hour treatment group had the statistically significant treatment effect, using a two-sided significance level of 0.05/3=0.0167 (Bonferroni adjustment).

Table 82. Study AR004: Survival at Day 28 by treatment group

	Placebo (N=9)	ETI-204 10 mg IV 24 hrs PC (N=10)	ETI-204 10 mg IV 36 hrs PC (N=10)	ETI-204 10 mg IV 48 hrs PC (N=7)
n (%)	0	8 (80.0)	5 (50.0)	3 (42.9)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.8 [0.402, 0.975] 0.0001*	0.5 [0.084, 0.813] 0.010	0.429 [0.012, 0.816] 0.0226
Adjusted 95% confidence interval		0.303, 0.986	-0.017, 0.856	-0.084, 0.865

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025/3=0.0083

Figure 46. Study AR004: Kaplan-Meier curve and 95% confidence band by treatment group

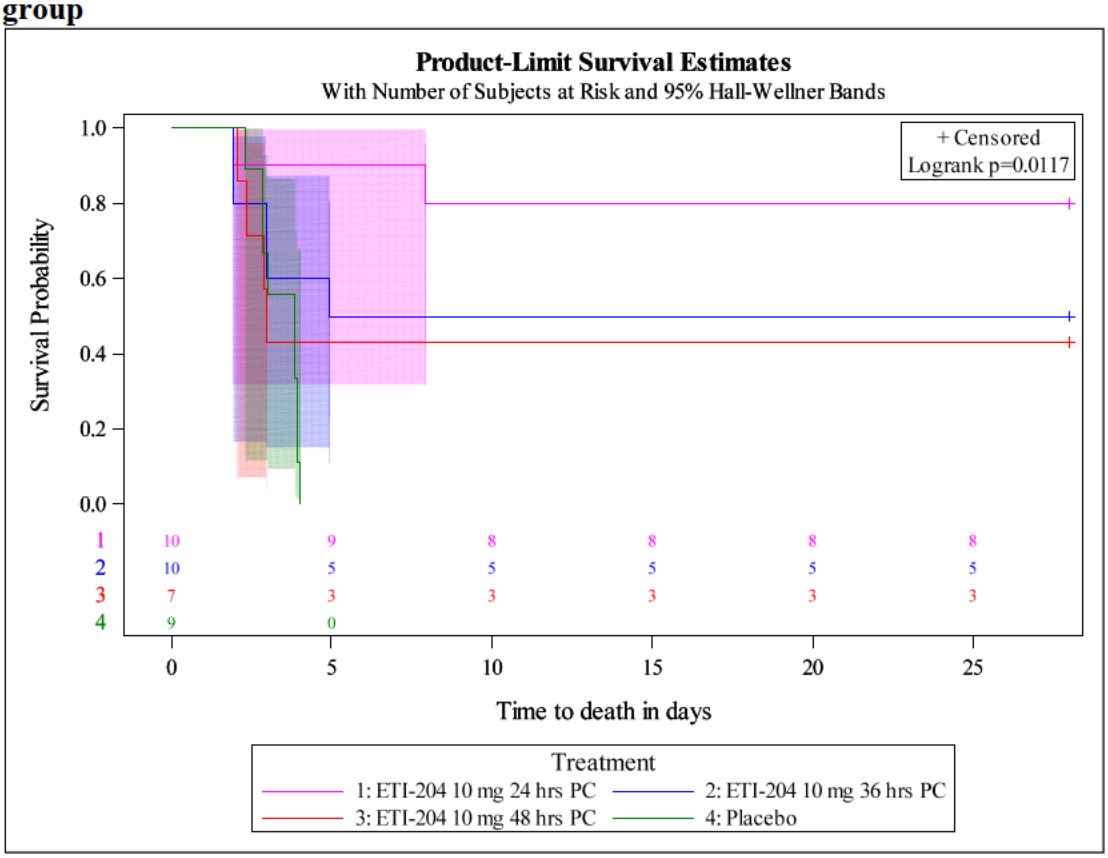


Table 83. Study AR004: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

ETI-204 10 mg24 hrs PC (N=10)	ETI-204 10 mg 36 hrs PC (N=10)	ETI-204 10 mg 48 hrs PC (N=7)
0.0001*	0.040	0.277

*Statistically significant at a one-sided significance level of 0.05/3=0.0167

Tissue bacterial assessments and pathological findings in the brain

No positive tissue bacterial loads were found in the tissues tested (lymph node, lung, and spleen) in surviving animals.

No pathological findings in the brain were reported.

Subgroup Analysis Results

Subgroup analysis results are shown in the following table. The sample sizes in some cells were too small to make meaningful conclusions.

Table 84. Study AR004: Survival at Day 28 by gender and challenge dose

	Placebo (N= 9)	ETI-204 10 mg IV 24 hrs PC (N= 10)	ETI-204 10 mg IV 36 hrs PC (N= 10)	ETI-204 10 mg IV 48 hrs PC (N= 7)	Total (N= 36)
Gender					
Female	0/4	4/5 (80%)	1/5 (20%)	1/3 (33.3%)	6/17 (35.3%)
Male	0/5	4/5 (80%)	4/5 (80%)	2/4 (50%)	10/19 (52.6%)
Challenge dose (LD_{50})					
<250	0/8	6/8 (75%)	4/8 (50%)	3/7 (42.9%)	13/31(41.9%)
250 or higher	0/1	2/2 (100%)	1/2 (50%)	0	3/5 (60.0%)

6.5.2.5 Conclusions

The 10 mg IV (approximately 4 mg/kg) administered 24 hours post challenge showed significant treatment effect compared with the placebo group (80% versus 0%), after using Bonferroni adjustment for multiple comparisons. Further delay of treatment to 36 or 48 hours post challenge, the survival proportion reduced to 50% and 43%. These treatment effects were not statistically significant after multiple comparison adjustment.

6.5.2 AR007

Test of ETI-204 in Rabbit Spore Challenge Model Post-Exposure with/without Levofloxacin

Conducted under (b) (4) Study No. 538-G005372

6.5.3.1 Study Design and Endpoints

Primary Objective

The primary objective was to demonstrate that post-exposure administration of ETI-204 leads to increased survival above that of Levaquin (levofloxacin) after an aerosolized *B. anthracis* (Ames strain) spore challenge.

Secondary Objective

The secondary objective was to collect serum and plasma for shipment to the applicant for sample analysis, bacteremia determinations, necropsies of moribund, euthanized and found dead rabbits, and clinical observations.

Study Design

This was a randomized, controlled, open-label, parallel group, factorial design study; dose received at 9 hours post anthrax exposure. This study was conducted at (b) (4) in 2005.

Animals were randomized to 6 different treatment groups: control, levofloxacin alone, ETI-204 10 mg/animal IV, ETI-204 20 mg/animal IM, and two arms of ETI-204 IV and IM in combination with levofloxacin. All groups contained 9 animals per group except for the levofloxacin alone arm which contained 12 animals. The statistical review by Dr. Ling Lan will address the effect of ETI-204 in combination with levofloxacin. This review will focus only on the comparisons of the ETI-204-alone arms to control. Note that 10 mg/animal is approximately 4 mg/kg and 20 mg/animal is approximately 8 mg/kg.

Animals were challenged with a targeted dose of approximately 200 LD_{50} (Ames) spores on Study Day 0. ETI-204 and its control (PBS) were administered approximately 9 hours (±3 hours) after anthrax challenge.

Animals were observed twice daily during the study.

Primary Endpoint

The primary endpoint was survival to 34 days post anthrax spore challenge.

6.5.3.2 Statistical Methodologies

Sample Size Calculation

It was considered that sample sizes of 9 control and 9 treated animals were sufficient to provide 80% power to detect a difference when the survival probabilities were 10% in the control group and 80% in the treated groups, using a one-sided Fisher's exact test.

Comment: If a one-sided type I error was 0.025 and the two survival probabilities were 0.1 and 0.8, the statistical power would be 83%.

Analysis Populations
No analysis population was defined in the protocol. In the analysis all randomized animals were included. No animals died prior to treatment.

Statistical Methods
One-sided Fisher's exact tests at the 0.05 significance level for each test were utilized by the sponsor to compare the survival rates between each individual antibody group and the control group, as well as each individual antibody group and the levofloxacin-only group.

6.5.3.3 Animal Disposition, Demographic and Baseline Characteristics

Demographic variables and baseline characteristics were well balanced in this study, as the following table shows.

Table 85. Study AR007: Demographic variables and baseline characteristics by treatment group

	Placebo (N=9)	**ETI-204 10 mg IV 9 hrs PC (N=9)**	**ETI-204 20 mg IM 9 hrs PC (N=9)**	**Total (N=27)**
Age (months)				
Mean (SD)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)
Range	4.0, 4.0	4.0, 4.0	4.0, 4.0	4.0, 4.0
Gender [n (%)]				
Female	5 (55.6)	5 (55.6)	4 (44.4)	14 (51.9)
Male	4 (44.4)	4 (44.4)	5 (55.6)	13 (48.1)
Body weight (kg)				
Mean (SD)	2.5 (0.1)	2.5 (0.1)	2.5 (0.1)	2.5 (0.1)
Range	2.2, 2.6	2.3, 2.7	2.3, 2.7	2.2, 2.7
Challenge dose (LD_{50})				
Mean (SD)	268.6 (47.5)	287.8 (69.5)	270.4 (38.4)	275.6 (52.1)
Range	153.0, 304.0	158.0, 400.0	201.0, 317.0	153.0, 400.0

6.5.3.4 Results

All control animals died at an average of 3.64 (SD 0.96) days, with a range of 2.35 to 4.93. All 18 treated animals survived to Day 28. In the reviewer's analysis, we focused on two comparisons (each of the two IM or IV ETI-204 IV groups versus placebo). After Bonferroni adjustment of the 2 comparisons, the difference in survival proportions was still statistically significant, as shown by the adjusted 95% confidence intervals.

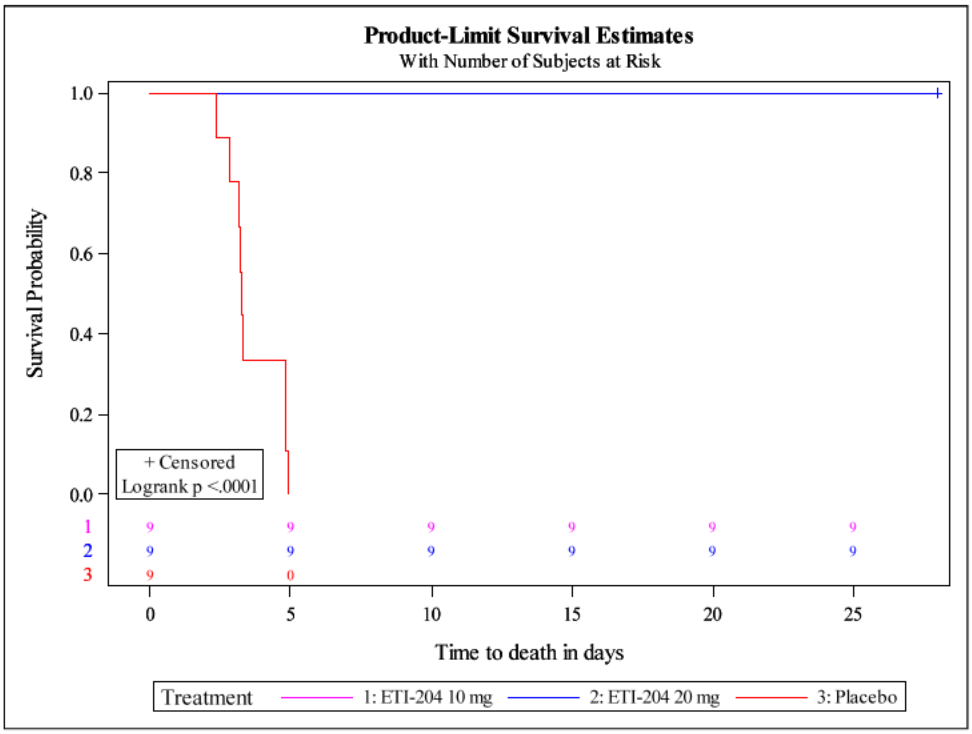
Table 86. Study AR007: Survival at Day 28 by treatment group

	Placebo (N=9)	ETI-204 10 mg 9 hrs PC IV (N=9)	ETI-204 20 mg 9 hrs PC IM (N=9)	Total (N=27)
n (%)	0	9 (100.0)	9 (100.0)	18 (66.7)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		1 [0.629, 1] <0.0001*	1 [0.629, 1] <0.0001*	
Adjusted 95% confidence interval		0.568, 1*	0.568, 1*	

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025/2=0.0125

As the following graph show, time to death analysis also demonstrated the significant treatment effect between any of the ETI-204 group and the placebo group.

Figure 47. Study AR007: Kaplan-Meier curve and 95% confidence band by treatment group



Tissue bacterial assessment and pathological findings in the brain

There was only spleen tested for bacteria. No positive bacterial findings in survivors were seen. Among non-survivors, only 7 animals from the control group had positive bacterial results in the spleen.

No animals had a pathological finding in the brain.

Subgroup Analysis Results

Because the survival proportions in the two treated groups were 100%, it is not possible to examine the effect of treatment in each subgroup.

Table 87. Study AR007: Survival at Day 28 by gender and challenge dose

	Placebo (N= 9)	**ETI-204 10 mg IV 9 hrs PC (N= 9)**	**ETI-204 20 mg IM 9 hrs PC (N= 9)**	**Total (N= 27)**
Gender				
Female	0/5	5/5 (100%)	4/4 (100%)	9/14 (64.3%)
Male	0/4	4/4 (100%)	5/5 (100%)	9/13 (69.2%)
Challenge dose (LD_{50})				
<250	0/2(0)	1/1 (100%)	2/2 (100%)	3/5 (60.0%)
250 or higher	0/7 (0)	8/8 (100%)	7/7 (100%)	15/22 (68.2%)

6.5.3.5 Conclusions

This study demonstrated that 10 mg IV (approximately 4 mg/kg IV) or 20 mg IM (approximately 8 mg/kg IM) administered 9 hours post challenge improved survival significantly. The survival proportion was 100% (9/9) in the two treated groups versus 0 (0/9) on placebo.

6.5.4 AR012

Rabbit Spore Challenge ETI-204 Post-exposure IV and IM Dose-Ranging Study
Conducted under (b) (4) Study 704-G005796

6.5.4.1 Study Design and Endpoints

Primary Objective

The primary objective was to determine the maximally-effective dose, optimally-effective dose, and lowest effective dose of ETI-204 when given by the IV and IM routes 24 hours post-exposure to *B. anthracis* spores.

Study Design

This was a randomized, placebo-controlled, parallel group, dose ranging study with treatment administered at fixed time, conducted at (b) (4) in 2007.

Animals were randomized to one of the following groups:

- Placebo
- ETI-204 2.5 mg/animal IV
- ETI-204 5 mg/animal IM
- ETI-204 10 mg/animal IV
- ETI-204 10 mg/animal IM
- ETI-204 20 mg/animal IV
- ETI-204 20 mg/animal IM
- ETI-204 40 mg/animal IM

All animals were challenged with a targeted 200 LD_{50} dose on Study Day 0. Treatment was administered 24 hour post challenge.

We considered this study as an open-label study because no blinding information was found. Animals were observed hourly for clinical signs of illness and survivability due to anthrax infection (e.g, moribund, respiratory distress, appetite, activity, and seizures) beginning approximately 18 hours after challenge time and until approximately 30 hours after challenge. Animals were observed for clinical signs twice daily through the end of the study.

Primary Endpoint

The primary endpoint was survival to 14 days post anthrax spore challenge.

6.5.4.2 Statistical Methodologies

Sample Size Calculation

It was stated in the protocol that sample sizes of 9 control and 9 treated animals were sufficient to provide greater than 82.4% power to detect a difference when the survival probabilities were 10% in the control group and 75% in the treated groups using a one-sided Fisher's exact test. With 12 treated animals there was 82.2% power for the sample comparison when the probability of survival was 70% in the treated group.

Comment: Apparently a one-sided type I error of 0.05 was used in the power calculations, which is higher than the one required.

Analysis Population

All randomized animals were the analysis population. The population was not defined in the protocol but this was gathered from the statistical methods section in the protocol and the study report.

Statistical Methods

One-sided Fisher's exact tests were utilized to compare the survival rates between the treated groups and the control group.

6.5.4.3 Animal Disposition, Demographic and Baseline Characteristics

Demographic variables were comparable. Age was reported as 3.8 months for all animals. Challenge dose was lower in the 20 mg IM group. The proportion of qualitative bacteremia at 24 hours post-challenge varied across different groups and there was no clear relationship with challenge dose, due to the small sample sizes (Table 88).

Time to bacteremia

Qualitative bacteremia data were available at 24, 27 hours and Day 14 after challenge in ADSL. Therefore, no time to bacteremia was included in this review, because an accurate time to bacteremia could not be determined, given these infrequent measurements.

6.5.4.4 Results

Survival

The applicant concluded that the 3 treatment groups (10 mg IV, 20 mg IM and IV) had significantly higher survival rates than the placebo group. Using a one-sided significance level of 0.025/7=0.0036 (Bonferroni adjustment for multiple comparisons), we concluded only the 20 mg IV group had a significantly higher survival rate than the placebo group. The adjusted exact 95%

confidence interval showed non-significant difference between the two groups, but the lower limit was very close to 0 (Table 89).

Table 88. Study AR012: Demographic variables and baseline characteristics by treatment group

	Placebo (N=9)	**ETI-204 2.5 mg IV (N=9)**	**ETI-204 5 mg IM (N=9)**	**ETI-204 10 mg IV (N=12)**	**ETI-204 10 mg IM (N=9)**	**ETI-204 20 mg IV** (N=12)	**ETI-204 20 mg IM (N=12)**	**ETI-204 40 mg IM (N=12)**	**Total (N=84)**
Gender [n (%)]									
Female	4 (44)	5 (55)	5 (56)	6 (50)	4 (44)	6 (50)	6 (50)	6 (50)	42 (50)
Male	5 (56)	4 (44)	4 (44)	6 (50)	5 (56)	6 (50)	6 (50)	6 (50)	42 (50)
Body weight (kg)									
Mean (SD)	2.63 (0.11)	2.62 (0.13)	2.61 (0.11)	2.56 (0.15)	2.61 (0.11)	2.59 (0.12)	2.59 (0.14)	2.62 (0.12)	2.60 (0.12)
Range	2.49, 2.80	2.46, 2.79	2.49, 2.75	2.26, 2.81	2.43, 2.74	2.40, 2.81	2.36, 2.76	2.38, 2.84	2.26, 2.84
Challenge dose (LD_{50})									
Mean (SD)	205.7 (47.4)	193.2 (34.3)	187.2 (32.3)	189.8 (27.3)	230.7 (87.5)	218.5 (117.2)	180.7 (46.4)	201.9 (62.6)	200.5 (64.3)
Range	111, 258	126, 239	149, 248	151, 243	167, 432	136, 567	111.0, 269.0	131.0, 357.0	111.0, 567.0
Challenge dose (LD_{50}) (n(%))									
<200	3 (33.3)	4 (44.4)	5 (55.6)	9 (75.0)	5 (55.6)	8 (66.7)	10 (83.3)	7 (58.3)	51 (60.7)
200 or higher	6 (66.7)	5 (55.6)	4 (44.4)	3 (25.0)	4 (44.4)	4 (33.3)	2 (16.7)	5 (41.7)	33 (39.3)
Positive qualitative bacteremia prior to treatment (n(%))	4 (44.4)	7 (77.8)	6 (66.7)	6 (50.0)	2 (22.2)	4 (33.3)	5 (41.7)	8 (66.7)	42 (50.0)

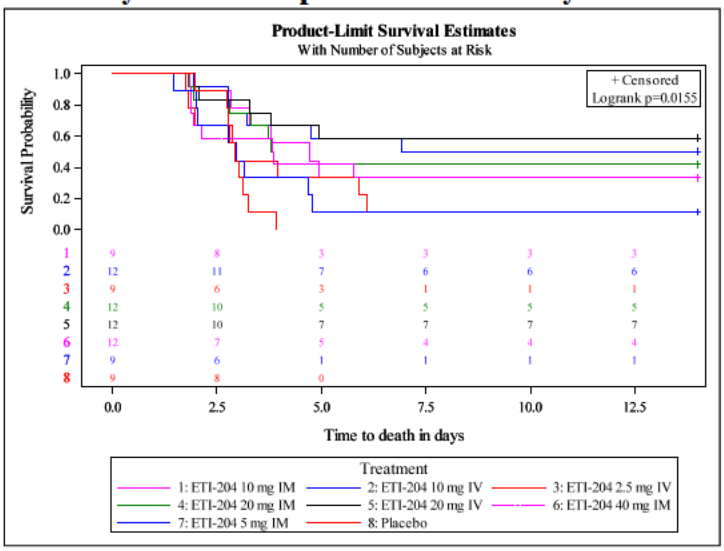
Table 89. Study AR012: Survival at Day 28 by treatment group

	Placebo (N=9)	ETI-204 2.5 mg IV (N=9)	ETI-204 5 mg IM (N=9)	ETI-204 10 mg IV (N=12)	ETI-204 10 mg IM (N=9)	ETI-204 20 mg IV (N=12)	ETI-204 20 mg IM (N=12)	ETI-204 40 mg IM (N=12)
All animals								
n(%)	0	1 (11.1)	1 (11.1)	6 (50)	3 (33.3)	7 (58.3)	5 (41.7)	4 (33.3)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.111 [-0.224, 0.483] 0.4073	0.111 [-0.224, 0.483] 0.4073	0.5 [0.094, 0.789] 0.0074	0.333 [-0.071, 0.701] 0.049	0.583 [0.187, 0.848] 0.0026*	0.417 [0.034, 0.725] 0.0186	0.333 [-0.066, 0.655] 0.051
Adjusted exact 95% confidence interval		-0.436, 0.610	-0.436, 0.610	-0.057, 0.859	-0.238, 0.794	-0.018, 0.904	-0.134, 0.806	-0.217, 0.749
Only qualitatively bacteremic animals								
N (%)	0/4	1/7 (14.3)	0/6	2/6 (33.3)	0/2	0/4	1/5 (20)	1/8 (12.5)

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025/7=0.0036
For bacteremic population, confidence intervals were not reported, because no significant differences were observed.

The following Kaplan-Meier curves show an overall treatment effect compared with the placebo group.

Figure 48. Study AR012: Kaplan-Meier curve by treatment group



Using a two-sided significance level of 0.05/7=0.00714, pairwise log-rank tests in Table 90 demonstrated that the groups of 10 mg IV, 10 mg IM and 20 mg IV had significant treatment effect on survival time.

Table 90. Study AR012: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

ETI-204 2.5 mg/kg IV (N=9)	ETI-204 5 mg IM (N=9)	ETI-204 10 mg IV (N=12)	ETI-204 10 mg IM (N=9)	ETI-204 20 mg IV (N=12)	ETI-204 20 mg IM (N=12)	ETI-204 40 mg IM (N=12)
0.190	0.333	0.0049	0.0068	0.0009	0.0143	0.1211

Pathological findings in the brain

No tissue bacterial load data were available. Among dead animals, only 1 animal from each of the 20 mg IM and the 40 mg IM groups (14.3%, and 12.5%) had positive pathological findings in the brain. No survivors had positive pathological results in the brain.

Subgroup Analysis Results

The following table shows the results of subgroup analyses. The sample sizes were too small to see a reliable trend by each grouping variable.

Table 91. Study AR012: Survival at Day 28 by gender and challenge dose

	Placebo (N= 9)	ETI-204 2.5 mg IV (N= 9)	ETI-204 5 mg IM (N= 9)	ETI-204 10 mg IV (N= 12)	ETI-204 10 mg IM (N= 9)	ETI-204 20 mg IV (N= 12)	ETI-204 20 mg IM (N= 12)	ETI-204 40 mg IM (N= 12)	Total (N= 84)
Gender									
Female	0/4	1/5 (20%)	0/5	4/6 (66.7%)	1/4 (25%)	3/6 (50%)	2/6 (33.3%)	3/6 (50.0%)	14/42 (33.3%)
Male	0/5	0/4	1/4 (25%)	2/6 (33.3%)	2/5 (40%)	4/6 (66.7%)	3/6 (50%)	1/6 (16.7%)	13/42 (31.0%)
Challenge dose (LD_{50})									
<250	0/8	1/9 (11.1%)	1/9 (11.1%)	6/12 (50%)	3/6 (50%)	7/10 (70.0%)	5/10 (50%)	3/10 (30%)	26/74 (35.1%)
250 or higher	0/1				0/3	0/2	0/2	1/2 (50%)	1/10 (10%)

6.5.4.5 Conclusions

In this exploratory dose ranging study, there were many groups included with the purpose to identify an appropriate dose for future studies. Using the Bonferroni adjustment for multiple comparisons, we concluded that the 20 mg (or approximately 8 mg/kg) IV group had significantly higher survival rates than the placebo group.

6.5.5 AR034 – Phase I

Re-challenge of Rabbits Treated Previously for Inhalational Anthrax with Intravenous ETI-204 to Assess Protective Immunity

Conducted under (b) (4) Study No. 2637-100012211

6.5.5.1 Study Design and Endpoints

This study was conducted to provide evidence that a single IV dose of ETI-204, either as a monotherapy or in combination with multiple doses of an antibiotic, did not interfere with the development of protective endogenous immunity to PA. Since in Phase I ETI-204 was administered 30 hours post-challenge and did not require the development of symptoms (typically occurring around 30 hours) prior to treatment, this study is described under the post-exposure study section. Phase II survival after secondary challenge in the absence of treatment, the primary focus of this study, will also be discussed in the re-challenge section of this appendix.

Primary Objective

The primary objective was to demonstrate that ETI-204 administered intravenously or alone in combination with antibiotics following primary challenge with spores of *B. anthracis* results in development of protective immunity as measured by increased survival in the absence of treatment following secondary challenge.

Secondary Objectives

There were several secondary objectives:

- To determine whether rabbits treated with ETI-204 alone, or in combination with levofloxacin following primary challenge were more likely to survive a secondary challenge with spores of *B. anthracis* as compared to rabbits treated with antibiotics alone
- To determine whether rabbits treated with ETI-204 alone or in combination with levofloxacin following primary challenge demonstrated longer time to death following secondary challenge with spores of *B. anthracis* as compared to rabbits treated with antibiotics alone
- To determine whether rabbits treated with ETI-204 alone or in combination with levofloxacin following primary challenge have significantly higher levels of circulating anti-PA IgGs at the time of secondary challenge as compared to rabbits treated with antibiotics alone.

Study Design

This was a randomized, controlled, open-label study; dose received at 30 hours post first anthrax exposure in Phase 1; then 9 months later survivors from the treated groups were challenged with anthrax spores in Phase 2. The study was conducted at (b) (4) in 2013.

In Phase 1 animals were randomized to one of four treatment arms, ETI-204 16 mg/kg IV alone, Levofloxacin for 3 days alone, a combination of ETI-204 and levofloxacin, or vehicle control. The 3 active treatment arms contained 20 animals each while the control contained 8 animals. Treatment started at 30 hours after challenge. This review of Phase 1 will focus on the results of ETI-204 compared to control. For an assessment of ETI-204 in combination with levofloxacin, see review by Dr. Ling Lan.

The test product was manufactured at the Lonza facility.

In Phase 1, animals were randomized by sex and weight to the four study groups. Animals were assigned to one of the two challenge days. Within each challenge day, animals were assigned a random challenge order (order numbers 1 through 34).

On Phase I Day 0, animals were exposed to aerosolized *B. anthracis* (Ames) spores (target 200 LD_{50}s). Animals were treated at 30 (±4) hours post challenge.

Phase II included treated animals that survived Phase I and 12 out of 14 naïve animals (13 males and 1 female) assigned to the Phase II control group, respectively. Animals were then randomized into two challenge days. Each challenge day was then assigned a challenge order. Phase II animals were exposed to aerosolized *B. anthracis* (Ames) spores (target 200 LD_{50}s, secondary challenge or re-challenge). No treatment was administered in Phase II.

Primary Endpoint

The primary endpoint was survival proportion of the Phase II dataset (survival to 21 days postsecondary challenge). Survival to 28 days post challenge will be considered for Phase I.

6.5.5.2 Statistical Methodologies

Sample Size Calculation

Assuming the Phase II survival rate was at least 55% for the treated groups (Group 1-3) and 5% for the control group (Group 4), the sample size of 14 per treatment group resulted in 80.7% power to detect a difference in survival rates between a treated group and the control group. Power calculations were for two-sided, 0.05 level Fisher's exact tests with no adjustment for multiple comparisons.

Analysis Populations

The following two populations were defined in the protocol:

***Phase I ITT*:** based on the treatment the animals received, including only animals surviving to receive treatment.

***Phase II ITT*:** including all animals that were challenged in Phase II. That is, all surviving animals from the treated groups in Phase I and newly added Phase II control group were included in the analysis population for the primary endpoint.

Statistical Methods

The protocol states that Phase II challenge will be considered successful if the mortality rate in the Phase II control population exceeds 90%. The survival data following the secondary challenge was used to compare Group 1 (ETI-204 IV) and Group 3 (ETI-204 + levofloxacin) to the Phase II control group (Group 4) using a one-sided Fisher's exact test (p=0.025 level). The p-value was only stated in the study report, not in the protocol. The study report also states that these two tests were performed with a Bonferroni-Holm adjustment for multiple comparisons. Because we considered the comparison of ETI-204 and its control group and the comparison of ETI-204 + levofloxacin and its control levofloxacin as two separate analyses, we did not adjust for multiple comparisons.

6.5.5.3 Animal Disposition, Demographic and Baseline Characteristics

Demographic and baseline variables are included in the following table. In Phase I, challenge dose, bacteremia, and PA-ELISA were slightly numerically higher in the treated group. In Phase II, all naïve control animals were male and the survivors from Phase II were older. The mean challenge doses in the two groups were much higher than 200 LD_{50}s. The mean bacteremia level in the placebo group was slightly higher than in the survivor group, indicting a possible immunity generated from Phase I exposure for the animals in the ETI-204 group.

Table 92. Study AR034: Demographic variables and baseline characteristics by treatment group

	Phase I		**Phase II**	
	Placebo (N=8)	**ETI-204 16 mg/kg 30 hrs PC (N=20)**	**Placebo (N=12)**	**ETI-204 16 mg/kg (N=13) Phase I Survivors**
Age (months)				
Mean (SD)	8.0 (0.0)	8.0 (0.0)	11.3 (1.0)	17.0 (0.0)
Range	8.0, 8.0	8.0, 8.0	10.0, 12.0	17.0, 17.0
Gender [n (%)]				
Female	4 (50.0)	10 (50.0)	0	8 (61.5)
Male	4 (50.0)	10 (50.0)	12 (100.0)	5 (38.5)
Body weight (kg)				
Mean (SD)	3.2 (0.3)	3.3 (0.3)	3.9 (0.1)	3.8 (0.4)
Range	2.8, 3.7	2.8, 4.1	3.8, 4.2	3.4, 4.9
Challenge dose (LD_{50})				
Mean (SD)	221.9 (47.0)	238.1 (58.6)	316.3 (69.2)	314.5 (87.3)
Range	150.0, 279.0	136.0, 367.0	238.0, 421.0	220.0, 520.0

	Phase I		Phase II	
Challenge dose (LD_{50}) (n(%))				
<200	3 (37.5)	4 (20.0)	0	0
200 or higher	5 (62.5)	16 (80.0)	12 (100.0)	13 (100.0)
Positive quantitative bacteremia (n(%))	4 (50)	17 (85)	8 (66.7)	0
Log_{10} bacteremia				
Mean (SD)	1.39 (1.33)	2.77 (1.46)	2.14 (1.66)	1.7 (0.00)
Range	0.30, 3.73	0.30, 5.20	0.00, 4.81	0.3, 0.3
	Placebo (N=8)	**ETI-204 16 mg/kg 30 hrs PC (N=20)**	**Placebo (N=12)**	**ETI-204 16 mg/kg (N=13) Phase I Survivors**
Bacteremia (cfm/mL)				
Geometric mean	24.4	594.7	126	2
95% confidence interval	1.9, 313.5	123.9, 2854.8	11.2, 1415.3	NA
Mean (SD) of log_{10} bacteremia	1.39 (1.33)	2.77 (1.46)	2.14 (1.66)	1.7 (0.00)
PA-ELISA positivity (n(%))	0	4 (20)	2 (16.7)	1 (7.7)
PA-ELISA (ng/mL)				
N	8	19	12	13
Geometric mean	0.68	6.8	6.05	5.60
95% confidence interval		4.8, 9.6	4.18, 8.76	4.07, 7.7
Mean (SD) of log_{10} PA	0.68 (0.00)	0.83 (0.31)	0.78 (0.25)	0.75 (0.23)

Bacteremia and PA measurements were prior to treatment in Phase I and 24 hours post challenge in Phase II

Time to bacteremia

The time to qualitative bacteremia is shown in the following table. The placebo group in Phase I had a longer time to qualitative bacteremia, because two animals did not have bacteremia until the terminal visit (at 94 and 139 hours). If these two animals were excluded, the time to bacteremia would be 27 hours. The two outliers increased the mean time significantly.

Table 93. Study AR034: Time to qualitative bacteremia

	Phase I		Phase II	
	Placebo (N=8)	**ETI-204 16 mg/kg 30 hrs PC (N=20)**	**Phase II placebo (N=12)**	**ETI-204 16 mg/kg (N=13) Phase I Survivors**
Time to qualitative bacteremia (hours)				
N	7	17	12	1
Mean (SD)	62.7 (46.8)	28.0 (1.32)	44.2 (31.7)	71.2
Range	25.7, 139.2	25.98, 30.1	22.9, 118.2	

6.5.5.4 Results

Survival

In the ETI-204 group, 13 out of 20 animals survived to Phase II. In Phase II, the survival proportion of this group was 100%, compared with 0 in the control group. In Phase I among

bacteremic animals, there was no statistically significant difference. In Phase II, no survivors from the ETI-204 group had positive bacteremia 24 hours post-challenge. Therefore no comparison in bacteremic population could be performed.

Table 94. Study AR034: Survival at Day 28 in Phase I and Day 21 in Phase II by treatment group

	Phase I		**Phase II**	
	Placebo	**ETI-204 16 mg/kg 30 hrs PC**	**Placebo**	**ETI-204 16 mg/kg Survivors**
All animals				
n/N (%)	0/8	13/20 (65.0)	0/12	13/13 (100)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.65 [0.156, 0.846] 0.0008*		1 [0.724, 1] <0.0001*
Including only bacteremic animals prior to treatment in Phase I and 24 hours post-challenge in Phase II				
n/N(%)	0/4 (0)	10/17 (58.82)	0/8 (0)	NA
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.588 [-0.072, 0.822] 0.0236*		

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025.

As shown in the following two graphs, survival (time-to-death) analysis also showed a statistically significant difference in survival in each phase. The difference in the first phase can be attributed to ETI-204, and the difference in Phase II can be attributed to the development of protective immunity, because there was no quantifiable concentration of ETI-204 in samples in any of the ETI-204 treated animals prior to the second challenge.

Figure 49. Study AR034: Kaplan-Meier curves by treatment group in Phase I

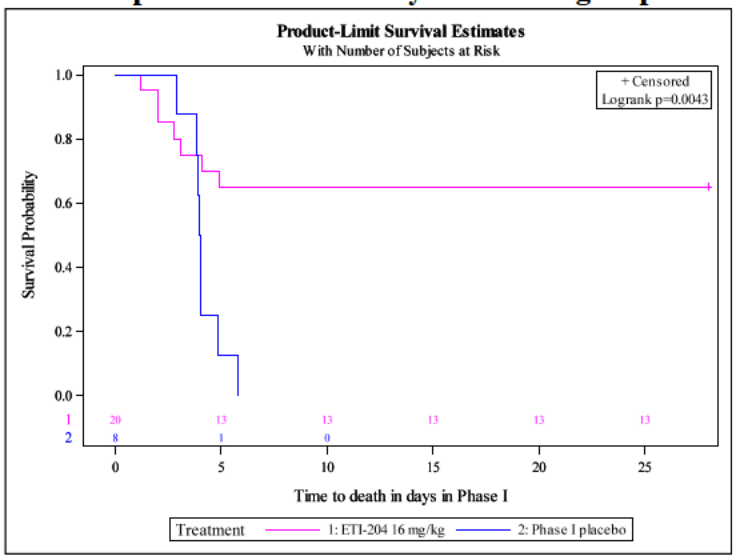
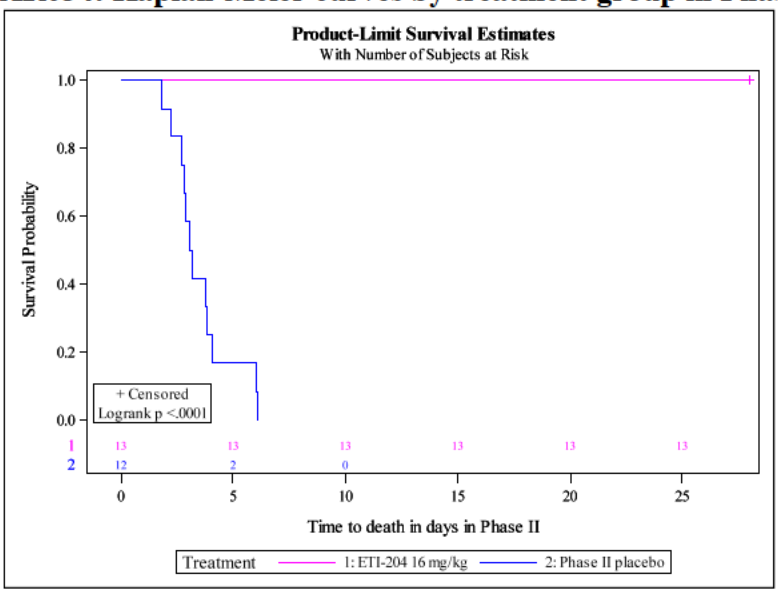


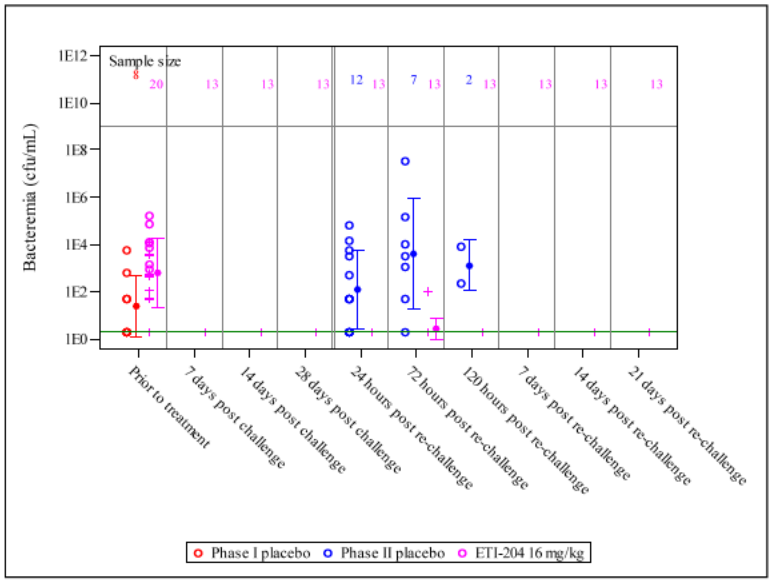
Figure 50. Study AR034: Kaplan-Meier curves by treatment group in Phase II



Bacteremia over time by phase

In Phase I, the ETI-204 treated group had higher bacteremia levels than the Phase I control group prior to treatment and reached a level below the LOD from Day 7. In Phase II, all survivors from the ETI-204 group had a lower bacteremia level than Phase II control animals at the same time point, due to immunity generated from Phase I exposure. From Day 5 after re-challenge, all survivors had a bacteremia level below the LOD, indicating the effect of generated immunity.

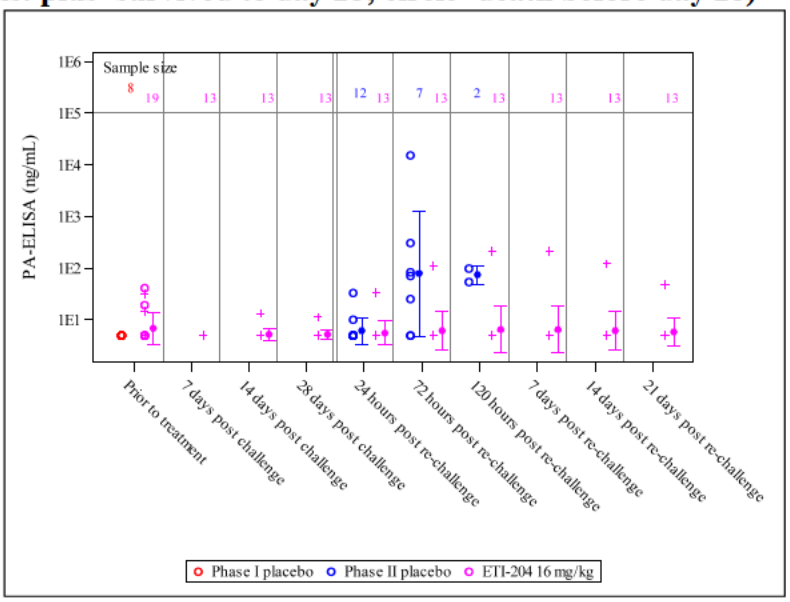
Figure 51. Study AR034: PA-ELISA by visit with geometric mean and standard deviation (by survival status: plus=survived to day 28; circle=death before day 28)



PA-ELISA over time by phase

As shown in the following graph, the ETI-204 treated group had a comparable PA level in Phase II compared with it in Phase I. One animal in the treated group (L40836) had a higher PA level, but survived in Phase II. The new naïve placebo animals in Phase II had a higher PA level 24 hours post re-challenge and all died within 6 days.

Figure 52. Study AR034: PA-ELISA by visit with geometric mean and standard deviation (by survival status: plus=survived to day 28; circle=death before day 28)

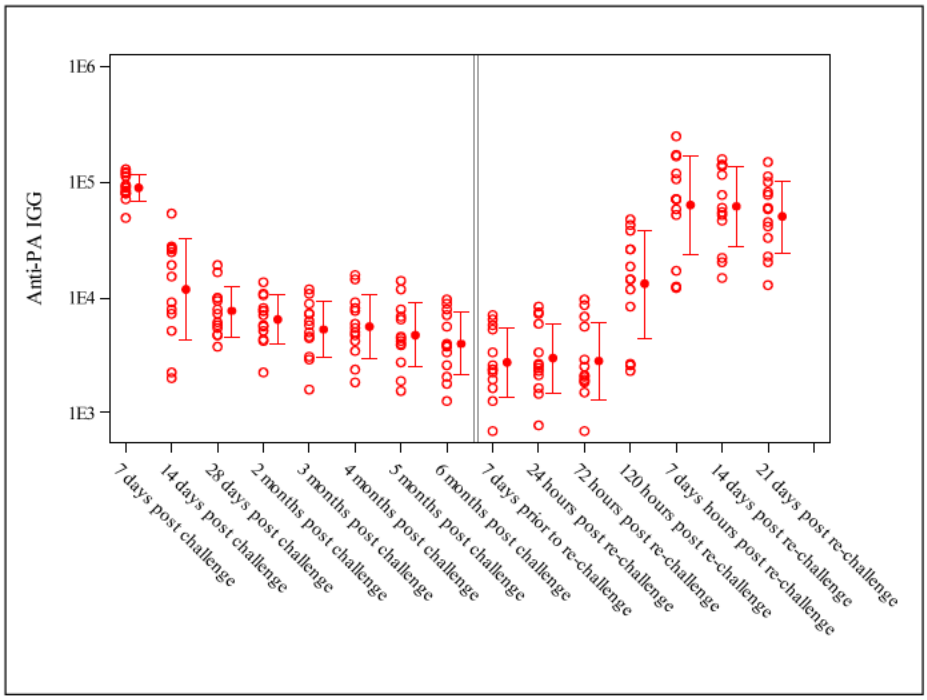


Immune response: anti-PA-IgG and TNA

The development of an immune response for the animals was assessed through the measurements of antibodies to PA (anti-PA IgG levels) and the functional ability of serum to neutralize *B. anthracis* lethal toxin activity (TNA primary endpoints: Effective Dilution-50 and the neutralization Factor-50 (ED_{50}/NF_{50}) titers). The ED_{50} is the reciprocal of the dilution of a serum sample that results in 50% neutralization of anthrax lethal toxin, and it is defined as the reciprocal of the dilution corresponding to the inflection point ('c' parameter) of a 4-parameter logistic log fit of the curve. The NF_{50} is the quotient of the ED_{50} of the test sample and the ED_{50} of the reference serum. The NF_{50} serves as a relative measure of toxin neutralization. A higher value of these measures indicated a higher lethal toxin naturalization activity.

Controls were excluded from following analyses because no data were available (except for anti-PA- IgG 2 days prior to primary challenge (all blow the LOD)). Anti-PA-IgG, TNA ED50, and NF50 data were only available for survivors in the treated groups. The following figure shows the anti-PA- IgG levels over time for those 13 surviving ETI-204 treated animals from both Phase I and Phase II. Two days prior to the first challenge, the levels were below the LLOQ=50 ng/mL. The anti-PA- IgG levels were higher at Day 7 post-challenge, then gradually reduced to a lower level until 5 days post re-challenge. Then the levels increased to a level similar to the level at Day 7 post first challenge. This demonstrated the developed immune response to re-challenge.

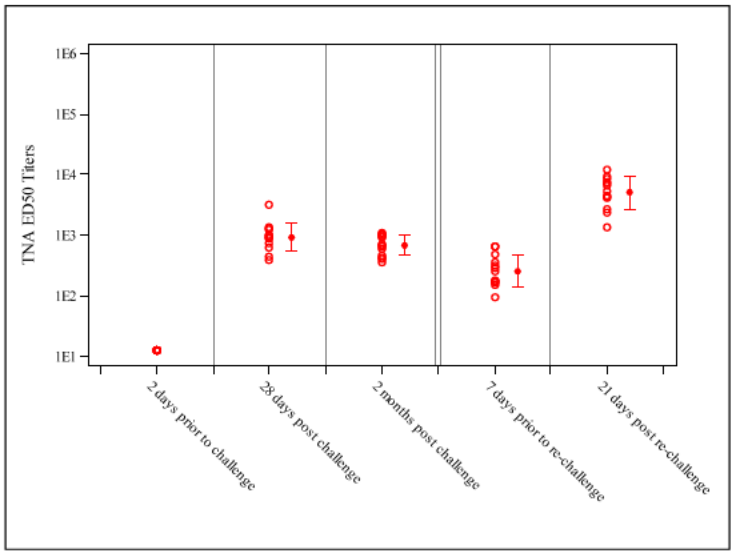
Figure 53. Study AR034: Anti-PA-IgG with geometric mean and standard deviation for 13 surviving treated animals from both Phase I and Phase II



LLOQ=50 ng/ml

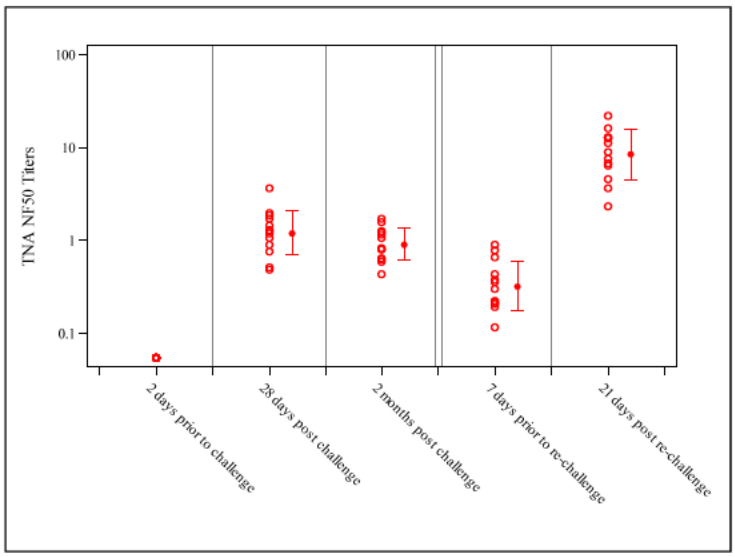
The following two figures show a summary of the geometric mean and 95% confidence intervals for the TNA ED_{50} and NF_{50} titers for 13 ETI-203 treated animals and in Phase I and Phase II. As stated before, these variables measured the functional ability of serum to neutralize *B. anthracis* lethal toxin activity. Two days prior to the first challenge, The ED_{50} levels were below the LOD=23. These survivors consistently exhibited an elevated ED_{50} or NF_{50} titer following primary challenge. In addition, the titer increased by the end of the secondary challenge in-life period compared to Day 7 prior to re-challenge.

Figure 54. Study AR034: TNA ED_{50} with geometric mean and standard deviation for 13 surviving treated animals from both Phase I and Phase II



LOD=23

Figure 55. Study AR034: TNA NF_{50} with geometric mean and standard deviation for 13 surviving treated animals from both Phase I and Phase II



LOD=0.054

Tissue bacterial assessments and pathological findings in the brain

All surviving animals in the ETI-204 groups had negative results in the brain, liver, lymph node, and spleen). Among dead animals, all control animals had a positive result in the brain, compared with 5 out of 7 (71.4%) in the ETI-204 16mg/kg group.

There were no positive pathological findings in the brain in both surviving and non-surviving animals.

Subgroup Analyses

The following table shows the results of subgroup analyses. In Phase I, a higher bacteremia or PA level prior to treatment was associated with a higher survival in the treated group. In other subgroups, the numbers were too small to make a conclusion.

Table 95. Study AP034: Survival at Day 28 by challenge dose, bacteremia, and PA-ELISA

	Phase I		**Phase II**	
	Placebo (N= 8)	**ETI-204 8 mg/kg IV (N= 20)**	**Placebo (N=12)**	**ETI-204 16 mg/kg (N=13) Phase I Survivors**
Gender				
Female	0/3	8/10 (80%)		8/8 (100%)
Male	0/3	5/10 (50%)	0/12	5/5 (100%)
Challenge dose (LD_{50})				
<250	0/6	7/13 (53.8%)	0/3	3/3 (100%)
250 or higher	0/2	6/7 (85.7%)	0/9	10/10 (100%)
Bacteremia prior to treatment (cfu/mL)				
$<10^2$	0/6	6/6 (100%)	0/7	13/13 (100%)
10^2 - $<10^4$	0/2	6/9 (66.7%)	0/3	
10^4 or higher		1/5 (20%)	0/2	
PA-ELISA (ng/mL)				
Missing		1/1 (100%)		
0 - < 10	0/8	11/15 (66.7%)	0/12	12/12 (100%)
10 - < 50		2/4 (50%)	0/2	1/1 (100%)

Bacteremia and PA measurements were prior to treatment in Phase I and 24 hours post challenge in Phase II

6.5.5.5 Conclusions

This re-challenge study demonstrated that ETI-204 16 mg/kg IV alone 30 hours following the primary challenge with *B. anthrax* spores statistically significantly improved survival not only following the first challenge, but also following the secondary challenge in the absence of treatment.

6.5.6 AR035

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

Conducted under [redacted] (b) (4) Study No. FY12-033

6.5.6.1 Study Design and Endpoints

Primary Objective

The primary objective was to assess the pharmacokinetics (PK) of ETI-204 following a single IM dose in rabbits infected via inhalation with *B. anthracis* spores and to identify the optimal window of protection when ETI-204, administered IM could effectively reduce the mortality rate in anthrax-infected rabbits.

Study Design

This was a randomized, open-label, placebo-controlled, IM ETI-204 dose-ranging study, dosing at 18, 24, and 30 hours following *B. anthracis* spore exposure, conducted at [redacted] (b) (4) in 2012.

There were four study groups:

- Placebo (vehicle) 18 hrs PC, IM
- ETI-204 16 mg/kg 18 hrs PC, IM
- ETI-204 16 mg/kg 24 hrs PC, IM
- ETI-204 16 mg/kg 30 hrs PC, IM

The test product was manufactured at the Lonza facility.

Animals were randomized by body weight into one of these groups. Animals were assigned to two exposure cohort based on numerical order of study IDs and were challenged with a target dose of 200±50 LD_{50} *B. anthracis* Ames spores.

Clinical observations were performed at least twice daily and more often during the first 7 days: hourly between 18 and 72 hours post challenge and every 6 hours between 78 hours and day 7.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.5.6.2 Statistical Methodologies

Sample Size Calculation

The sample size of the study (10 per group) was considered in the protocol to be adequate to demonstrate data trending to support the utility of the rabbit model of anthrax

Analysis Populations

There were two analysis populations mentioned in the protocol primary analysis:

1. All animals that received treatment.
2. All animals that were confirmed infected either by blood bacteremia or by the detection of circulating endogenous anti-PA antibodies.

Statistical Methods

The statistical report stated that a one-sided 0.025 level Fisher's exact test would be used to compare survival rates in ETI-204 treated groups to that in the control group. To address the multiple treatment arms, we will use Bonferroni adjustment for multiple comparisons.

6.5.6.3 Animal Disposition, Demographic and Baseline Characteristics

Table 96. Study AR035: Demographic variables and baseline characteristics by treatment group

	Placebo (N=10)	**ETI-204 16 mg/kg 18 hrs PC IM (N=10)**	**ETI-204 16 mg/kg 24 hrs PC IM (N=10)**	**ETI-204 16 mg/kg 30 hrs PC IM (N=8)**	**Total (N=38)**
Body weight (kg) Mean (SD) Range	 3.3 (0.2) 3.1, 3.6	 3.3 (0.2) 3.1, 3.7	 3.3 (0.2) 3.0, 3.7	 3.2 (0.2) 3.0, 3.5	 3.3 (0.2) 3.0, 3.7
Challenge dose (LD_{50}) Mean (SD) Range	 283.1 (84.9) 151.0, 427.0	 281.7 (84.2) 151.0, 424.0	 281.7 (84.4) 151.0, 423.0	 297.9 (89.4) 150.0, 424.0	 285.5 (82.2) 150.0, 427.0
Challenge dose (LD_{50}) (n(%)) <200 200 or higher	 1 (10.0) 9 (90.0)	 1 (10.0) 9 (90.0)	 1 (10.0) 9 (90.0)	 1 (12.5) 7 (87.5)	 4 (10.5) 34 (89.5)
Positive quantitative bacteremia prior to treatment (n(%))	0	0	4 (40.0)	7 (87.5)	11 (28.9)
Bacteremia prior to treatment (cfu/mL) Geometric mean 95% confidence interval Mean (SD) of log_{10} bacteremia	 2.0 NA 0.30 (0.00)	 2.0 NA 0.30 (0.00)	 8.5 1.2, 59.7 0.93 (1.19)	 32574.7* 197.2, 5382200.8 4.51 (2.65)	 22.5 4.5, 111.8 1.35 (2.12)

*One animal's bacteremia was truncated at 3E7 because the value was >3E7. NA: not available because all animals had the same value.

Forty male animals were randomized and challenged. All of the animals were 6-7 months old males. Two animals assigned to the 30-hour post-challenge group died or were moribund sacrificed prior to drug administration. Therefore these two animals were not included in the following table. These variables in Table 96 were comparable across treatment group. Only in the last two treatment groups some animals were bacteremic prior to treatment. Note that exclusions of the two animals that did not survive to treatment could have potentially biased the results in favor of the 30 hour treatment group. However, given the poor results in the 30 hour post challenge group, this is not a concern with the analysis.

Time to bacteremia

The time to quantitative bacteremia was comparable across different groups, as shown in the following table.

Table 97. Study AR035: Time to quantitative bacteremia

	Placebo	ETI-204 16 mg/kg 18 hrs PC IM	ETI-204 16 mg/kg 24 hrs PC IM	ETI-204 16 mg/kg 30 hrs PC IM	Total
Time to quantitative bacteremia (hours)					
N	10	7	7	8	32
Mean (SD)	30.0 (13.3)	25.7 (4.5)	26.6 (3.2)	27.0 (4.5)	27.6 (8.0)
Range	24, 66	24, 36	24, 30	24, 36	24, 66

6.5.6.4 Results

Survival

Survival to 28 days is shown in the following table. One animal in the 18-hour treatment group was humanely euthanized on Study Day 20 and the death was not considered to be attributed to anthrax. The applicant considered this animal as survivor, but in the following table it was considered as a death, to be conservative. For the last treatment group, the applicant's analysis included two more animals which died prior to treatment. The following table excludes these two animals because they died before receiving treatment. If they were included, the survival proportion was still 0 for the last treatment group.

Using a one-sided significance level of 0.025/3=0.0083, there were statistically significant differences between the 18- and 24-hour treatment groups and the placebo group. Administration of ETI-204 at 16 mg/kg IM at 30 hours post-challenge was too late to be effective.

Table 98. Study AR035: Survival at Day 28 by treatment group

	Placebo (N=10)	ETI-204 16 mg/kg 18 hrs PC IM (N=10)	ETI-204 16 mg/kg 24 hrs PC IM (N=10)	ETI-204 16 mg/kg 30 hrs PC IM (N=8)
n (%)	0	6 (60.0)	6 (60.0)	0
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.60 [0.213, 0.878] 0.0018*	0.60 [0.213, 0.878] 0.0018*	0 [-0.309, 0.369]
Adjusted exact 95% confidence interval		0.119, 0.912	0.119, 0.912	-0.387, 0.480

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025/3=0.0083

In the survival (time to death) analysis, using a two-sided significance level of 0.05/3=0.0167, the 18- and 24-hour treatment groups had significantly improved survival, compared with the placebo group.

Figure 56. Study AR035: Kaplan-Meier curve by treatment group

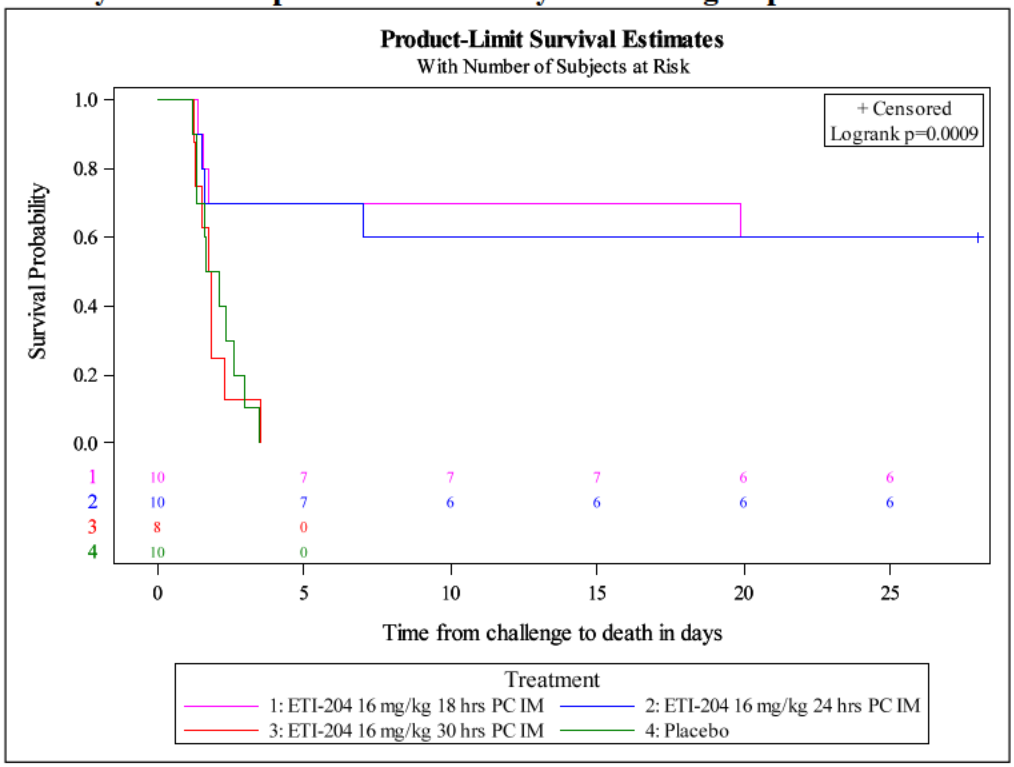


Table 99. Study AR035: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

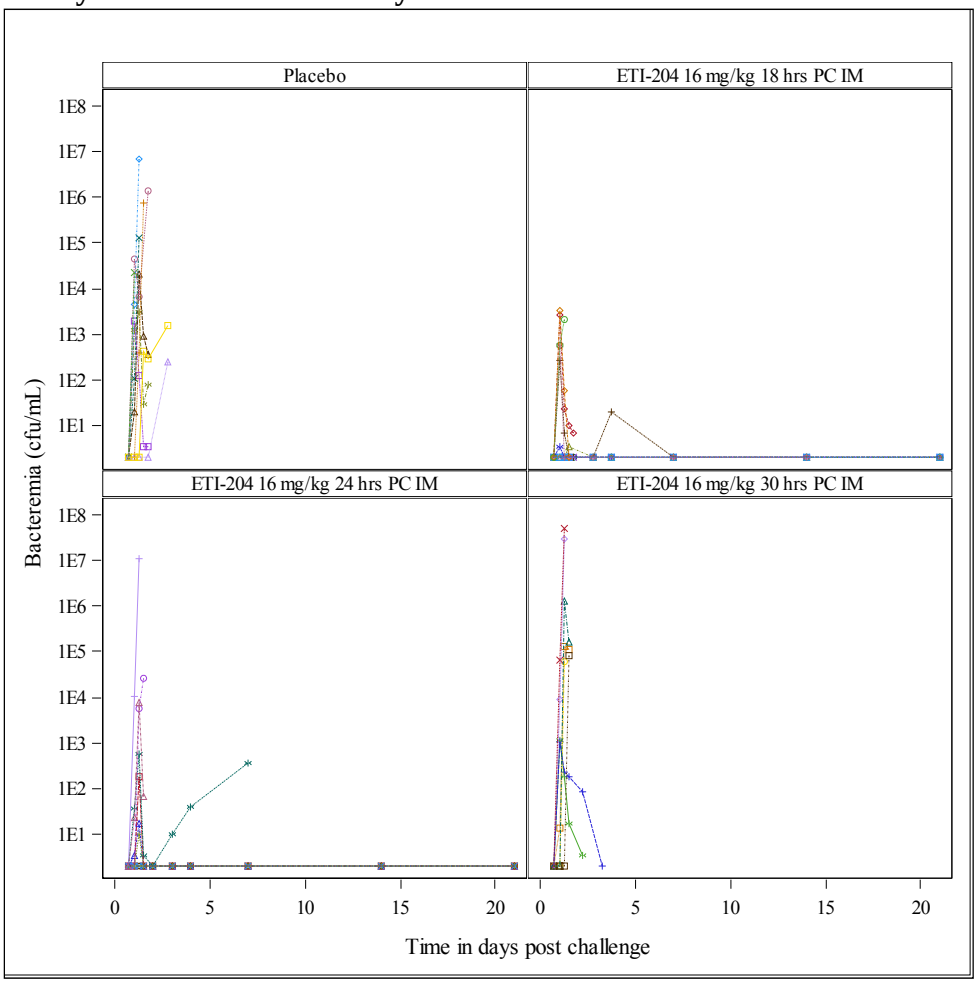
ETI-204 16 mg/kg 18 hrs PC IM (N=10)	ETI-204 16 mg/kg 24 hrs PC IM (N=10)	ETI-204 16 mg/kg 30 hrs PC IM (N=8)
0.0033*	0.0054*	0.894

*Statistically significant at a two-sided significance level of 0.05/3=0.0167

Bacteremia over time

The bacteremia levels are shown in the following figure. Before treatment the bacteremia levels increased post challenge then most treated animals had a reduced bacteremia level. At 2 days post challenge the bacteremia levels in surviving animals in the ETI-204 groups were very low. Since 10 days post challenge, all surviving animals had a bacteremia level below the LOD.

Figure 57. Study AR035: Bacteremia by treatment and animal



Tissue bacterial assessments and pathological findings in brain
The bacterial load in all tissues tested (brain, heart, kidney, lung, spleen) in surviving were below the detection level.

Only one dead animal from the 16 mg/kg 30 hours post challenge had a positive pathological finding in the brain.

Subgroup analysis results

Only male monkeys were included in this study. Therefore subgroup analysis for gender was not applicable. The sample sizes were too small to see a reliable trend by each grouping variable.

Table 100. Study AR035: Survival at Day 28 by gender and challenge dose

	Placebo (N= 10)	**ETI-204 16 mg/kg 18 hrs PC IM (N= 10)**	**ETI-204 16 mg/kg 24 hrs PC IM (N= 10)**	**ETI-204 16 mg/kg 30 hrs PC IM (N= 8)**	**Total (N= 38)**
Challenge dose (LD_{50})					
<250	0/5	2/5 (40%)	3/5 (60%)	0/3	5/18 (27.8%)
250 or higher	0/5	4/5 (80%)	3/5 (60%)	0/5	7/20 (35%)
Bacteremia prior to treatment (cfu/mL)					
$<10^2$	0/10	6/10 (60%)	6/9 (66.7%)	0/1	12/30 (40%)
10^2 - 10^4	0	0	0	0/2	0/2
10^4 - $<10^6$	0	0	0/1	0/2	0/3

6.5.6.5 Conclusions

This study demonstrated that 16 mg/kg ETI-204 administered IM 18 or 24 hours post-challenge significantly improved survival. Further delay of the IM administration of ETI-204 did not provide any protection. The product Lonza was used in this study, which provided supportive evidence for use of this product in the treatment of anthrax.

6.5.7 AR037

Evaluating the Post-Exposure Effect of Intramuscularly Administered ETI-204 in Inhalational Anthrax Challenged Rabbits
Conducted under (b) (4) Study No. FY12-097

6.5.7.1 Study Design and Endpoints

Primary Objective

The primary objective was to assess the effect of a single IM dose of ETI-204 administered at 24 hours post challenge with a lethal dose of *B. anthracis* spores given by inhalation in NZW rabbits.

Secondary Objectives

Secondary objectives were to assess time to death and to evaluate the dose response of ETI-204 on overall mortality rate, time to death, bacteremia, tissue bacteremia burden, and free circulating PA level.

Study Design

This was a randomized, open-label, placebo-controlled IM ETI-204 dose-ranging study, conducted at (b) (4) in 2012.

Animals were randomized into the following groups

- Placebo (vehicle)
- 8 mg/kg, IM
- 16 mg/kg, IM
- 32 mg/kg, IM

The test product was manufactured at the Lonza facility.

Animals were randomized by sex and body weight and then assigned to 4 challenge days based on numerical order by group. Although a few animals were mis-dosed (4 animals were switched between Groups 3 and 4), the imbalance of animal numbers among the challenge cohorts had minimal impact on the study, because body weight, gender, and challenge dose were well balanced across groups, as described in the next section.

Animals were challenged with approximately 200±50 LD_{50} *B. anthracis* Ames spores via aerosol on Day 0, and were administered placebo or ETI-204 24 hours post challenge. Clinical observations were performed twice daily (AM and PM).

Primary Endpoint
The primary endpoint was survival to 28 days post anthrax spore challenge.

6.5.7.2 Statistical Methodologies

Sample Size Calculation

The sample size of the study (minimum of 10/group) animals was considered adequate in the protocol to demonstrate data trending to support the utility of the rabbit model of anthrax for application to a therapeutic setting. According to the protocol, data analysis would use a Fisher's exact test (one-sided, one sample) using a 0.05 level of significance.

Analysis Populations

The population defined in the protocol was the ITT dataset including all challenged animals that received treatment. The population consisting of animals that were confirmed infected either by blood bacteremia or by the detection of circulating endogenous anti-PA antibodies was added in the primary analysis section of the study report.

Comment: As stated previously, 4 animals were switched between the two highest dose groups. The applicant analyzed the data based on the treatment the animals received. Since this study did not yield any significant results, we did not conduct any additional analyses based on the ITT principle. We report the applicant's results in the result section.

Statistical Methods

According to the protocol, the primary analysis only included descriptive statistics for the primary endpoint and comparison of survival rates with control group was one secondary analysis. In the study report, it was stated that one-sided 0.025 level Fisher's exact test used to compare survival rate in ETI-204 treated group to that in the control group.

6.5.7.3 Animal Disposition, Demographic and Baseline Characteristics

Demographic variables and baseline characteristics by treatment group are shown in the following table. The groups were based on the treatment received, not randomized, because 4 randomized animals were switched between the highest dose groups. These variables were comparable in general. There were slightly higher proportions of animals with bacteremia and positive PA in the ETI-204 groups, which was not an issue for efficacy evaluation.

Table 101. Study AR037: Demographic variables and baseline characteristics by treatment group

	Placebo (N=10)	ETI-204 8 mg/kg 24 hrs PC IM (N=16)	ETI-204 16 mg/kg 24 hrs PC IM (N=16)	ETI-204 32 mg/kg 24 hrs PC IM (N=16)	Total (N=58)
Age (weeks)					
Mean (SD)	28.2 (1.2)	28.1 (1.2)	27.4 (0.9)	28.9 (0.8)	28.1 (1.1)
Range	26.6, 29.6	26.6, 29.6	26.6, 28.7	27.6, 29.6	26.6, 29.6

	Placebo (N=10)	ETI-204 8 mg/kg 24 hrs PC IM (N=16)	ETI-204 16 mg/kg 24 hrs PC IM (N=16)	ETI-204 32 mg/kg 24 hrs PC IM (N=16)	Total (N=58)
Gender [n (%)]					
Female	5 (50.0)	8 (50.0)	8 (50.0)	8 (50.0)	29 (50.0)
Male	5 (50.0)	8 (50.0)	8 (50.0)	8 (50.0)	29 (50.0)
Body weight (kg)					
Mean (SD)	3.5 (0.3)	3.5 (0.3)	3.5 (0.3)	3.5 (0.3)	3.5 (0.3)
Range	3.0, 3.9	2.9, 4.0	2.9, 4.0	3.0, 3.9	2.9, 4.0
Challenge dose (LD_{50})					
Mean (SD)	153.1 (50.5)	142.1 (44.7)	156.2 (54.0)	124.7 (22.6)	143.1 (44.6)
Range	101.0, 271.0	76.0, 268.0	76.0, 269.0	64.0, 151.0	64.0, 271.0
Challenge dose (LD_{50}) (n(%))					
<200	8 (80.0)	15 (93.8)	13 (81.3)	16 (100.0)	52 (89.7)
200 or higher	2 (20.0)	1 (6.3)	3 (18.8)	0	6 (10.3)
Positive quantitative bacteremia 24 hours post challenge (n(%))	2 (20.0)	5 (31.3)	5 (31.3)	6 (37.5)	18 (31.0)
Bacteremia 24 hours post challenge (cfu/mL)					
Geometric mean	5.1	12.7	21.3	14.4	13.0
95% confidence interval	1.2, 20.7	2.2, 72.2	2.8, 162.3	2.9, 71.6	5.7, 29.6
Mean (SD) of log_{10} bacteremia	0.70 (0.85)	1.10 (1.42)	1.33 (1.65)	1.16 (1.31)	1.11 (1.36)
PA-ELISA Positivity 24 hours post challenge	0	1 (6.3)	3 (18.8)	3 (18.8)	7 (12.1)
PA-ELISA 24 hours post challenge (ng/mL)					
Geometric mean	5.0	5.3	8.3	7.0	6.4
95% confidence interval	NA	4.7, 5.9	4.3, 16	4.7, 10.4	5.2, 7.9
Mean (SD) of log_{10} PA	0.70 (0.00)	0.72 (0.09)	0.92 (0.53)	0.85 (0.32)	0.81 (0.33)

Time to quantitative bacteremia

The time to quantitative bacteremia was slightly shorter in the 16 mg/kg and 32 mg/kg groups, compared with the placebo group.

Table 102. Study AR037: Time to quantitative bacteremia

	Placebo (N= 10)	ETI-204 8 mg/kg 24 hrs PC IM (N= 16)	ETI-204 16 mg/kg 24 hrs PC IM (N= 16)	ETI-204 32 mg/kg 24 hrs PC IM (N= 16)	Total (N= 58)
Time to quantitative bacteremia (hours)					
N	9	14	11	11	45
Mean (SD)	40.0 (14.7)	38.6 (20.6)	30.5 (6.3)	34.9 (21.1)	36.0 (17.0)
Range	24, 72	24, 96	24, 36	24, 96	24, 96

6.5.7.4 Results

Survival

Using a one-sided significance level of 0.025/3=0.0083, there was no statistically significant difference between any treatment groups and the placebo, as shown in the following table. In bacteremic only animals at 24 hours post-challenge, no animals survived. Therefore no analysis for bacteremic population was needed.

Table 103. Study AR037: Survival at Day 28 by treatment group

	Placebo (N=10)	ETI-204 8 mg/kg 24 hrs PC IM (N=16)	ETI-204 16 mg/kg 24 hrs PC IM (N=16)	ETI-204 32 mg/kg 24 hrs PC IM (N=16)
n (%)	0	5 (31.3)	5 (31.3)	5 (31.3)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.313 [-0.019, 0.587] 0.033	0.313 [-0.019, 0.587] 0.033	0.313 [-0.019, 0.587] 0.033

Two-sided 95% confidence interval and one-sided p-values were calculated by the reviewer

Three animals with anti-PA IgG 7 days prior to challenge (2 in the 8 mg/kg group and 1 in the placebo group) succumbed to anthrax on Study Day 2 or 4. The baseline IgG did not have an effect on improving survival.

Survival analyses of time to death did not demonstrate any statistically significant difference using a two-sided significance level of 0.05/3=0.0167, as the following figure and table show.

Figure 58. Study AR037: Kaplan-Meier curve by treatment group

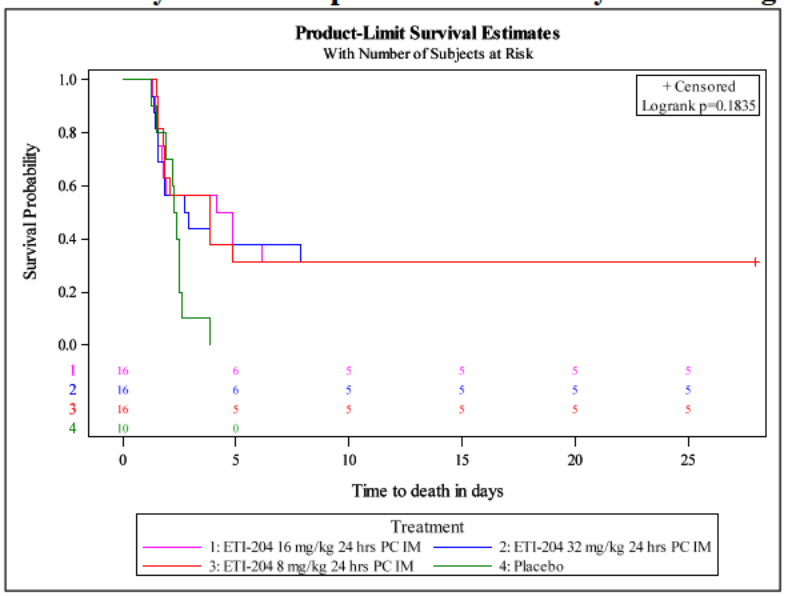


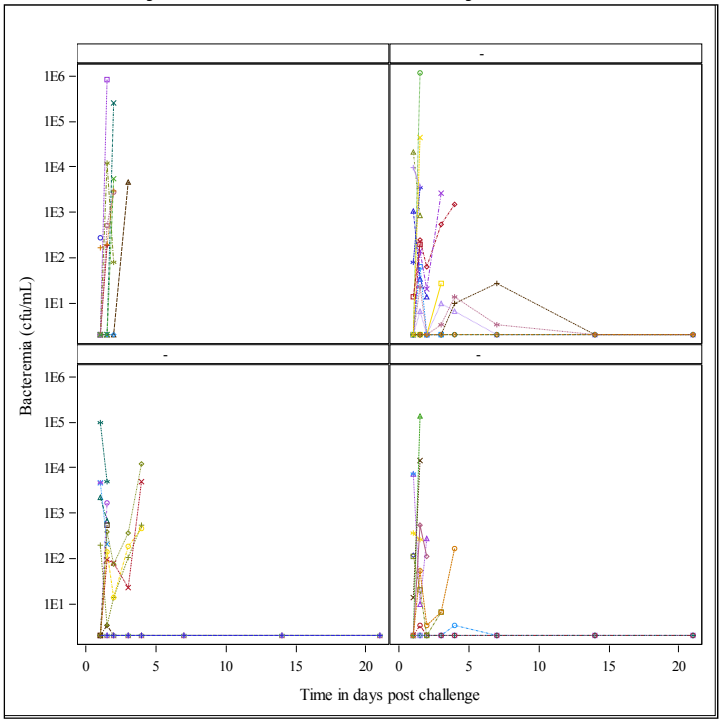
Table 104. Study AR037: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

ETI-204 8 mg/kg 24 hrs PC IM (N=16)	ETI-204 16 mg/kg 24 hrs PC IM (N=16)	ETI-204 32 mg/kg 24 hrs PC IM (N=16)
0.0478	0.0393	0.0668

Bacteremia level over time

As the following figure shows, from 24 to 36 hours post-challenge, bacteremia levels increased for most animals in all groups. Then the treated groups had decreased bacteremia levels, but did not reach a level close to the LOD until 7 days post challenge. Most deaths occurred between 36 hours and 7 days post challenge. Based on data, all surviving animals were not bacteremic 24 hours post-challenge.

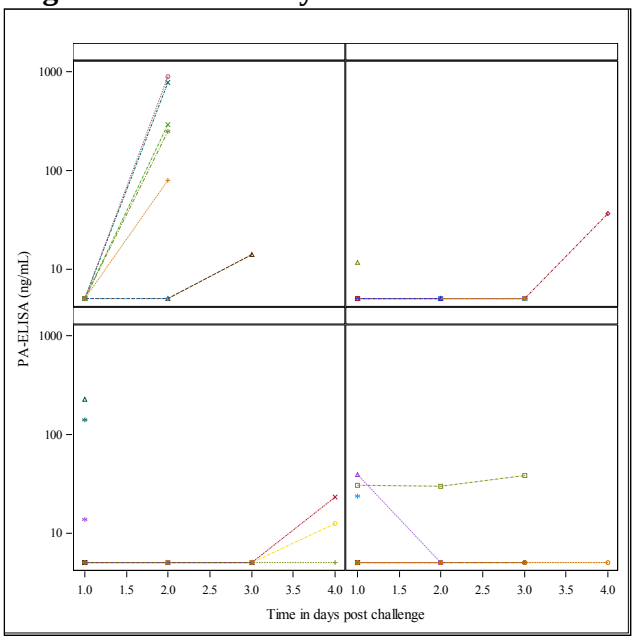
Figure 59. Study AR037: Bacteremia by treatment and animal



PA-ELISA level over time

PA was not measured frequently between 24 hours and 48 hours post challenge, when more than 30% of animals died. After 2 days post challenge, PA levels were low in the treatment group. However, 2 surviving animals in the 16 mg/kg group had an increased PA level compared with the previous visit. Not every surviving animal had PA data at Day 4 and no PA level was measured after this time point, so it was not possible to explore the effect of PA on survival beyond this time point.

Figure 60. PA level by treatment and animal



Tissue bacterial assessments and pathological findings in the brain

In all surviving animals, all tissues tested (brain, kidney, liver, lung, lymph node, and spleen) had results below the detection limit.

Only 3, 2, 1 dead animals from 8, 16, 32 mg/kg groups had positive pathological findings in the brain. No positive results were recorded for surviving animals.

Subgroup Analysis

The following table shows the results of the subgroup analyses. The sample sizes were too small to see a reliable trend by gender and challenge dose. All surviving animals had the lowest category of bacteremia and PA prior to treatment.

Table 105. Study AR037: Survival at Day 28 by challenge dose, bacteremia, and PA

	Placebo (N= 10)	**ETI-204 8 mg/kg 24 hrs PC IM (N= 16)**	**ETI-204 16 mg/kg 24 hrs PC IM (N= 16)**	**ETI-204 32 mg/kg 24 hrs PC IM (N= 16)**	**Total (N= 58)**
Gender					
Female	0/5	2/8 (25.0%)	3/8 (37.5%)	0/8	5/29 (17.2%)
Male	0/5	3/8 (37.5%)	2/8 (25.0%)	5/8 (62.5%)	10/29 (34.5%)
Challenge dose (LD_{50})					
<250	0/9	4/15 (26.7%)	5/14 (35.7%)	5/16 (31.3%)	14/54 (25.9%)
250 or higher	0/1	1/1 (100%)	0/2	0	1/4 (25.0%)

	Placebo (N= 10)	ETI-204 8 mg/kg 24 hrs PC IM (N= 16)	ETI-204 16 mg/kg 24 hrs PC IM (N= 16)	ETI-204 32 mg/kg 24 hrs PC IM (N= 16)	Total (N= 58)
Bacteremia prior to treatment (cfu/mL)					
$<10^2$	0/8	5/13 (38.5%)	5/11 (45.5%)	5/11 (45.5%)	15/43 (34.9%)
10^2 - 10^4	0/2	0/2	0/4	0/5	0/13
10^4 - $<10^6$	0	0/1	0/1	0	0/2
PA prior to treatment (ng/mL)					
0 - < 10	0/10	5/15 (33.3%)	5/13 (38.5%)	5/13 (38.5%)	15/51 (29.4%)
10 - < 50	0	0/1	0/1	0/3	0/5
50 or higher	0	0	0/2	0	0/2

6.5.7.5 Conclusions

There was no statistically significant differences between Lonza ETI-204 8, 16, or 32 mg/kg administered IM 24 hours post challenge and the control group. The reason was not clear. Bacteremia and PA levels prior to treatment were not so high to explain the lower survival proportion in this study. Subgroup analyses showed that survival proportion in the lowest bacteremia category in the 16 mg/kg group were also lower than in the same dose group in Study AR035 (45.5% versus 66.7%). This study used the Lonza product, as did study AR035.

6.5.8 AR0315

An Evaluation of the Efficacy of ETI-204 When Administered Intramuscularly in a Rabbit Post-Exposure Spore Challenge Model

Conducted under (b) (4) Study 1142-G924203

6.5.8.1 Study Design and Endpoints

Primary Objective

The primary objective was to evaluate the survival of NZW rabbits when ETI-204 was given IM at either 18 or 24 hours following inhalation exposure to *B. anthracis* spores.

Study Design

This was a randomized, placebo-controlled, parallel group, dose ranging with treatment administered at a fixed time.

Animals were randomized into four groups of 12 and one group (placebo) of 10 animals.

- Placebo, 24 hrs
- 4 mg/kg ETI-204 IM, 18 hrs
- 16 mg/kg ETI-204 IM, 18 hrs
- 4 mg/kg ETI-204 IM, 24 hrs
- 16 mg/kg ETI-204 IM, 24 hrs

The test product was manufactured at the Baxter facility.

All rabbits were aerosol challenged on Study Day 0 with a targeted 200 LD_{50} dose of *B. anthracis* spores (Ames).

Clinical observations were performed twice daily.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.5.8.2 Statistical Methodologies

Sample Size Calculation

Sample sizes of 12 animals per treated group and 10 in the control group provided 80.8% power to compare the survival rates of 5% and 60%, with a two-sided 0.05 level Fisher's exact test, with no adjustment for multiple comparisons.

Analysis Population

All randomized animals were used for the comparison of each treatment group to the control group as mentioned in the protocol statistical methods section.

Statistical Methods

Two-sided Fisher's exact tests were utilized to compare the survival rates between the treated groups and the control group.

6.5.8.3 Animal Disposition, Demographic and Baseline Characteristics

Demographic variables and challenged dose were comparable across groups. As expected, bacteremia levels were higher when treatment was administered at 24 hours post challenge than at 18 hours post challenge.

Table 106. Study AR0315: Demographic variables and baseline characteristics by treatment group

	Placebo (N=10)	ETI-204 4 mg/kg 18 hrs PC IM (N=12)	ETI-204 4 mg/kg 24 hrs PC IM (N=12)	ETI-204 16 mg/kg 18 hrs PC IM (N=12)	ETI-204 16 mg/kg 24 hrs PC IM (N=12)	Total (N=58)
Age (years)						
Range	6-7	6-7	6-7	6-7	6-7	6-7
Gender [n (%)]						
Female	5 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	29 (50.0)
Male	5 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	6 (50.0)	29 (50.0)
Body weight (kg)						
Mean (SD)	2.9 (0.2)	3.0 (0.1)	3.0 (0.2)	2.9 (0.3)	2.9 (0.3)	2.9 (0.2)
Range	2.4, 3.2	2.8, 3.2	2.7, 3.2	2.0, 3.3	2.1, 3.2	2.0, 3.3
Challenge dose (LD_{50})						
Mean (SD)	245.5 (16.2)	235.3 (27.6)	221.6 (15.7)	223.5 (31.0)	255.7 (53.0)	236.0 (33.7)
Range	218.0, 270.0	197.0, 278.0	197.0, 253.0	141.0, 261.0	150.0, 337.0	141.0, 337.0
Challenge dose (LD_{50}) (n(%))						
<200	0	1 (8.3)	1 (8.3)	1 (8.3)	2 (16.7)	5 (8.6)
200 or higher	10 (100)	11 (91.7)	11 (91.7)	11 (91.7)	10 (83.3)	53 (91.4)
Positive quantitative bacteremia prior to treatment (n(%))	5 (50.0)	5 (41.7)	11 (91.7)	5 (41.7)	11 (91.7)	37 (63.8)

	Placebo (N=10)	ETI-204 4 mg/kg 18 hrs PC IM (N=12)	ETI-204 4 mg/kg 24 hrs PC IM (N=12)	ETI-204 16 mg/kg 18 hrs PC IM (N=12)	ETI-204 16 mg/kg 24 hrs PC IM (N=12)	Total (N=58)
Bacteremia prior to treatment (cfu/mL)						
Geometric mean	24.4	7.6	735.5	9.3	556.3	60.8
95% confidence interval	3.5, 167.4	2.7, 21.9	133.1, 4063.8	2.6, 33.6	89.7, 3449.4	26.9, 137.1
Mean (SD) of log_{10} bacteremia	1.39 (1.17)	0.88 (0.72)	2.87 (1.17)	0.97 (0.87)	2.75 (1.25)	1.78 (1.34)

Time to bacteremia

The time from challenge to quantitative bacteremia is shown in the following table. The two treatment groups administrated 24 hours post-challenge had a shorter time to bacteremia. Bacteremia levels were measured only at Days 1, 3, 5, 7, and 14, which were not frequent enough for accurately assessing the time to bacteremia. This limited the interpretation of the differences across different groups.

Table 107. Study AR0315: Time from challenge to bacteremia

	Placebo	ETI-204 4 mg/kg 18 hrs PC IM	ETI-204 4 mg/kg 24 hrs PC IM	ETI-204 16 mg/kg 18 hrs PC IM	ETI-204 16 mg/kg 24 hrs PC IM	Total
Time to quantitative bacteremia (hours)						
N	10	9	12	5	12	48
Mean (SD)	47.7 (25.0)	49.7 (47.7)	28.0 (13.9)	18.0 (0.5)	28.1 (13.8)	35.2 (27.0)
Range	23.8, 72.4	17.6, 160.7	23.6, 72.3	17.2, 18.4	23.7, 72	17.2, 160.7

6.5.8.4 Results

Survival

Using a one-sided significance level of 0.025/4=0.00625 (Bonferroni adjustment method), the 4 mg/kg 18 hours post-challenge group and two 16 mg/kg groups significantly improved survival.

Table 108. Study AR0315: Survival at Day 28 by treatment group

	Placebo (N=10)	ETI-204 4 mg/kg 18 hrs PC IM (N=12)	ETI-204 4 mg/kg 24 hrs PC IM (N=12)	ETI-204 16 mg/kg 18 hrs PC IM (N=12)	ETI-204 16 mg/kg 24 hrs PC IM (N=12)
n (%)	0	11 (91.7)	5 (41.7)	11 (91.7)	8 (66.7)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.917 [0.535, 0.998] <0.0001*	0.417 [0.065, 0.723] 0.0131	0.9167 [0.535, 0.998] <0.0001*	0.667 [0.290, 0.901] 0.0005*
Adjusted exact 95% confidence interval		0.425, 1	-0.058, 0.786	0.425, 1	0.172, 0.934

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at a one-sided significance level of 0.025/4=0.0063

Kaplan-Meier survival curves are shown in the following graph. Using a two-sided significance level of 0.05/4=0.0125, pairwise log-rank tests in Table 109 demonstrated that the groups 4 mg/kg 18 hours IM post challenge and 16 mg/kg IM had significant treatment effect on survival time.

Figure 61. Study AR0315: Kaplan-Meier curve by treatment group

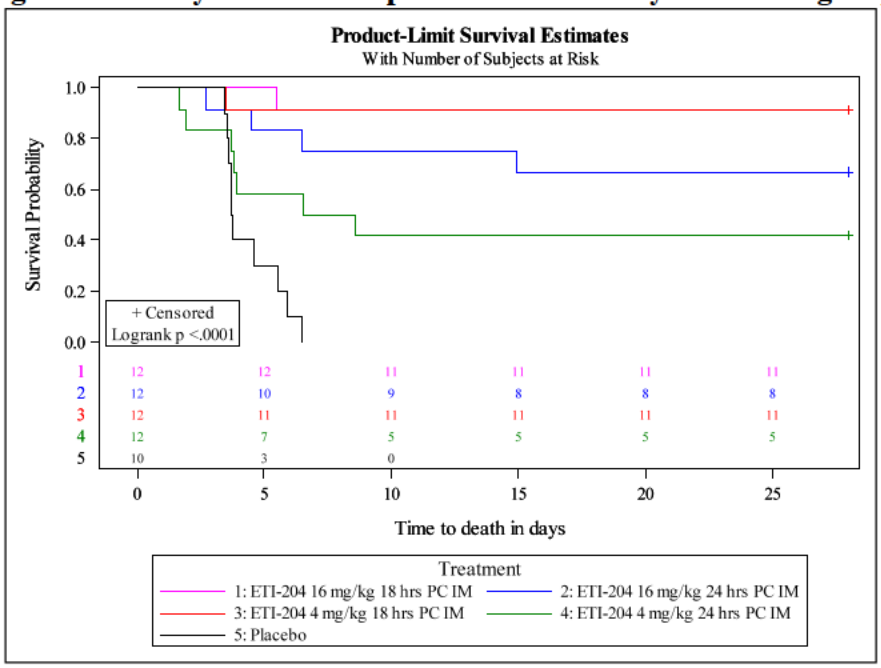


Table 109. Study AR0315: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

ETI-204 4 mg/kg 18 hrs PC IM (N=12)	ETI-204 4 mg/kg 24 hrs PC IM (N=12)	ETI-204 16 mg/kg 18 hrs PC IM (N=12)	ETI-204 16 mg/kg 24 hrs PC IM (N=12)
<0.0001*	0.0132	<0.0001*	0.0002*

*Significant at a one-sided significance level of 0.05/4=0.0125

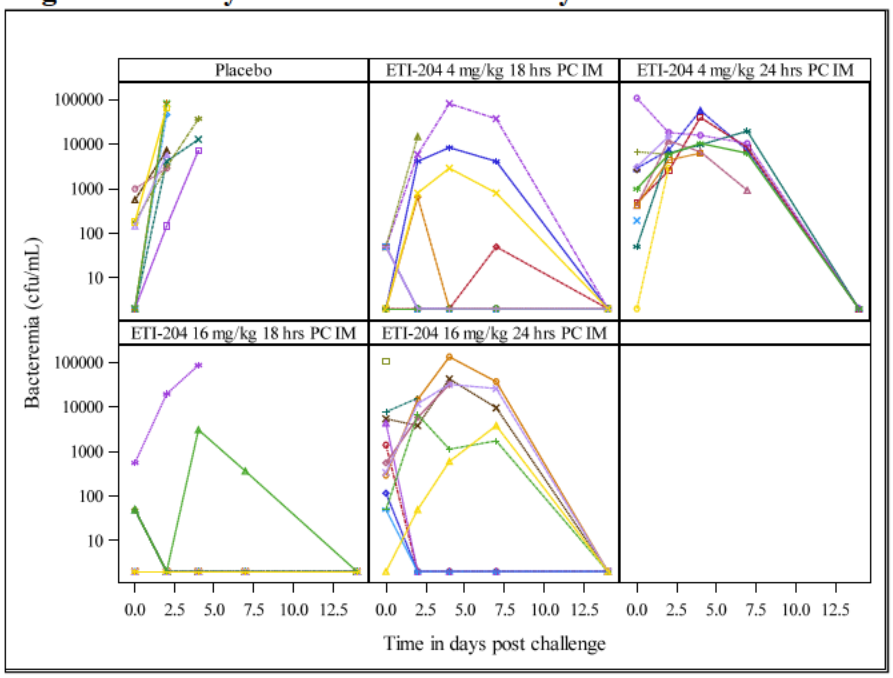
The following graph shows that the four treated groups had a reduced bacteremia level from Day 7 to Day 14 and all control animals died with a high bacteremia level.

Tissue bacterial assessments and pathological findings in the brain

In the two tissues tested (lymph node and spleen), among all surviving animals, only one animal in the 16 mg 24 hour post-challenge group had a positive load (0.5) in bronchial lymph node.

Among dead animals, only 2, 1, 1, and 1 from the placebo, 4 mg/kg 18 and 24 hours post challenge, and 16 mg/kg 24 hours post-challenge had positive pathological findings in the brain (discoloration(s), diffuse, etc). No survivors had recorded positive pathological findings in the brain.

Figure 62. Study AR0315: Bacteremia by treatment and animal



Subgroup Analysis Results

The following table shows the results of subgroup analyses. The sample sizes were too small to see a reliable trend by each grouping variable.

Table 110. Study AR0315: Survival at Day 28 by challenge dose

	Placebo (N= 10)	ETI-204 4 mg/kg 18 hrs PC IM (N= 12)	ETI-204 4 mg/kg 24 hrs PC IM (N= 12)	ETI-204 16 mg/kg 18 hrs PC IM (N= 12)	ETI-204 16 mg/kg 24 hrs PC IM (N= 12)	Total (N= 58)
Gender						
Female	0/5	5/6 (83.3%)	4/6 (66.7%)	5/6 (83.3%)	4/6 (66.7%)	18/29 (62.1%)
Male	0/5	6/6 (100.0%)	1/6 (16.7%)	6/6 (100.0%)	4/6 (66.7%)	17/29 (58.6%)
Challenge dose (LD_{50})						
<250	0/5	7/8 (87.5%)	4/11 (36.4%)	9/10 (90%)	2/4 (50%)	22/38 (57.9%)
250 or higher	0/5	4/4 (100%)	1/1 (100%)	2/2 (100%)	6/8 (75%)	13/20 (65%)
<200	0	1/1 (100%)	1/1 (100%)	1/1(100%)	1/2 (50%)	4/5 (80%)
Bacteremia prior to treatment (cfu/mL)						
$<10^2$	0/5	11/12 (91.7%)	1/2 (50%)	11/11 (100%)	3/3 (100%)	26/33 (78.8%)
10^2 - 10^4	0/5	0	3/9 (33.3%)	0/1	5/8 (62.5%)	8/23 (34.8%)
10^4 - $<10^6$	0	0	1/1 (100%)	0	0/1	1/2 (50%)

6.5.8.5 Conclusions

This study demonstrated that ETI-204 4 mg/kg administered IM 18 hours post-challenge and 16 mg/kg administered IM 18 or 24 hours improved survival significantly. ETI-204 4 mg/kg IM administered 24 hours did not improve survival significantly after using Bonferroni adjustment for multiple comparisons.

6.6 Monkey Pre-Exposure Prophylaxis Study

6.6.1 Summary of monkey pre-exposure prophylaxis study

There is one monkey pre-exposure prophylaxis (PrEP) study to assess the prophylactic effect of 16 mg/kg ETI-204 IM administered at different times (3, 2, and 1 day prior to challenge). This study was conducted by the applicant. The study used the Lonza product. The treated groups had a 100% survival, significantly higher than a 10% survival in the placebo group.

6.6.2 AP305

AP305: Study to Evaluate the Prophylactic Effect of a Single Intramuscular ETI-204 Dose Administered at Various Times Prior to Anthrax Challenge in a Cynomolgus Macaque Aerosol Challenge Model of *B. anthracis*

Conducted under (b) (4) Study 2778-100018326

6.6.2.1 Study Design and Endpoints

Primary Objective

The primary objective was to determine the duration of ETI-204 prophylactic efficacy when administrated IM to cynomolgus macaques at increasing times prior to exposure to *B. anthracis* spores.

Secondary Objective

The secondary objective was to perform a kinetic analysis of ETI-204 when administered IM.

Study Design

This was a randomized, blinded, placebo-controlled, time-ranging study with treatment received within 24, 48, and 72 hours before anthrax spore challenge, conducted at (b) (4) in 2013.

Monkeys were randomized into the following groups:

- Placebo IM, Day -3, Day -2, and Day -1
- ETI-204 IM, Day -1
- ETI-204 IM, Day -2
- ETI-204 IM, Day -3

The test product was manufactured at the Lonza facility.

Animals were randomized in three steps. Stratified by sex and body weight into 3 groups for each gender, they were randomized to each treatment group. Animals were then randomized to four challenge days and assigned a challenge order in a challenge day.

Monkeys were aerosol-challenged with a targeted 200 LD_{50} dose of *B. anthracis* (Ames) spores.

Group assignment was blinded for applicant, study director, QA study auditor, and staff who evaluate animals to make decision about animal care and euthanasia. In addition, group assignment was blinded to microbiologists and the study pathologist.

NHPs were observed twice daily (at least 6 hours apart) for clinical signs.

Primary Endpoint

The primary endpoint was survival to 56 days post anthrax spore challenge.

6.6.2.2 Statistical Methodologies

Sample Size Calculation

Assuming that the true probabilities of survival in the control and the group treated 48 hours pre-challenge with ETI-204 (Group 3) were 10% and 70% respectively, there was 83.1% power to detect a difference in survival rates between Group 3 (n=14) and the control group (n=10). Power calculation was for a one-sided, 0.025 level, Fisher's exact test with the planned implantation of sequential testing to adjust for multiple comparisons across the three tests, according to the Protocol Amendment No. 1 dated 3/14/2013.

Comment: We were able to replicate the calculation.

Analysis Population

In the protocol study population, it states that that all animals were assigned to groups based on the dose the animals received. So the analysis population should include all randomized animals that received treatment.

Statistical Methods

In the study protocol, for treatment group comparison, the survival data from each treatment group was to be compared to the control group using a one-sided, 0.025 level Fisher's exact test with no adjustment for multiple comparisons. According to the Protocol Amendment No 1, the principal of closed testing was used to test three hypotheses (for comparing Days -2, -1, and -3 versus control, respectively) sequentially using the following pre-specified order of testing: The second hypothesis was only tested if the first was significant and the third hypothesis was only tested if the first two were significant. There was no additional adjustment for multiple comparisons required. Thus, the overall significance level of 0.025 was maintained and there was no need to use other adjustment methods.

6.6.2.3 Animal Disposition, Demographic and Baseline Characteristics

All randomized animals received treatment and were included in the following table. Demographic variables and baseline characteristics were comparable. Only 3 (30%) and 1

(6.7%) animals in the first two groups were bacteremic 24 hours post challenge. It is not clear why the placebo group had such a low proportion of bacteremia at this time point. All placebo animals were bacteremic for at least one time point post challenge.

Table 111. Study AP305: Demographic variables and baseline characteristics by treatment group

	Placebo (N=10)	**ETI-204 16 mg/kg PrEP-3 (N=15)**	**ETI-204 16 mg/kg PrEP-2 (N=14)**	**ETI-204 16 mg/kg PrEP-1 (N=14)**	**Total (N=53)**
Age (years) Range	2.36-3.96	2.36-3.96	2.36-3.96	2.36-3.96	2.36-3.96
Gender [n (%)]					
Female Male	5 (50.0) 5 (50.0)	8 (53.3) 7 (46.7)	7 (50.0) 7 (50.0)	7 (50.0) 7 (50.0)	27 (50.9) 26 (49.1)
Body weight (kg) Mean (SD) Range	 2.7 (0.2) 2.3, 3.0	 2.5 (0.2) 2.3, 2.9	 2.5 (0.2) 2.3, 2.9	 2.5 (0.2) 2.2, 3.0	 2.6 (0.2) 2.2, 3.0
Challenge dose (LD_{50}) Mean (SD) Range	 217.8 (65.2) 144.0, 330.0	 220.2 (86.7) 126.0, 490.0	 209.3 (61.6) 103.0, 315.0	 237.3 (96.1) 138.0, 440.0	 221.4 (78.3) 103.0, 490.0
Challenge dose (LD_{50}) (n(%)) <200 200 or higher	 5 (50.0) 5 (50.0)	 7 (46.7) 8 (53.3)	 7 (50.0) 7 (50.0)	 6 (42.9) 8 (57.1)	 25 (47.2) 28 (52.8)
Positive quantitative bacteremia 24 hours post challenge (n(%))	3 (30.0)	1 (6.7)	0	0	4 (7.5)
Bacteremia 24 hours post challenge (cfu/mL) Geometric mean 95% confidence interval Mean (SD) of log_{10} bacteremia	 13.7 1.4, 132.5 1.14 (1.38)	 2.5 1.6, 3.9 0.39 (0.36)	 2.0 NA 0.30 (0.00)	 2.0 NA 0.30 (0.00)	 3.1 2, 4.7 0.49 (0.68)

NA: not available because only one value.

6.6.2.4 Results

Time to bacteremia

The time to quantitative bacteremia is shown in the following table. In the treatment groups, the sample sizes were too small. Therefore it is not possible to make a conclusive comparison for the time to bacteremia. Given the administration of ETI-204, most animals in the treated groups did not develop bacteremia. This also demonstrated the prophylactic effect of ETI-204.

Table 112. Study AP305: Time to quantitative bacteremia

	Placebo	ETI-204 16 mg/kg PrEP-3	ETI-204 16 mg/kg PrEP-2	ETI-204 16 mg/kg PrEP-1	Total
Time to quantitative bacteremia (hours)					
N	10	2	3	1	16
Mean (SD)	44.3 (14.5)	39.7 (22.3)	54.3 (0.8)	95.6 (0)	48.8 (18.3)
Range	22.3, 55.3	23.9, 55.4	53.4, 54.9	95.6, 95.6	22.3, 95.6

Survival

As the following table shows, the survival proportions in the treatment groups were 100%. Using the closed-testing procedure, all treatment groups were statistically significant. There was one surviving animal in the control group, which received a challenge dose of 330 LD_{50} spores, had a bacteremia of 400 cfu/mL at 24 hours post challenge and was non-bacteremic on Day 7, 14, 28 and 56.

Table 113. Study AP305: Survival at Day 56 by treatment group

	Placebo (N=10)	ETI-204 16 mg/kg PrEP-3 (N=15)	ETI-204 16 mg/kg PrEP-2 (N=14)	ETI-204 16 mg/kg PrEP-1 (N=14)
n (%)	1 (10.0)	15 (100.0)	14 (100.0)	14 (100.0)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.90 [0.554, 0.998] <0.0001*	0.90 [0.555, 0.998] <0.0001*	0.90 [0.555, 0.998] <0.0001*

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Significant at an overall one-sided significance level of 0.025

Survival analyses demonstrated that each treatment group significantly improved survival compared with the control group.

Figure 63. Study AP305: Kaplan-Meier curve by treatment group

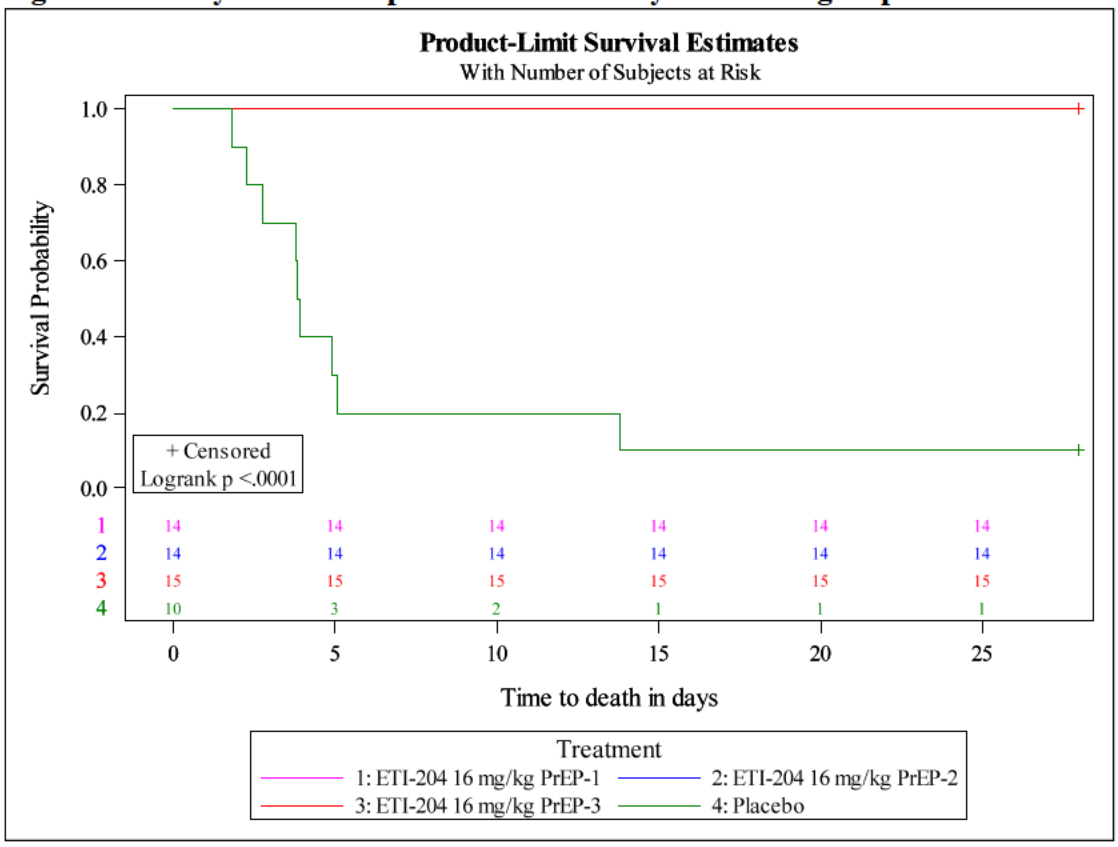


Table 114. Study AP305: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

ETI-204 16 mg/kg PrEP-3 (N=15)	ETI-204 16 mg/kg PrEP-2 (N=14)	ETI-204 16 mg/kg PrEP-1 (N=14)
<0.0001*	<0.0001*	<0.0001*

*Significant at a two-sided significance level of 0.05

Bacteremia over time

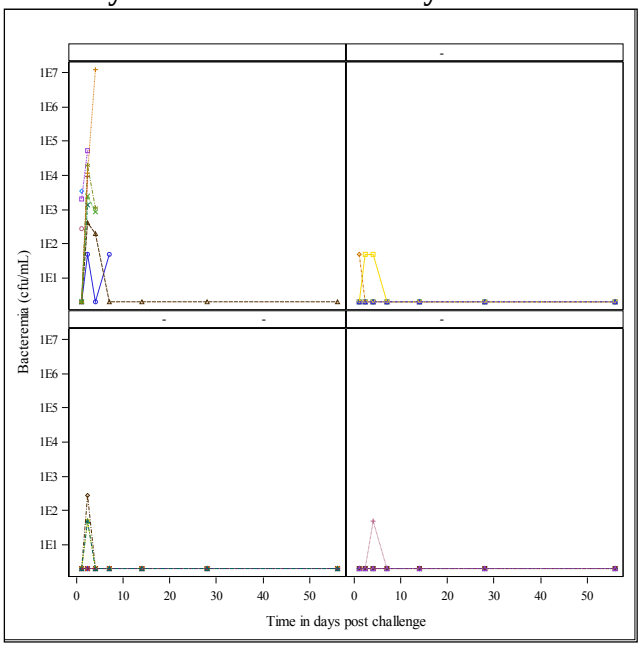
As shown in the following graph, the bacteremia levels in control group were higher at 54 hours post-challenge than at 24 hours. In contrast, all the bacteremia levels in the treatment groups remained at a lower level. From 7 days post-challenge on, all surviving animals in the treatment groups had a bacteria level below LOD. The surviving animal in the control group only had a bacteremia (400 and 200 cfu/mL) at 54 and 96 hours post-challenge among all visits shown in the graph and were negative at other visits.

Tissue bacterial assessments and pathological findings in the brain

In all surviving animals, there were no bacteria found in the brain, lymph node, liver, and spleen. Eight out of 9 dead animals in the placebo group had a positive result in the brain.

Only one dead animal (11.1%) from the placebo group had a positive microscopic pathological result (discoloration(s)).

Figure 64. Study AP305: Bacteremia by treatment and animal



Subgroup Analyses

The survival proportions were comparable across different subgroups, because the survival proportions in the treatment groups were 100%. The only surviving animal in the control group was male, challenged with a 330 LD_{50} dose.

Table 115. Study AP305: Survival at Day 56 by gender and challenge dose

	Placebo (N= 10)	**ETI-204 16 mg/kg PrEP-3 (N= 15)**	**ETI-204 16 mg/kg PrEP-2 (N= 14)**	**ETI-204 16 mg/kg PrEP-1 (N= 14)**	**Total (N= 53)**
Gender					
Female	0/5	8/8 (100%)	7/7 (100%)	7/7 (100%)	22/27 (81.5%)
Male	1/5 (20%)	7/7 (100%)	7/7 (100%)	7/7 (100%)	22/26 (84.6%)
Challenge dose (LD_{50})					
<250	0/6	11/11 (100%)	10/10 (100%)	9/9 (100.0%)	30/36 (83.3%)
250 or higher	1/4 (25%)	4/4 (100%)	4/4 (100%)	5/5 (100%)	14/17 (82.4%)
Bacteremia prior to treatment (cfu/mL)					
$<10^2$	1/7 (14.3%)	15/15 (100%)	14/14 (100%)	14/14 (100%)	44/50 (88%)
10^2 - 10^4	0/3				0/3

6.6.2.5 Conclusion

This study demonstrated that 16 mg/kg IM of ETI-204 administered 1 to 3 days prior to challenge provided significant prophylactic protection against anthrax infection. The Lonza product was used in this study.

6.7 Rabbit Pre-Exposure Prophylaxis Studies

6.7.1 Summary of rabbit pre-exposure prophylaxis studies

There are two rabbit studies to assess the pre-exposure prophylactic effect of ETI-204 with varying doses administered IV or IM within 45 minutes prior to challenge. The product used in the two studies was manufactured at the Elusys facility. All doses of no less than 5 mg IV or a dose of 20 mg IM statistically significantly improved survival compared with a 0 survival proportion in the control groups.

6.7.2 AR001

Assessment of the Effectiveness of a Monoclonal anti-PA Antibody Candidate as Therapeutic Protection Against a *Bacillus anthracis* Aerosol Challenge in the Rabbit Model
Conducted under (b) (4) Study No. 357-G004819

6.7.2.1 Study Design and Endpoints

Primary Objective

The primary objective was to examine the efficacy of the (b) (4) anti-PA monoclonal antibody (ETI-204), when administered as a therapeutic treatment, against lethality due to inhalational exposure to *B. anthracis* spores in rabbits.

Study Design

This was a randomized, placebo-controlled, pre-exposure (dosing 30-45 minutes prior to exposure), with treatment administered at a fixed dose, conducted at (b) (4) in 2003.

Animals were randomized by weight into the following groups:

- Placebo (phosphate-buffered saline, PBS) IV
- ETI-204 10 mg/animal (approximately 4 mg/kg) IV (one animal received 8.13 mg)

The test product was from the Elusys facility.

After receiving a single IV dose, all animals were challenged with a targeted aerosol dose of 100 LD_{50}s on Study Day 0. Clinical observations were performed twice daily.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.7.2.2 Statistical Methodologies

Sample Size Calculation

Sample sizes of 5 control and 10 treated animals were considered in the protocol sufficient to provide greater than 80% power to detect a difference when the survival probabilities were 10% in the control group and 80% in the treated group, using a one-sided Fisher's exact test. In the study report, a 5% significance level was mentioned.

Comment: We could replicate this calculation using a one-sided type I error of 0.05. However, type I rate of 0.025 should be used.

Analysis Population

In the protocol the study population was not defined and in the analysis all randomized animals were included.

Statistical Method

One-sided Fisher's exact test was used to compare the survival rates between the antibody group and the control group.

6.7.2.3 Animal Disposition, Demographic and Baseline Characteristics

Age was estimated using a range. Gender and challenge doses were comparable. Because the targeted challenge dose was 100 LD_{50}s, about 80% of animals received a dose less than 200 LD_{50}s. No animals had qualitative bacteremia 24 hours post challenge.

Table 116. Study AR001: Demographic variables and baseline characteristics by treatment group

	Placebo (N=5)	**ETI-204 8.13 or 10.16 mg 30-45 min IV PrEP (N=9)**	**Total (N=14)**
Age (weeks) Range	 13-17	 13-17	 13-17
Gender [n (%)] Female Male	 2 (40.0) 3 (60.0)	 4 (44.4) 5 (55.6)	 6 (42.9) 8 (57.1)
Body weight (kg) Mean (SD) Range	 2.4 (0.2) 2.2, 2.6	 2.3 (0.1) 2.2, 2.4	 2.3 (0.1) 2.2, 2.6

	Placebo (N=5)	ETI-204 8.13 or 10.16 mg 30-45 min IV PrEP (N=9)	Total (N=14)
Challenge dose (LD_{50})			
Mean (SD)	171.9 (55.2)	156.0 (43.9)	161.7 (46.7)
Range	96.4, 244.0	106.1, 217.5	96.4, 244.0
Challenge dose (LD_{50}) (n(%))			
<200	4 (80.0)	8 (88.9)	12 (85.7)
200 or higher	1 (20.0)	1 (11.1)	2 (14.3)

6.7.2.4 Results

Time to bacteremia

The mean time to bacteremia in the control group was 72 hours. No animals developed bacteremia in the treatment group.

Table 117. Study AR001: Time to bacteremia

	Placebo (N=5)	ETI-204 8.13 or 10.16 mg 30-45 min IV PrEP (N=9)	Total (N=14)
Time to qualitative bacteremia (hours)			
N	5	NA	5
Mean (SD)	72.0 (33.9)		72.0 (33.9)
Range	48, 120		48, 120

Survival

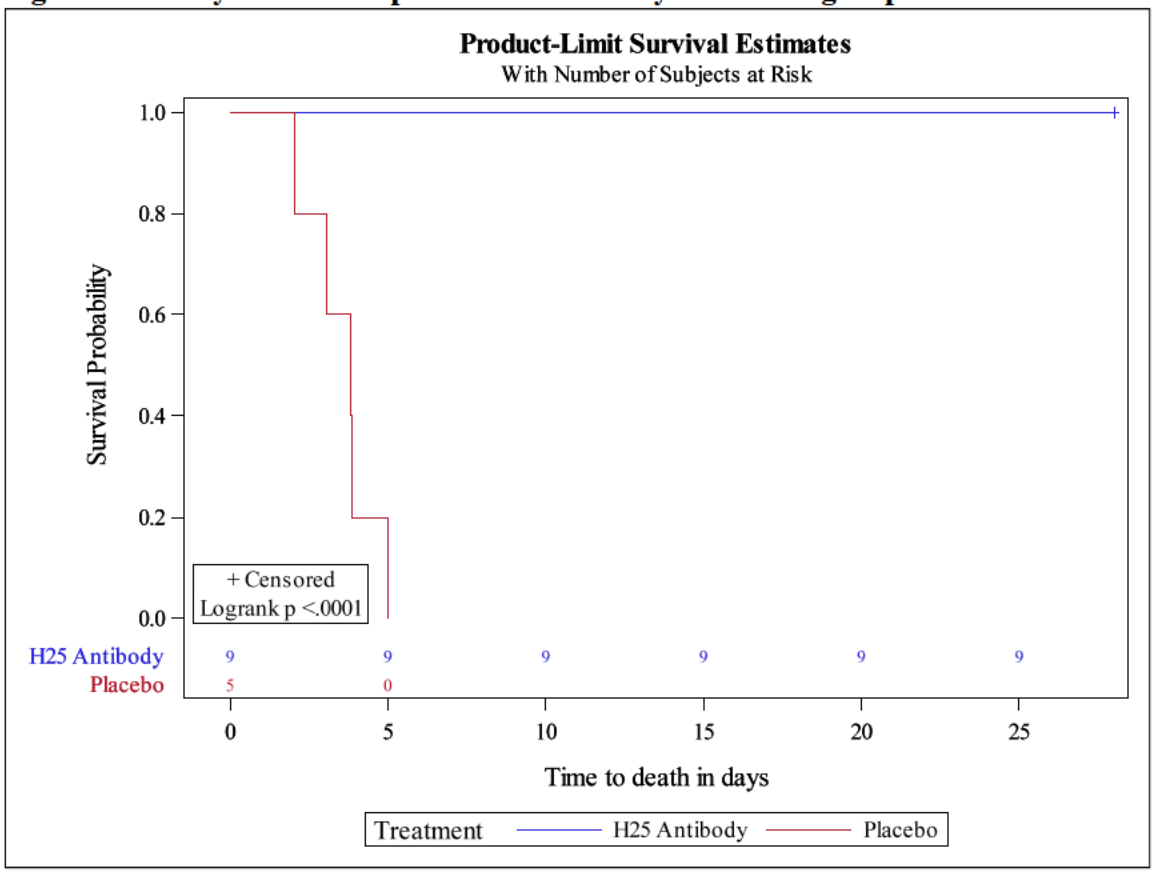
There was a statistically significant difference in survival proportions between the two groups, as the following table and graph show.

Table 118. Study AR001: Survival at Day 28 by treatment group

	Placebo (N=5)	ETI-204 8.13 or 10.16 mg 30-45 min IV PrEP (N=9)
n (%)	0 (0.0)	9 (100.0)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-values		1.00 0.474, 1 0.0001*

Two-sided 95% confidence interval and one-sided p-values were calculated by the reviewer
*Significant at a one-sided significance level of 0.025

Figure 65. Study AR001: Kaplan-Meier curve by treatment group



Bacteremia

Three control animals (out of 5) developed qualitative bacteremia on Day 2 (48 hours post-challenge) and no data were available after this visit. No animals in the treated group developed qualitative bacteremia on Days 1, 2, 7, 10, 14, 21, and 28.

Tissue bacterial assessments and pathological findings in the brain

Sections of the spleen, lung and intra-thoracic lymph nodes from each surviving animal on Study Day 28 were cultured for the presence or absence of bacteremia. Out of all the tissues cultured, two lung samples and one lymph node sample, each from a different animal had a positive culture for bacteremia while all other tissue samples were negative.

There were no positive pathological findings in the brain.

Subgroup analyses

There was no gender-related survival difference. All animals had a challenge dose less than 250 LD_{50}s, so it was not possible to examine the trend using this cut-off point for challenge dose.

Table 119. Study AR001: Survival at Day 28 by gender and challenge dose

	Placebo (N=5)	ETI-204 8.13 or 10.16 mg 30-45 min PrEP (N=9)	Total (N=14)
Gender			
Male	0/3	5/5 (100%)	5/8 (62.5%)
Female	0/2	4/4 (100%)	4/6 (66.7%)

6.7.2.5 Conclusion

This study shows that 10 mg ETI-204 (approximately 4 mg/kg) administered IV 30-45 minutes prior to challenge provided significant prophylactic protection from anthrax infection. This study used the Elusys product.

6.7.3 AR003

Minimum Effective Dose of the (b) (4) Monoclonal anti-PA Antibody when Administered Immediately Prior to Challenge Against a Aerosolized Anthrax in the NZW Rabbit Model
Conducted under (b) (4) Study No. 397-G004957

6.7.3.1 Study Design and Endpoints

Primary Objective

The primary objective was to examine the efficacy of varying doses of the (b) (4) anti-PA monoclonal antibody ETI-204 delaying or preventing death in rabbits from anthrax when administered as a therapeutic treatment at various dose concentrations and routes immediately (within 35 minutes) prior to an inhalational exposure to *B. anthracis*.

Study Design

This was a randomized, placebo-controlled, parallel group, pre-exposure (dosing within 35 minutes prior to exposure), dose ranging study with treatment administered at fixed doses, conducted at (b) (4) in 2004.

Animals were randomized into the one of the following groups:

Group	Dose (mg/animal) [mg/kg]	Number of Animals Planned
Placebo (PBS)	0	8
ETI-204 IV	1.25 [0.05]	8
ETI-204 IV	2.5 [1]	8
ETI-204 IV	5 [2]	8
ETI-204 IV	10 [4]	8
ETI-204 IM	20 [8]	8

PBS: phosphate-buffered saline

The test product was manufactured at the Elusys facility.

Immediately (within 35 minutes), all animals were challenged with a targeted dose of approximately 200 LD_{50}'s. Clinical observations were performed daily.

Primary Endpoint

The primary endpoint was survival to 28 days post anthrax spore challenge.

6.7.3.2 Statistical Methodologies

Sample Size Calculation

The sample size of 8 rabbits in each arm provided 80% power at a 5% significant level to detect the difference in survival rates between the treatment arms and the vehicle control arm.

Comment: The assumed survival proportions in the two groups were not provided.

Analysis Populations

In the protocol the study population was not defined and in the analysis all randomized animals were included.

Statistical Methods

One-sided Fisher's exact test at the 0.05 was used to compare the survival rates between each individual antibody group and the control group.

6.7.3.3 Animal Disposition, Demographic and Baseline Characteristics

Demographic variables and baseline characteristics were comparable across different groups, except for challenge dose in the placebo group, which had more variability and a higher proportion of less than 200 LD_{50}s (Table 120). This was not a concern because all control animals succumbed to anthrax and challenge doses were high enough in the treated groups.

Table 120. Study AR003: Demographic variables and baseline characteristics by treatment group

	Placebo (N=8)	ETI-204 1.25 mg IV (N=8)	ETI-204 2.5 mg IV (N=8)	ETI-204 5 mg IV (N=8)	ETI-204 10 mg IV (N=8)	ETI-204 20 mg IM (N=8)	Total (N=48)
Age (weeks) range	13-17	13-17	13-17	13-17	13-17	13-17	13-17
Gender [n (%)]							
Female	4 (50.0)	3 (37.5)	5 (62.5)	4 (50.0)	4 (50.0)	4 (50.0)	24 (50.0)
Male	4 (50.0)	5 (62.5)	3 (37.5)	4 (50.0)	4 (50.0)	4 (50.0)	24 (50.0)
Body weight (kg)							
Mean (SD)	2.5 (0.0)	2.4 (0.2)	2.4 (0.1)	2.4 (0.1)	2.5 (0.1)	2.5 (0.1)	2.5 (0.1)
Range	2.4, 2.6	2.2, 2.6	2.2, 2.6	2.3, 2.5	2.3, 2.5	2.4, 2.6	2.2, 2.6

	Placebo (N=8)	ETI-204 1.25 mg IV (N=8)	ETI-204 2.5 mg IV (N=8)	ETI-204 5 mg IV (N=8)	ETI-204 10 mg IV (N=8)	ETI-204 20 mg IM (N=8)	Total (N=48)
Challenge dose (LD_{50})							
Mean (SD)	301.0 (117.8)	282.1 (84.8)	296.6 (53.1)	303.8 (78.0)	269.8 (99.6)	268.1 (56.6)	286.9 (81.4)
Range	163.2, 434.6	91.8, 358.8	228.1, 401.5	180.3, 413.7	106.2, 404.6	187.1, 352.5	91.8, 434.6
Challenge dose (LD_{50}) (n(%))							
<200	3 (37.5)	1 (12.5)	0	1 (12.5)	2 (25.0)	2 (25.0)	9 (18.8)
200 or higher	5 (62.5)	7 (87.5)	8 (100)	7 (87.5)	6 (75.0)	6 (75.0)	39 (81.3)
Positive qualitative bacteremia 24 hours post challenge (n(%))	2 (25.0)	0	1 (12.5)	1 (12.5)	0	0	4 (8.3)

6.7.3.4 Results

Time to bacteremia

As shown in the following table, the 1.25 mg group had a longer time to bacteremia than the placebo group. The sample sizes in other groups were too small to make a conclusion about the time to bacteremia. But only a few treated animals with a dose no less than 2.5 mg developed bacteremia, which indicated the prophylactic effect of the product.

Table 121. Study AR003: Time to qualitative bacteremia

	Placebo (N= 8)	ETI-204 1.25 mg IV (N= 8)	ETI-204 2.5 mg IV (N= 8)	ETI-204 5 mg IV (N= 8)	ETI-204 10 mg IV (N= 8)	ETI-204 20 mg IM (N= 8)	Total (N= 48)
Time to qualitative bacteremia (hours)							
N	7	6	1	1	NA	NA	15
Mean (SD)	65.1 (18.1)	128 (29.1)	192	168			
Range	48, 96	96, 168					

*Derived from study visits, from specimen collection day

Survival

Using a one-sided type I error of 0.025/5=0.005, all treatment groups except for the 1.25 mg group had a statistically significant difference compared with the placebo group (p-values from a Boschloo's test in Table 122).

Table 122. Study AR003: Survival at Day 28 by treatment group

	Placebo (N= 8)	ETI-204 1.25 mg IV (N= 8)	ETI-204 2.5 mg IV (N= 8)	ETI-204 5 mg IV (N= 8)	ETI-204 10 mg IV (N= 8)	ETI-204 20 mg IM (N= 8)
n (%)	0	1 (12.5)	5 (62.5)	5 (62.5)	7 (87.5)	8 (100)
Difference in survival proportion compared with placebo [exact 95% confidence interval] one-sided p-value		0.125 [-0.292, 0.527] 0.5 0.402	0.625 [0.173, 0.915] 0.004*	0.625 [0.173, 0.915] 0.004*	0.875 [0.395, 0.997] 0.0003*	1 [0.588, 1] <0.0001*
Adjusted exact 95% confidence interval		-0.427, 0.632	0.019, 0.953	0.019, 0.953	0.237, 0.999	0.436, 1

Two-sided 95% confidence interval and one-sided Boschloo's p-values were calculated by the reviewer
*Significant at a two-sided significance level of 0.025/5=0.005

All control animals died by Day 4. There was a statistically significant difference between all of the treatment groups and the placebo group, using a two-sided significance level of 0.05/5=0.01 (Figure 66 and Table 123).

Figure 66. Study AR003: Kaplan-Meier curve by treatment group

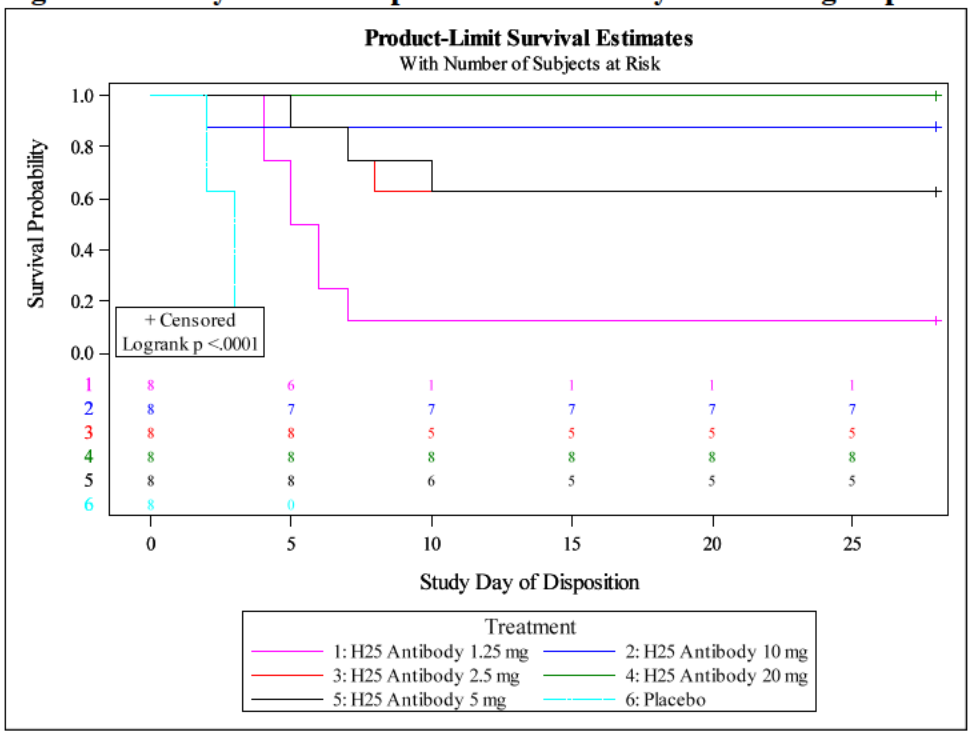


Table 123. Study AR003: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

ETI-204 1.25 mg IV (N= 8)	ETI-204 2.5 mg IV (N= 8)	ETI-204 5 mg IV (N= 8)	ETI-204 10 mg IV (N= 8)	ETI-204 20 mg IM (N= 8)
0.0002*	<0.0001*	<0.0001*	0.0009*	<0.0001*

*Significant at a two-sided significance level of 0.05/5=0.01

Tissue bacterial assessment

No positive bacterial results were reported from both non-survivors and survivors. No microscopic results were reported.

Subgroup Analysis Results

The following table shows the results of subgroup analyses. The sample sizes were too small to see a reliable trend by gender and challenge dose.

Table 124. Study AR003: Survival at Day 28 by gender and challenge dose

	Placebo (N= 8)	ETI-204 1.25 mg IV (N= 8)	ETI-204 2.5 mg IV (N= 8)	ETI-204 5 mg IV (N= 8)	ETI-204 10 mg IV (N= 8)	ETI-204 20 mg IM (N= 8)	Total (N= 48)
Gender							
Female	0/4	0/3	4/5 (80%)	3/4 (75%)	4/4 (100%)	4/4 (100%)	15/24 (62.5%)
Male	0/4	1/5 (20%)	1/3 (33.3%)	2/4 (50%)	3/4 (75%)	4/4 (100%)	11/24 (45.8%)
Challenge dose (LD_{50})							
<250	0/4	0/1	0/1	2/2 (100%)	2/2 (100%)	2/2 (100%)	6/12 (50%)
250 or higher	0/4	1/7 (14.3%)	5/7 (71.4%)	3/6 (50%)	5/6 (83.3%)	6/6 (100%)	20/36 (55.6%)

6.7.3.5 Conclusions

This study demonstrated that ETI-204 administered IV with a dose no less than 2.5 mg/animal (approximately 1 mg/kg) or IM 20 mg/animal (approximately 8 mg/kg) within 35 minutes prior to challenge significantly improved survival. This study used the Elusys project.

6.8 Re-challenge study (AR034 Phase II)

There was one re-challenge study (AR034) to demonstrate that ETI-204 administered intravenously alone or in combination with antibiotics following primary challenge with spores of *B. anthracis* results in development of protective immunity as measured by increased survival in the absence of treatment following secondary challenge. This study was conducted by the applicant and the Lonza product was used. There were two phases in this study. In Phase I, rabbits were challenged and treated with ETI-204 alone, levofloxacin, ETI-204 + levofloxacin, or placebo. In Phase 2 surviving animals from the treated groups were re-challenged with no treatment.

Section 6.5.5 reviews the study design and the results from Phase I ETI-204 alone compared to the placebo. The review of Phase I comparing the combination to the levofloxacin alone arm was covered in the review by Dr. Ling Lan. In this section we briefly review the results from Phase II, the re-challenge portion of the study.

In Phase I, animals were challenged and treated with ETI-204 16 mg/kg (IV), levofloxacin (50 mg/kg/day for 3 days), ETI-204 and levofloxacin, or placebo. Surviving animals were re-challenged and new control animals were challenged 9 months later in Phase II. No treatment was administered in Phase 2. The analysis population included all animals that were spore challenged in Phase II. The primary endpoint was survival to day 21 in phase II.

The study results from this study by Phase are in Table 125. The survival proportions in Phase II were 100% in the ETI-204 alone re-challenged group, 89% in the ETI-204 and levofloxacin re-challenged group, and 95% in the levofloxacin -alone re-challenged group. All were statistically significantly different than the Phase II control group with no surviving animals. This demonstrated that ETI-204 with or without co-administration with levofloxacin provided a statistically significant post-exposure prophylactic effect after first exposure to anthrax spores and the ETI-204 treated animals in Phase I could develop protective immunity after a secondary exposure to anthrax spores.

Table 125. Study AR034: Survival at the end of each phase by treatment group

	Control n/N(%)	**ETI-204** n/N(%)	**Levo** n/N(%)	**ETI-204 and Levo** n/N(%)
Phase I	0/8 (phase I controls)	13/20	20/20	19/20
Phase II	0/12 (phase II controls)	13/13 (100%)	19/20 (95%)	17/19 (89%)
Phase II analysis (treatment – phase 2 control) p-value and 95% CI		<0.0001* (0.025) [0.724, 1]	<0.0001* (0.025) [0.695, 0.999]	<0.0001* (0.025) [0.615, 0.987]

Two-sided 95% confidence interval and one-sided p-values from Boschloo's test were calculated by the reviewer
*Statistically significant at the specified significant level

6.9 Summary of all reviewed monotherapy studies

The following table shows a summary of all monotherapy studies in this review. The adjusted 95% confidence interval was calculated based on the type I error based on the Bonferroni method for multiple comparisons if needed.

Table 126. Summary of all reviewed monotherapy studies

Study	Animals	Administration			Survival n/N (%)	Difference in survival [95% CI]
		Route	Time (hrs) from challenge	Dose		
Treatment						
AP202 Lonza & Baxter	Monkeys	IV	39	0	0/17 (0)	
				16 (Lonza)	5/16 (31)	0.31 [0.08, 0.59]*
				16 (Baxter)	6/17 (35)	0.35 [0.11, 0.66]*
AP203 Lonza	Monkeys	IV	37	0	2/16 (12.50)	
			36	8	1/16 (6.25)	-0.063 [-0.358, 0.238]
			38	32	6/16 (37.50)	0.25 [-0.114, 0.577]
AP204 Baxter	Monkeys	IV	39	0	1/16 (6.3)	
			40	4	4/16 (25.0)	0.188 [-0.090, 0.473]
			44	16	8/16 (50.0)	0.438 [0.070, 0.733]
AP201 Baxter	Monkeys	IV	45	0	2/14 (14.3)	
			41	4	11/14 (78.6)	0.643 [0.206, 0.898]*
			43	8	11/15 (73.3)	0.590 [0.162, 0.864]*
NIAID 1056 Baxter	Monkeys	IV			0/8 (0)	
			36		4/8 (50)	0.50 [0.058, 0.843]
AR021 Baxter	Rabbits	IV	32	0	1/10 (10)	
			28	1	4/10 (40.0)	0.3 [-0.219, 0.732]
			29	4	13/17 (76.5)	0.665 [0.155, 0.918]*
			30	16	16/17 (94.1)	0.841 [0.352, 0.989]*
AR033 Baxter	Rabbits	IV	27	0	0/14	
			28	1	4/14 (28.6)	0.286 [-0.077, 0.649]
			29	4	6/14(42.9)	0.429 [0.044, 0.769]*
			27	8	10/14 (71.4)	0.714 [0.312, 0.944]*
			28	16	9/14 (64.3)	0.643 [0.237, 0.909]*
NIAID 1030 Baxter	Rabbits			0	0/6 (0)	
		IV	32	8	12/16 (75)	0.75 [0.174, 0.941]*
NIAID 1045 Baxter	Rabbits			0	0/6 (0)	
		IV	73	8	7/11 (63.6)	0.636 [0.022, 0.911]*

Study	Animals	Adminstration			Survival n/N (%)	Difference in survival [95% CI]
		Route	Time (hrs) from challenge	Dose		
Post-exposure prophylaxis						
AP107 Baxter	Monkeys	IV or IM	24	0	1/6 (16.7)	
		IV	24	2	4/9 (44.4)	0.278 [-0.391, 0.765]
		IV	24	8	6/8 (75.0)	0.583 [-0.130, 0.941]
		IM	24	4	6/8 (75.0)	0.583 [-0.130, 0.941]
		IM	24	8	5/9 (55.6)	0.389 [-0.292, 0.835]
AP301 Lonza	Monkeys	IM	18	0	0/6 (0)	
			18	8	6/6 (100)	1 [0.438, 1]*
			18	16	6/6 (100)	1 [0.438, 1]*
			24	8	5/6 (83)	0.83 [0.196, 0.998]*
			24	16	5/6 (83)	0.83 [0.196, 0.998]*
			36	8	0/6 (0)	0
			36	16	3/6 (50)	0.5 [-0.069, 0.893]
AP307 Lonza	Monkeys	IM	24	0	1/10 (10)	
				16	13/14 (93)	0.83 [0.347, 0.987]*
AR004 Elusys	Rabbits	IV	48	0	0/9 (0)	
			24	4	8/10 (80.0)	0.80 [0.303, 0.986]*
			36	4	5/10 (50.0)	0.50 [-0.017, 0.856]
			48	4	3/7 (42.9)	0.429 [-0.084, 0.865]
AR007 (b) (4)	Rabbits	IV	9	0	0/9 (0)	
				4	9/9 (100)	1 [0.629, 1]*
		IM		8	9/9 (100)	1 [0.629, 1]*
AR012 Elusys	Rabbits	IM	24	0	0/9 (0)	
		IV		1	1/9 (11.1)	0.111 [-0.436, 0.610]
				4	6/12 (50)	0.50 [-0.057, 0.859]
		IV		8	7/12 (58.3)	0.583 [-0.018, 0.904]
		IM		2	1/9 (11.1)	0.111 [-0.436, 0.610]
				4	3/9 (33.3)	0.333 [-0.238, 0.794]
				8	5/12 (41.7)	0.417 [-0.134, 0.806]
				16	4/12 (33.3)	0.333 [-0.217, 0.749]
AR0315 Baxter	Rabbits	IM	24	0	0/10 (0)	
			18	4	11/12 (91.7)	0.917 [0.425, 1]*
AR0315 Baxter	Rabbits	IM	24	4	5/12 (41.7)	0.417 [-0.058, 0.786]
			18	16	11/12 (91.7)	0.917 [0.425, 1]*
			24	16	8/12 (66.7)	0.667 [0.172, 0.934]*
AR034 Lonza	Rabbits	IV	30	0	0/8	
			30	16	13/20 (65)	0.65 [0.300, 0.969]*
AR035 Lonza	Rabbits	IM	18	0	0/10 (0)	
			18	16	6/10 (60)	0.60 [0.119, 0.912]
			24	16	6/10 (60)	0.60 [0.119, 0.912]*
			36	16	0/8 (0)	0 [-0.387, 0.480]

Study	Animals	Administration			Survival n/N (%)	Difference in survival [95% CI]
		Route	Time (hrs) from challenge	Dose		
AR037 Lonza	Rabbits	IM	24	0	0/10	
				8	5/16 (31.3)	0.313 [-0.019, 0.587]
				16	5/16 (31.3)	0.313 [-0.019, 0.587]
				32	5/16 (31.3)	0.303 [-0.019, 0.587]
Pre-exposure prophylaxis						
AP305 Lonza	Monkeys	IM	72, 48, 24	0	1/10 (10)	
			72	16	15/15(100)	0.9 [0.554, 0.998]*
			48	16	14/14(100)	0.9 [0.554, 0.998]*
			24	16	14/14(100)	0.9 [0.554, 0.998]*
AR001 Elusys	Rabbits	IV	0.5 to 0.75	0	0/5 (0)	
				4	9/9 (100)	1 [0.474, 1]*
AR003 Elusys	Rabbits	IV	0.5	0	0/8 (0)	
				0.5	1/8 (12.5)	0.125 [-0.427, 0.632]
				1	5/8 (62.5)	0.625 [0.019, 0.953]*
				2	5/8 (62.5)	0.625 [0.019, 0.953]*
				4	7/8 (87.5)	0.875 [0.237, 0.999]*
		IM		8	8/8 (100)	1 [0.436, 1]*
Re-challenge						
AR034 Phase II Lonza	Rabbits			0	0/12 (0)	
		IV	9 months prior	16	13/13 (100)	1 [0.724, 1]*

Confidence interval reported in table is an adjusted 95% confidence interval constructed using the Bonferroni's method, if adjustment for multiple comparisons needed.
*Significant at an overall one-sided significance level of 0.025.

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/s/

XIANBIN LI
12/13/2015

KAREN M HIGGINS
12/14/2015
I concur.

TSAE YIN D LIN
12/14/2015