CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125509Orig1s000

STATISTICAL REVIEW(S)

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
Review Priority:	Standard
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Keywords: Animal efficacy studies

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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.

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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

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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.

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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.

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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

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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

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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

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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.

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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

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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

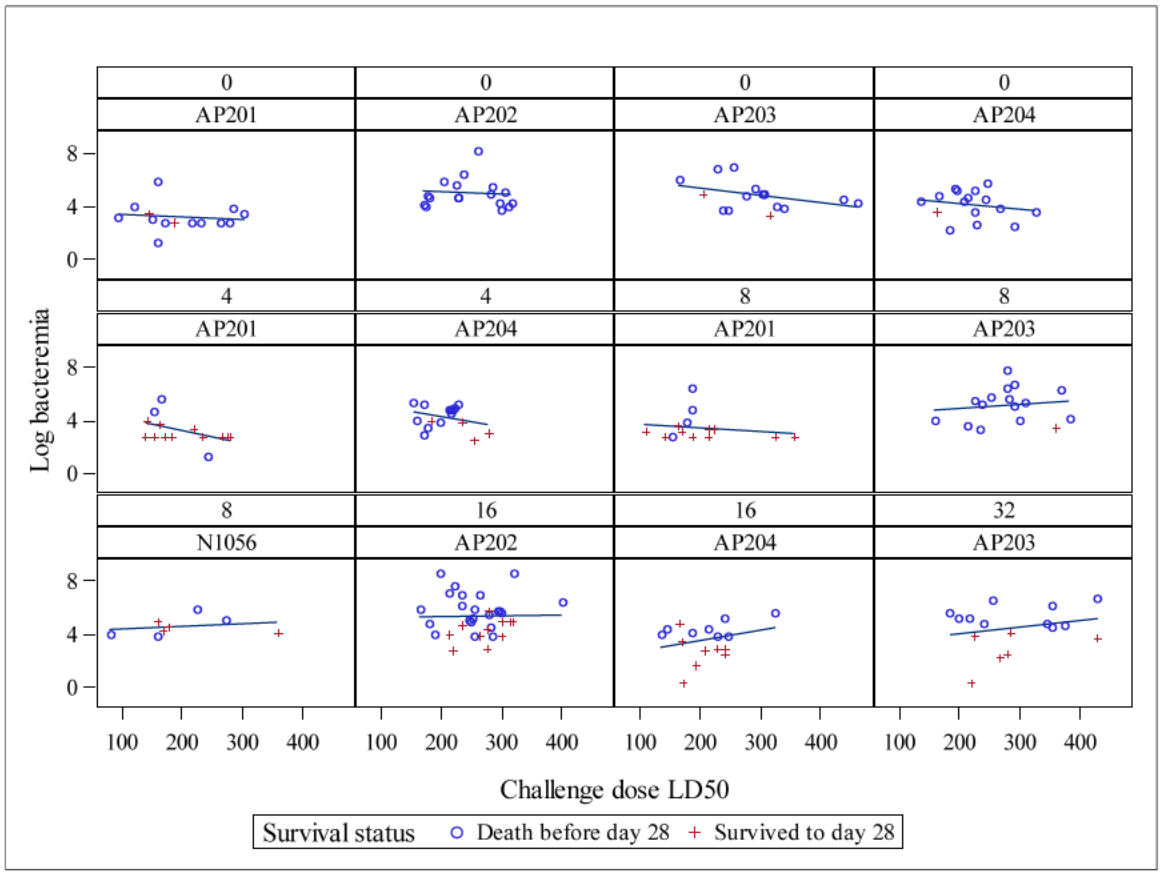

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Figure 2. Challenge dose and bacteremia prior to treatment by dose, study, and survival status in monkey treatment studies

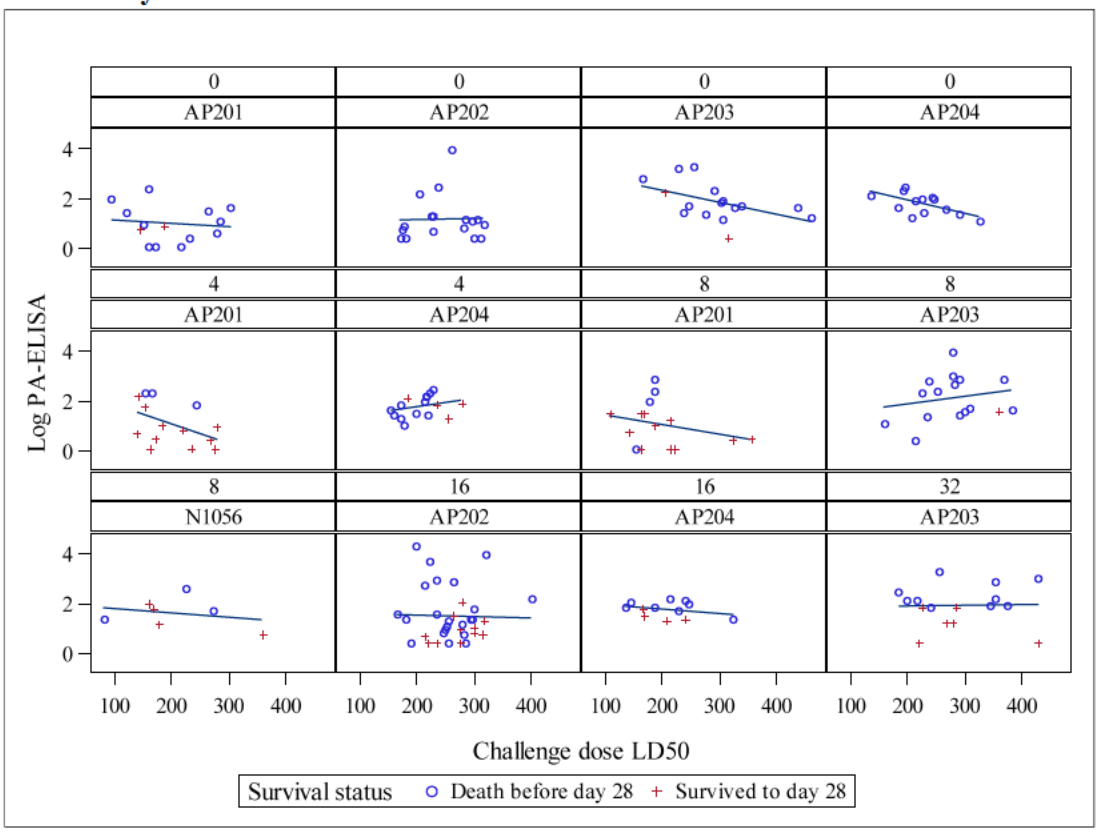

Figure 3 shows the relationship between bacteremia and PA-ELISA prior to treatment in the monkey treatment studies. The solid line in the middle is the regression line, and the dotted line is the 95% upper and lower confidence limits for individual predicted values and the shaded band is the 95% upper 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.

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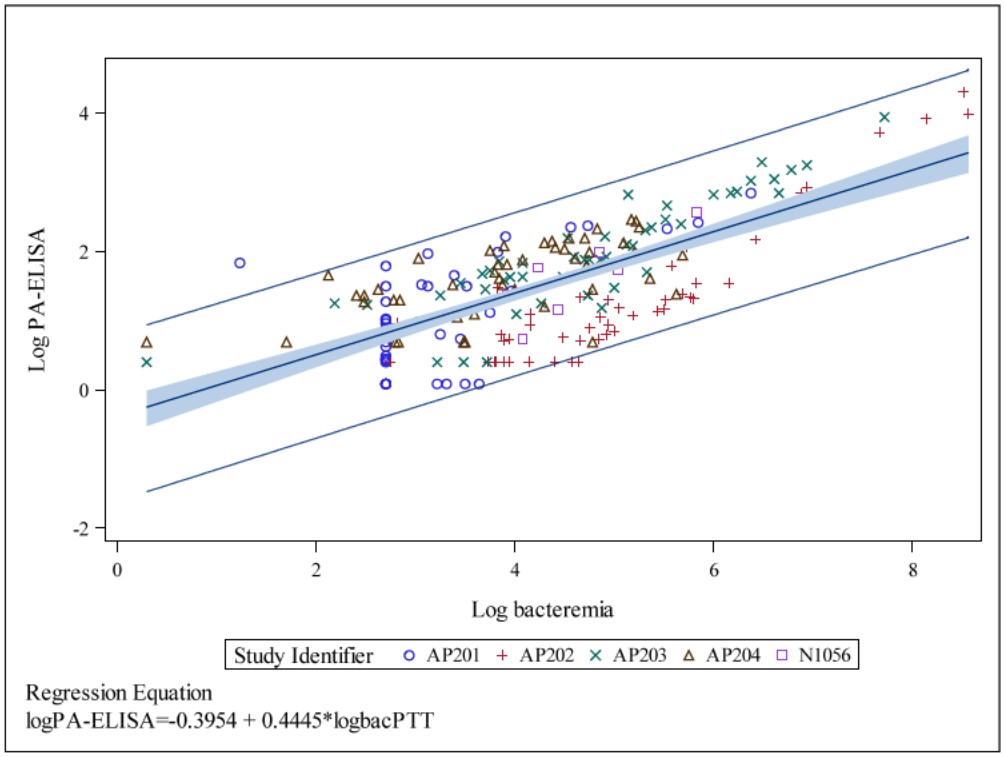
Figure 3. The relationship between bacteremia and PA-ELISA prior to treatment in monkey treatment studies



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.

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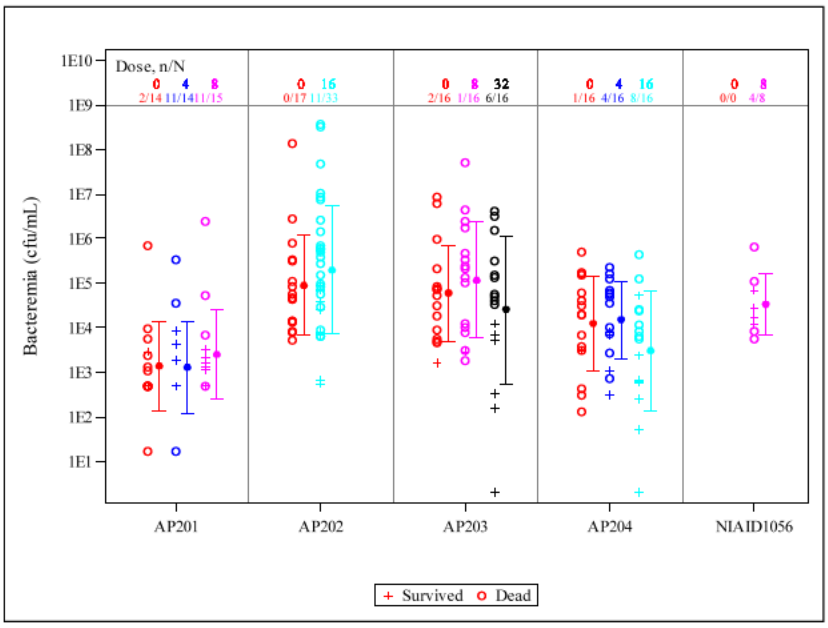
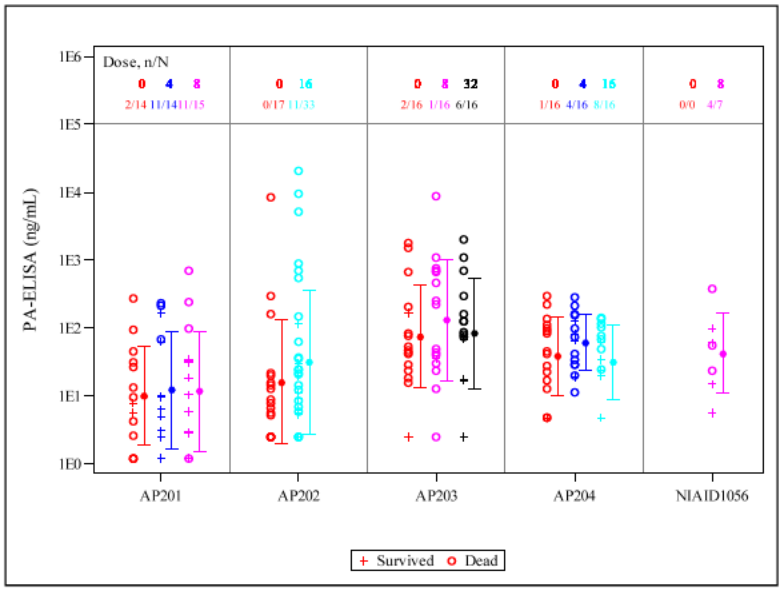
Figure 4. Bacteremia prior to treatment by study and treatment in monkey treatment studies



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

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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

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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

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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

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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

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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).

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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)

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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.

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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)

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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

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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

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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.

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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

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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.

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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.

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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.

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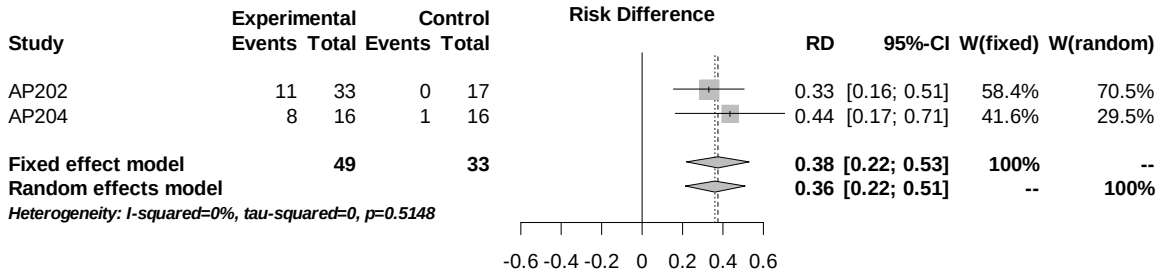
5.2.1 Meta-analysis of monkey and rabbits monotherapy studies

16 mg/kg IV in monkeys

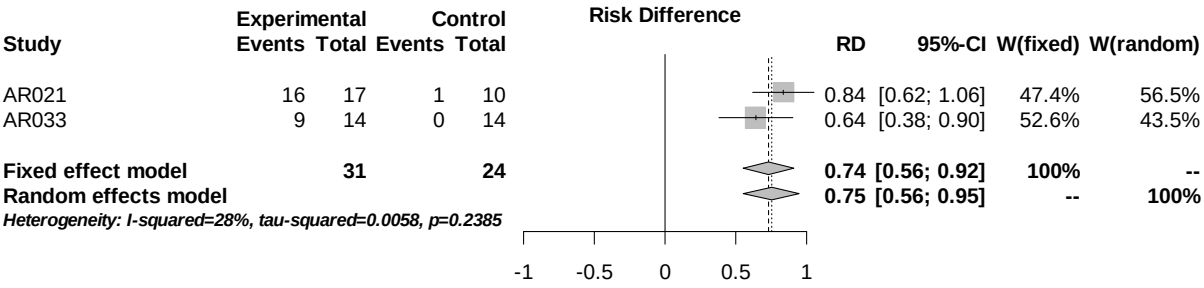
Because the 16 mg/kg dose is the proposed dose, this review will only include the meta-analysis results for this dose. Meta-analysis of 16 mg/kg IV in monkey studies is shown in the following graph.



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.

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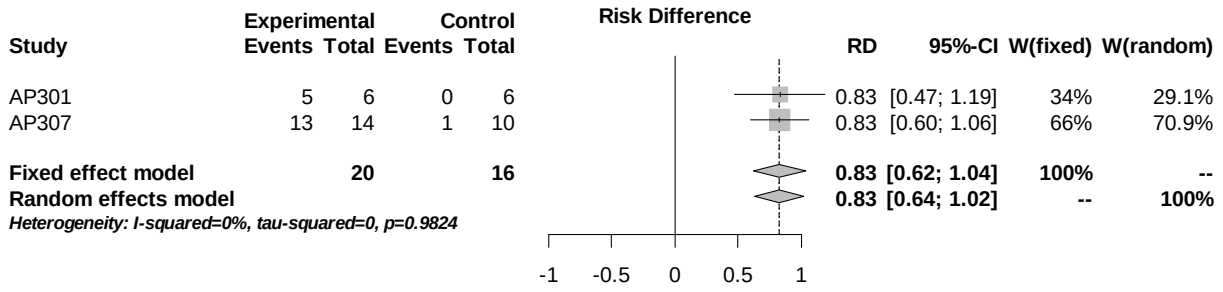
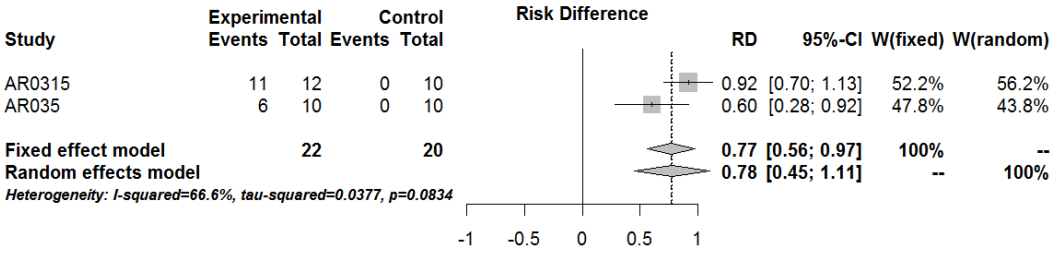
16 mg/kg IM 24 hours post challenge in monkeys

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.

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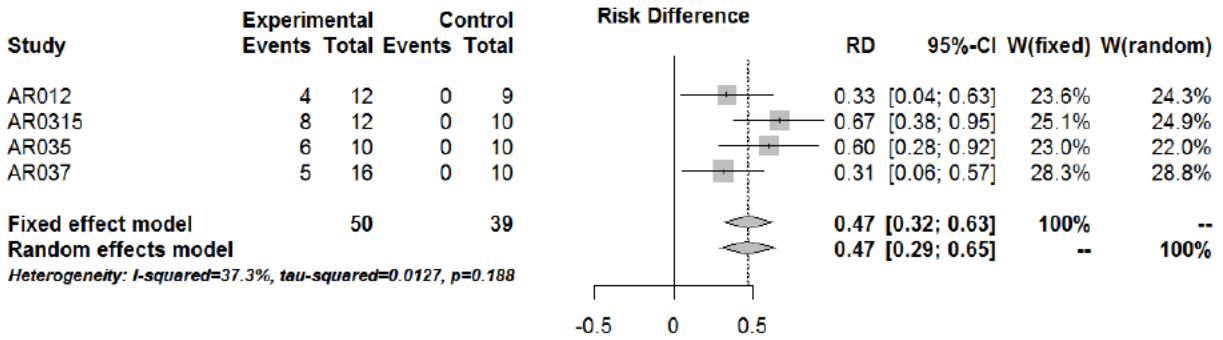
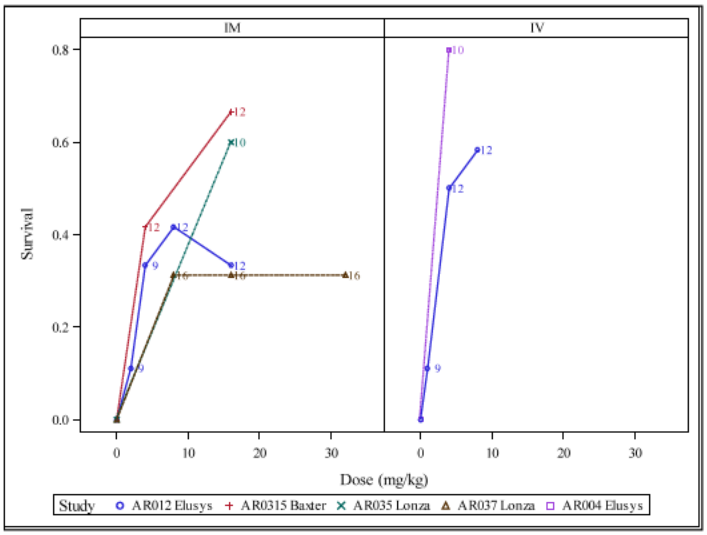


The following graph shows a summary of the PEP studies with ETI-204 administered 24 hours post challenge. The numbers in the graph indicate the group sample sizes. The survival proportions varied across studies and products. Note that the Lonza product administered via IM in study AR037 had the smallest 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

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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.

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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).

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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,

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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

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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.

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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.

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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.

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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

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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

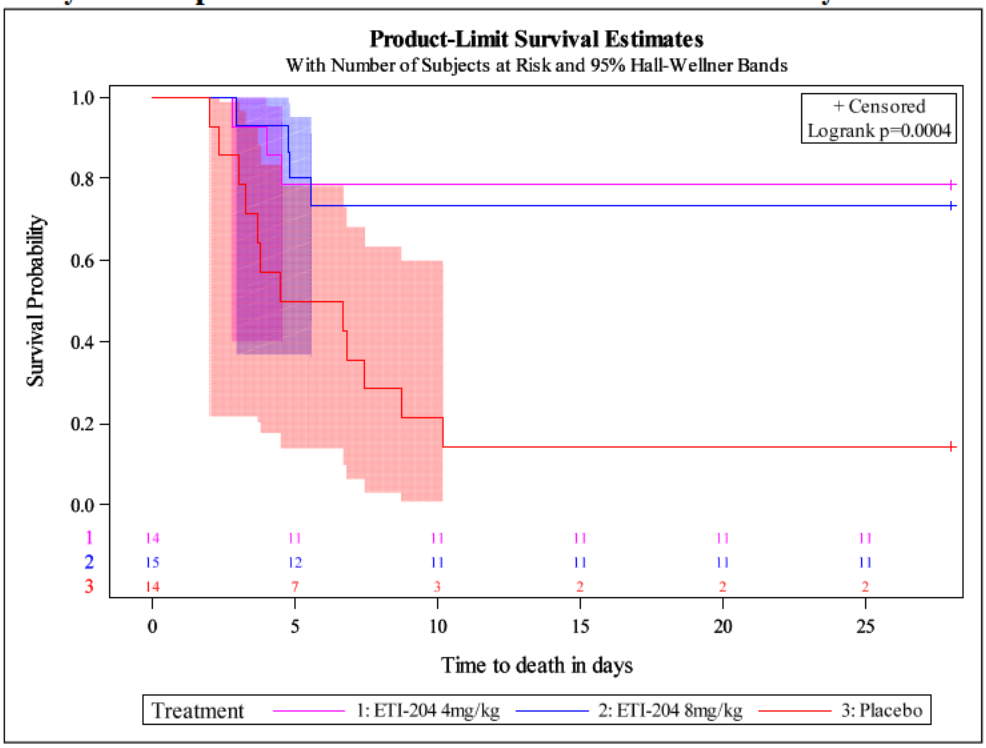

Table 24. Study AP201: two-sided p-values of pairwise log-rank tests comparing time from challenge to death among groups

	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.

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

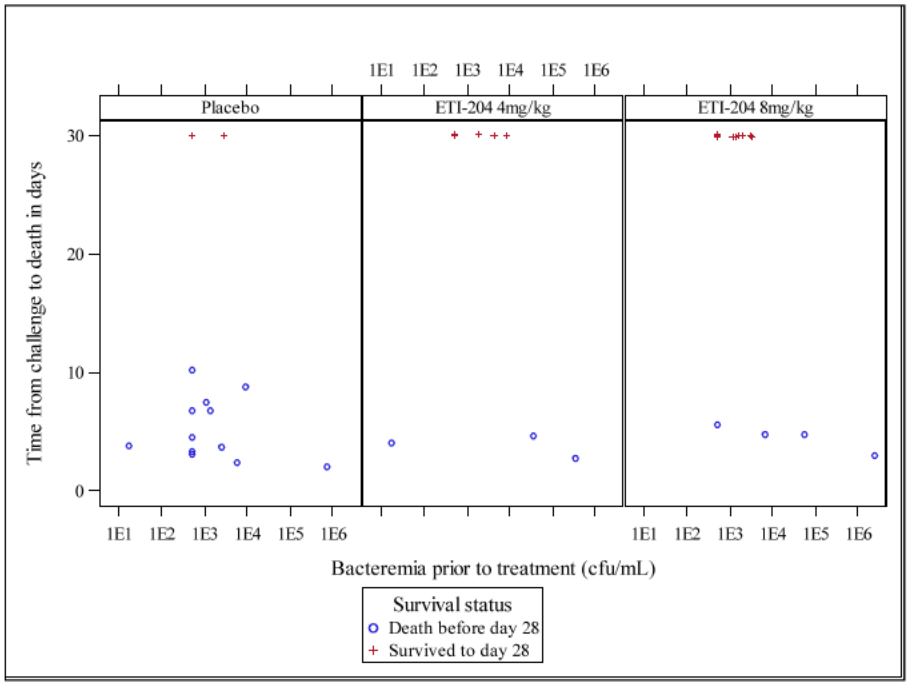
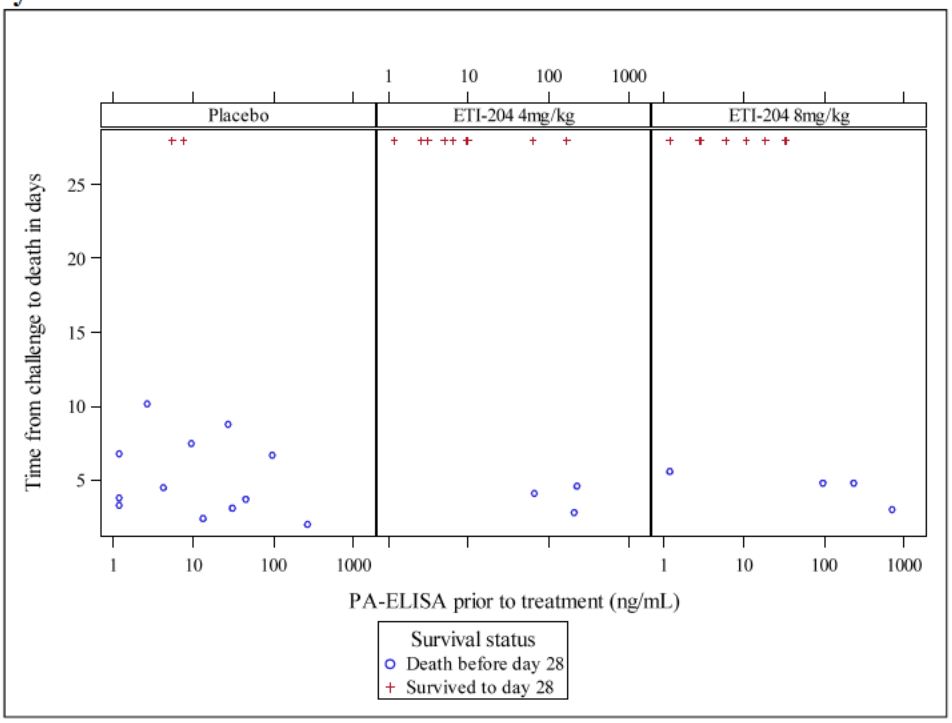


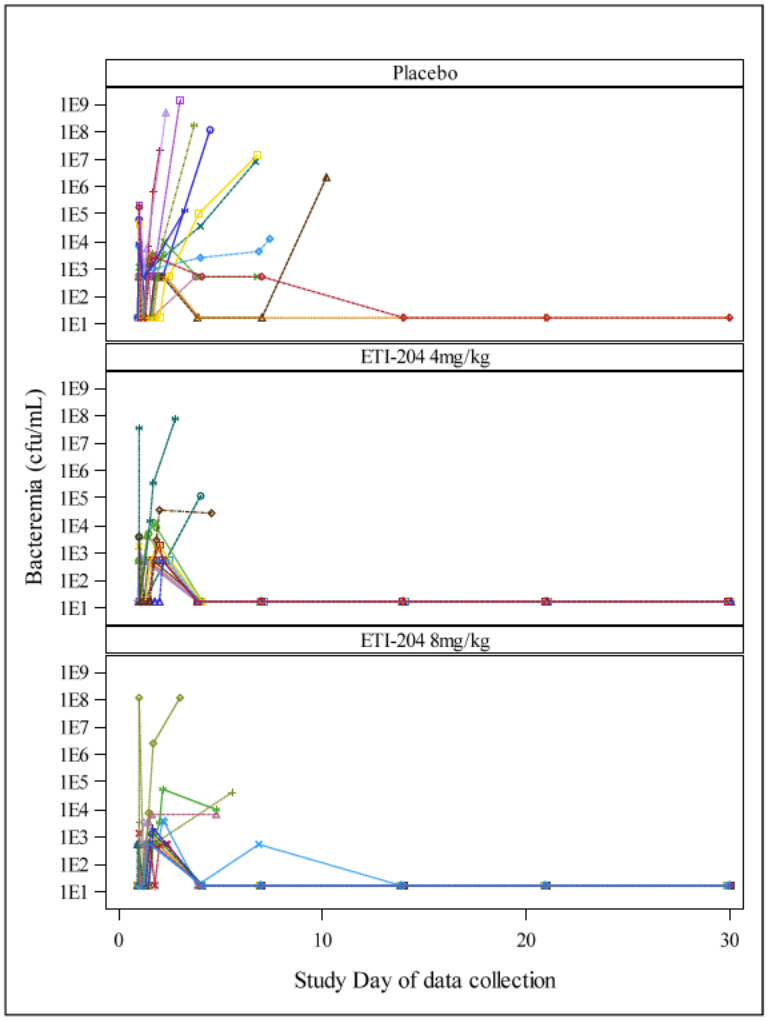
Figure 9. Study AP201: Time to death versus PA-ELISA prior to treatment by survival status at Day 28



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.

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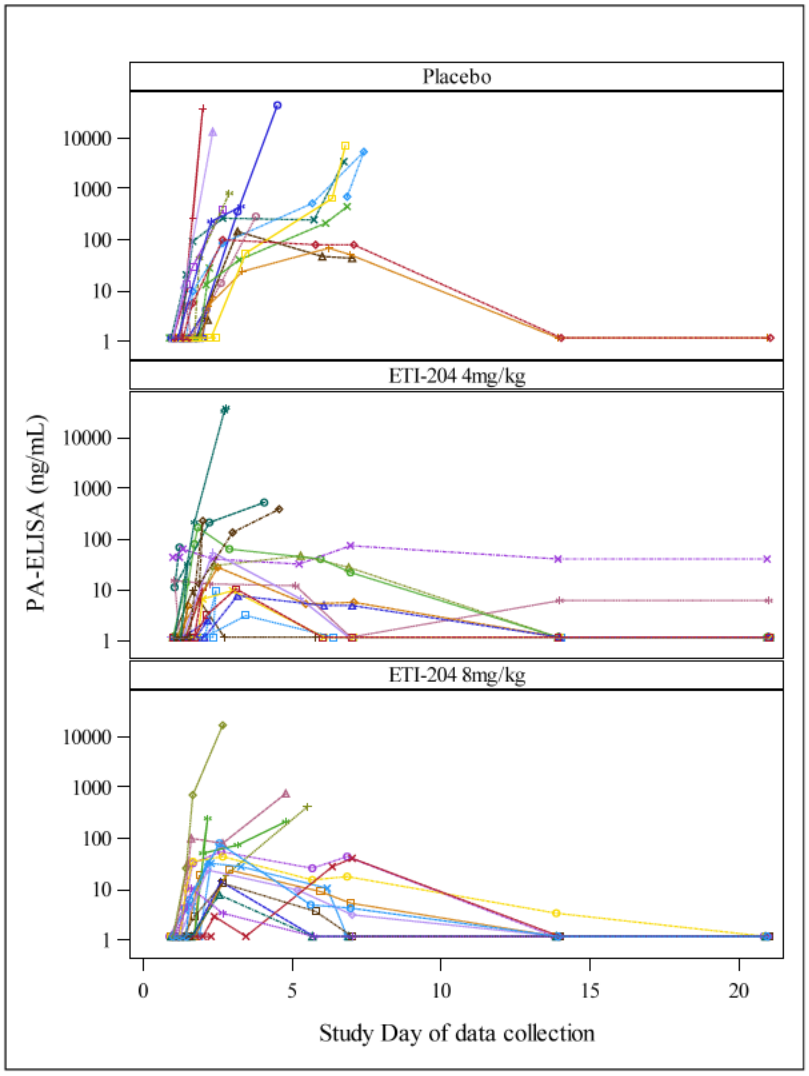
Figure 11. Study AP201: PA-ELISA over time by animal and treatment



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.

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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.

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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.

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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

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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])

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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

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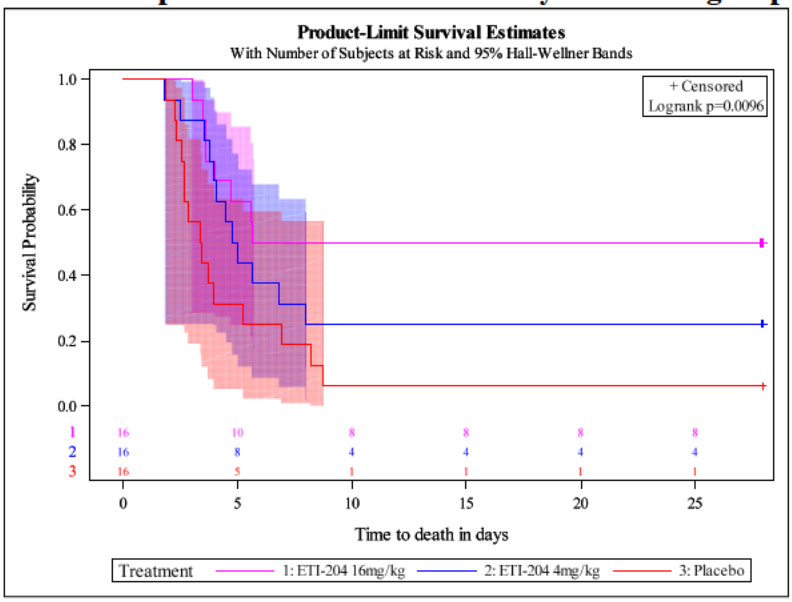
	Placebo (N=16)	ETI-204 4 mg/kg IV (N=16)	ETI-204 16 mg/kg IV (N=16)
Excluding one animal without bacteremia prior to treatment (primary)			
n/N (%)	Same as above	Same as above	7/15 (0.467)
Difference in survival proportion [exact 95% confidence interval] one-sided p-value compared with control		Same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

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

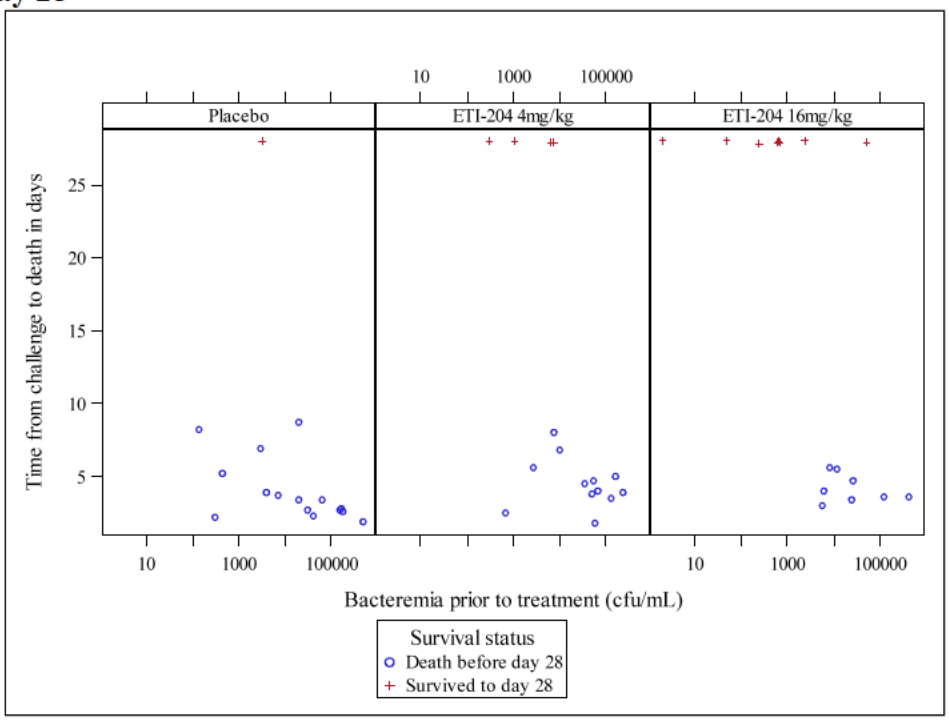
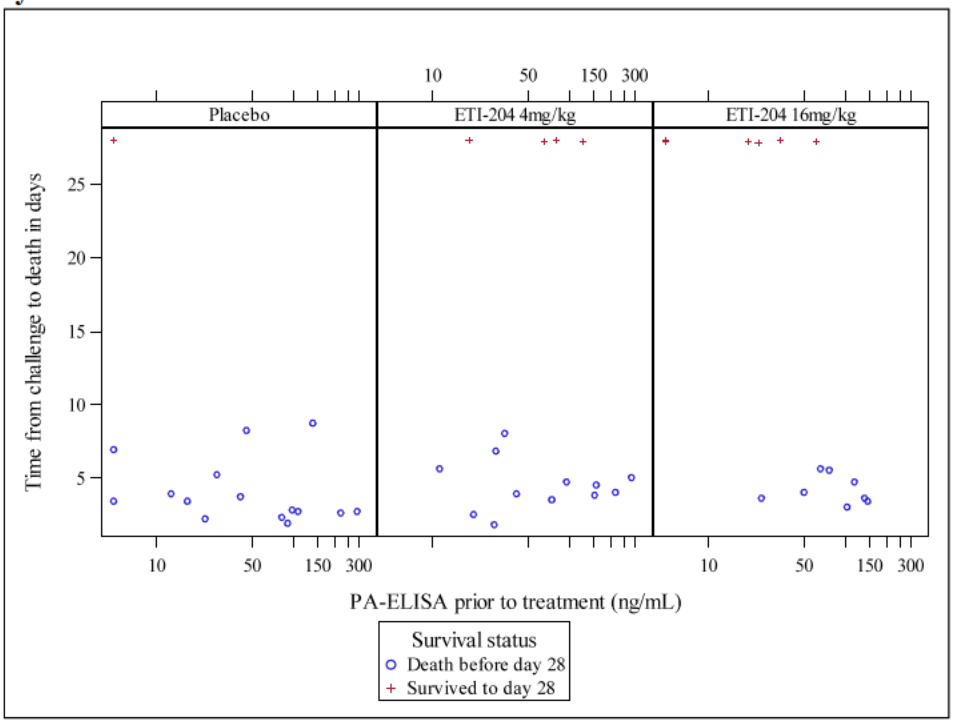
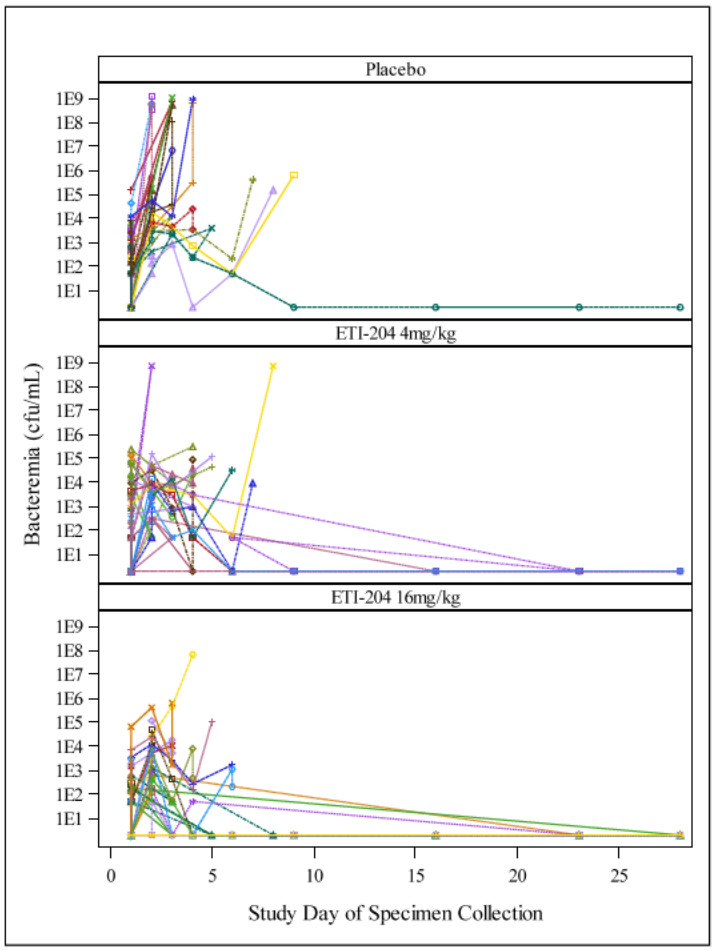


Figure 14. Study AP204: Time to death versus PA-ELISA prior to treatment by survival status at Day 28



Reference ID: 3859664

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

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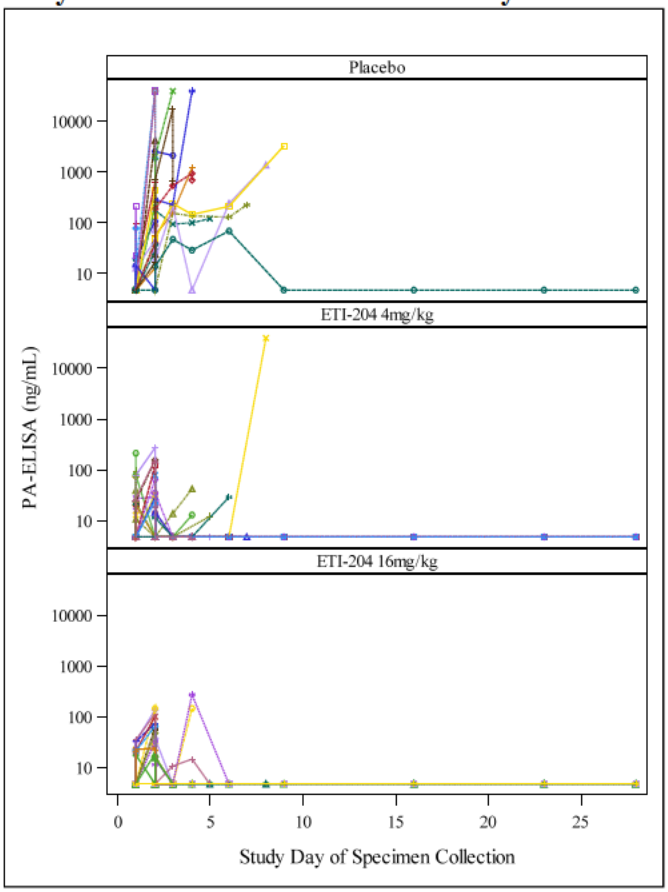
bacterial load in the brain, liver, kidney, and spleen. Only one animal in the 16 mg/kg group had a 0.5 bacterial load in bronchial lymph node.

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.

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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.

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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].

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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.

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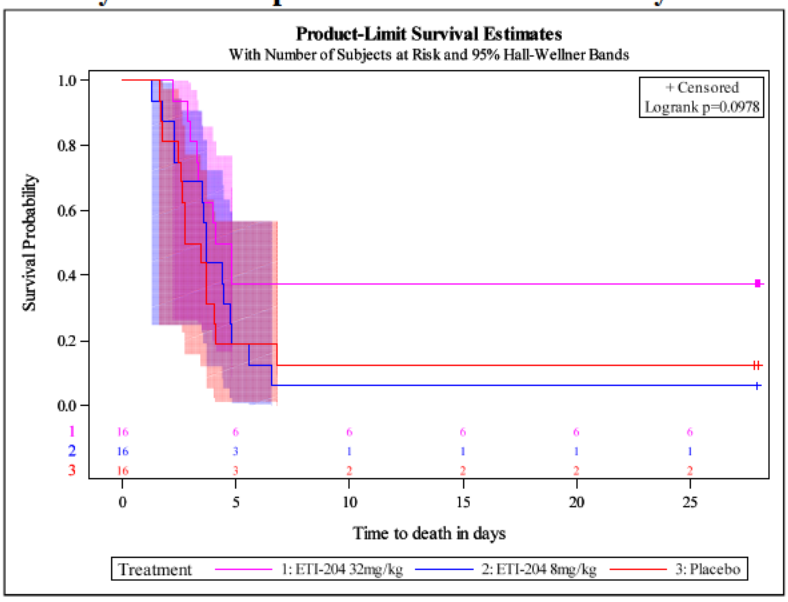
Table 32. Study AP203: Survival at Day 28 by treatment group

	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



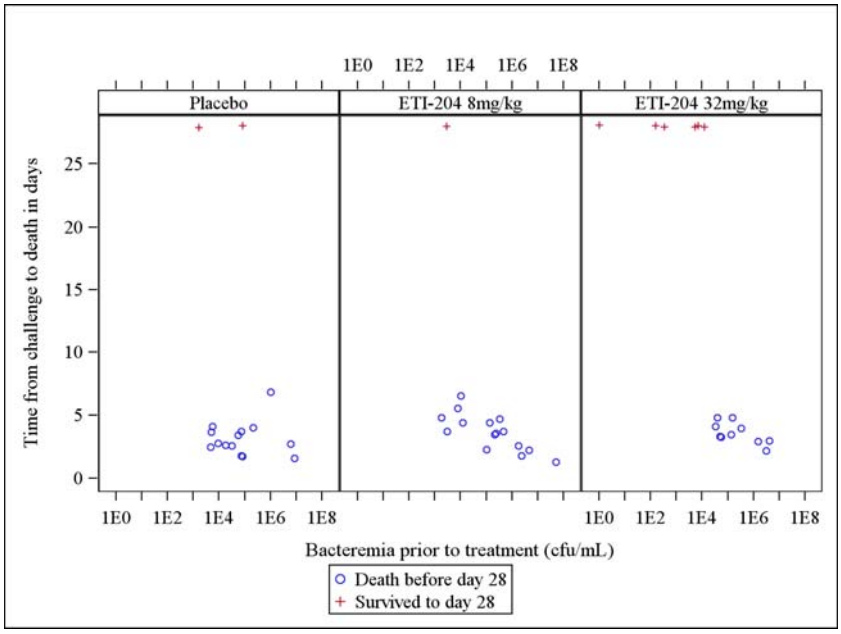
Reference ID: 3859664

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.

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

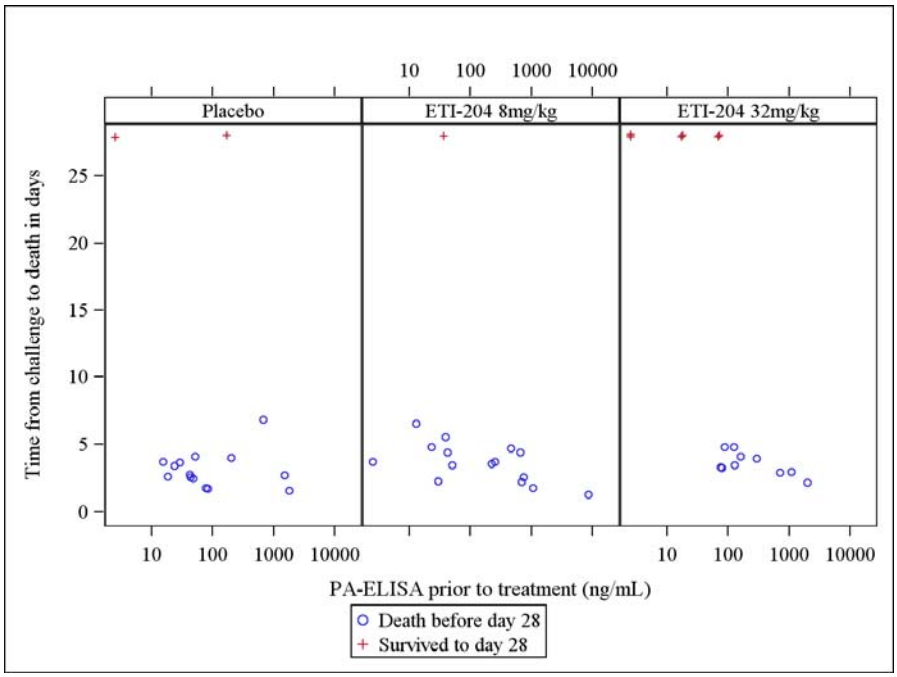

Table 34. Study AP203: Estimated odds ratio of survival at Day 28 associated with treatment and bacteremia or PA-ELISA prior to treatment from logistic regression on survival

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.

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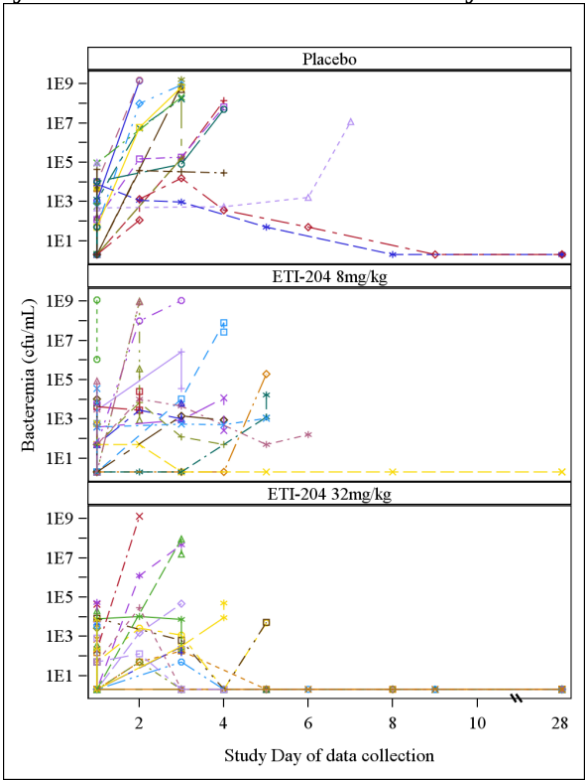
Figure 20. Study AP203: Bacteremia over time by treatment and animal



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

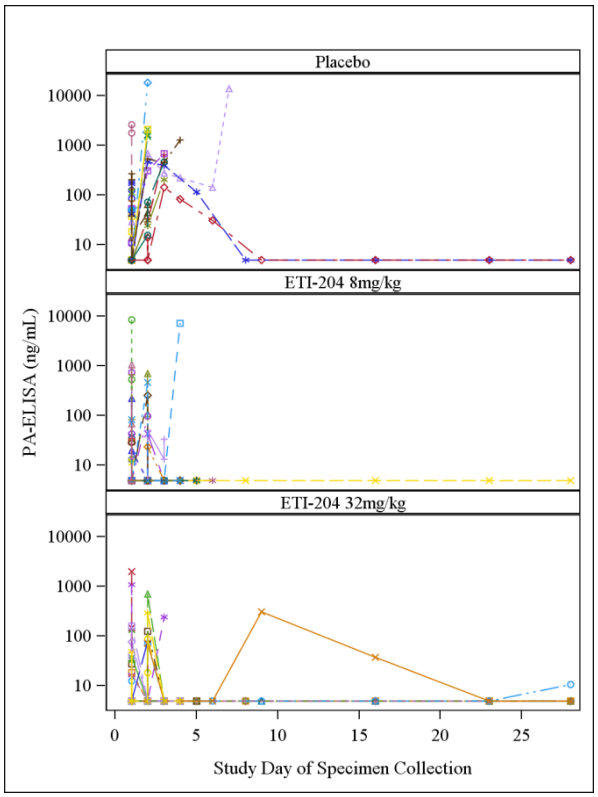


Table 35. Study AP203: Survival at Day 28 by gender, challenge dose, log_{10} bacteremia, PA prior to treatment

	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%)

Reference ID: 3859664

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.

Reference ID: 3859664

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.

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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.

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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.

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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

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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

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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

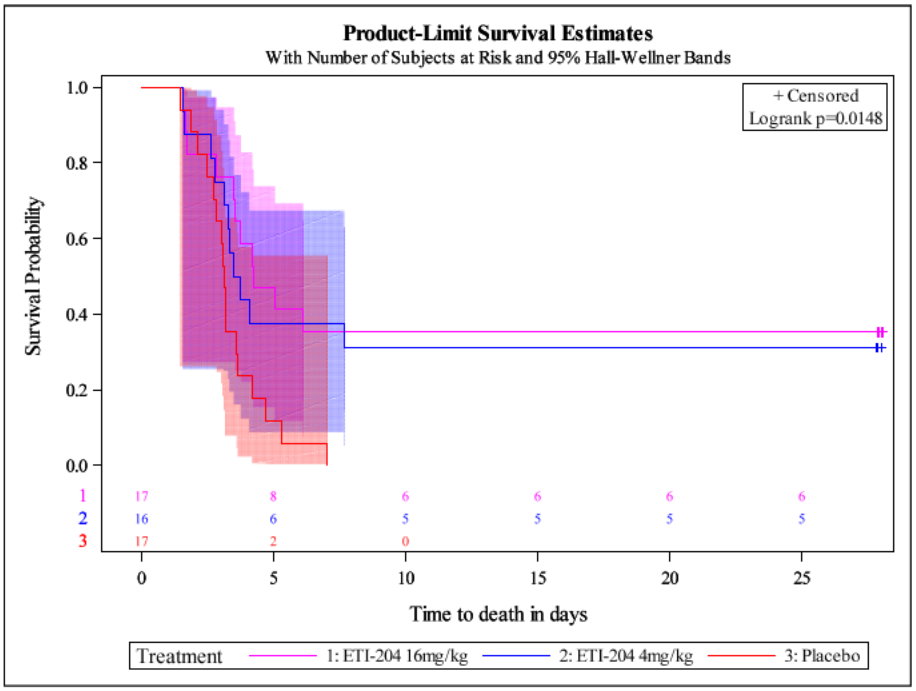


Table 39. Study AP202: two-sided p-values of pairwise log-rank tests comparing time from challenge to death among groups

	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

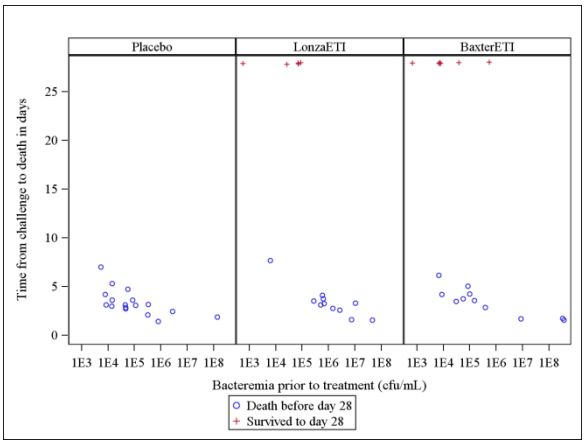
The following table shows the results for a proportional hazards model on time to death with treatment and $\log_{10}$ bacteremia prior to treatment. Compared with the placebo group, both treatment groups had a significantly reduced risk of death. Higher bacteremia prior to treatment was also significantly 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

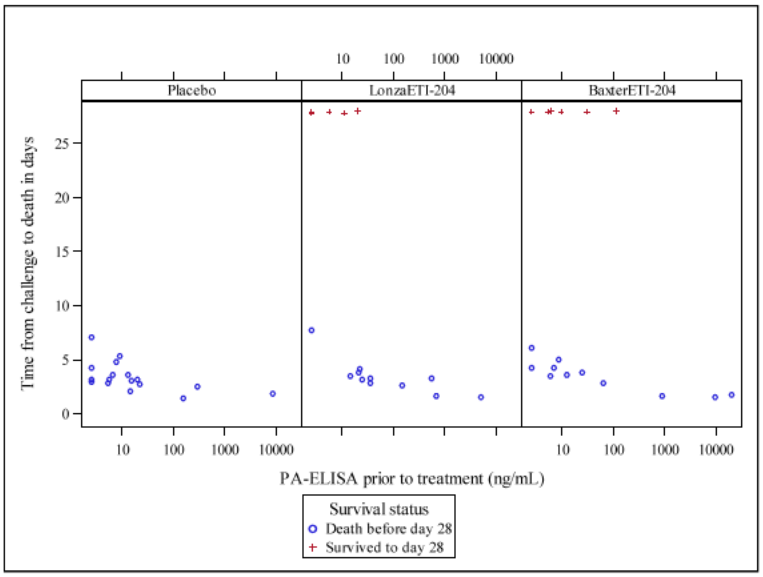
The following figure shows time to death versus bacteremia prior to treatment by treatment and survival status at day 28. It is evident that an animal with a higher level of bacteremia was more likely to die in the two treatment groups.

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).

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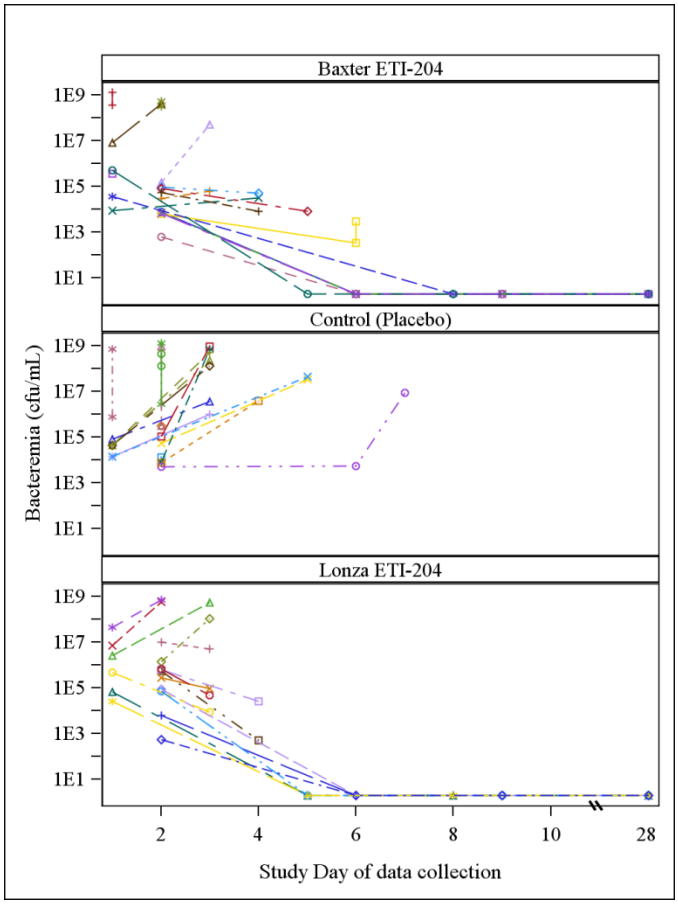
Figure 25. Study AP202: Study 202: Bacteremia over time by animal



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.

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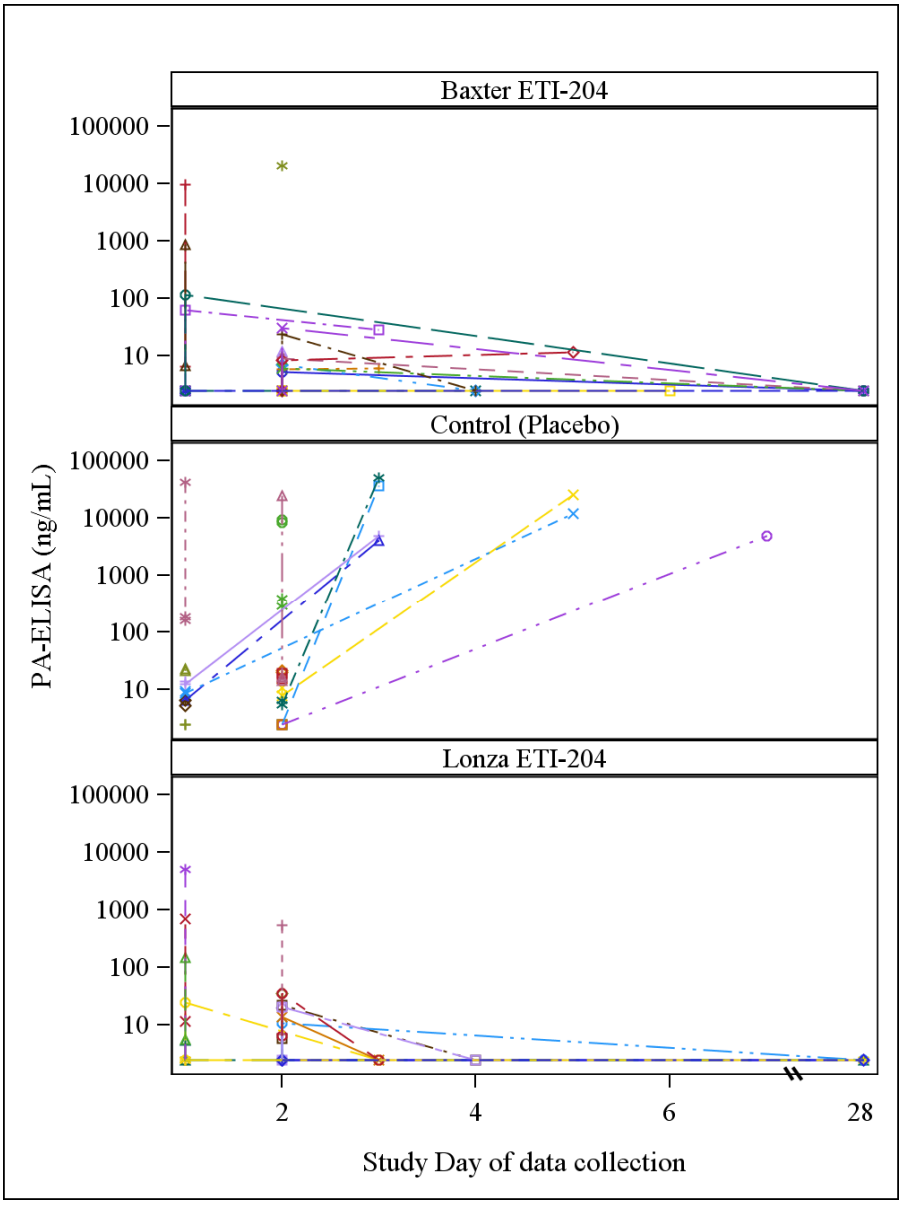
Figure 26. Study AP202: PA-ELISA over time by animal and treatment



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).

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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

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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.

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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.

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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

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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)

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	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.

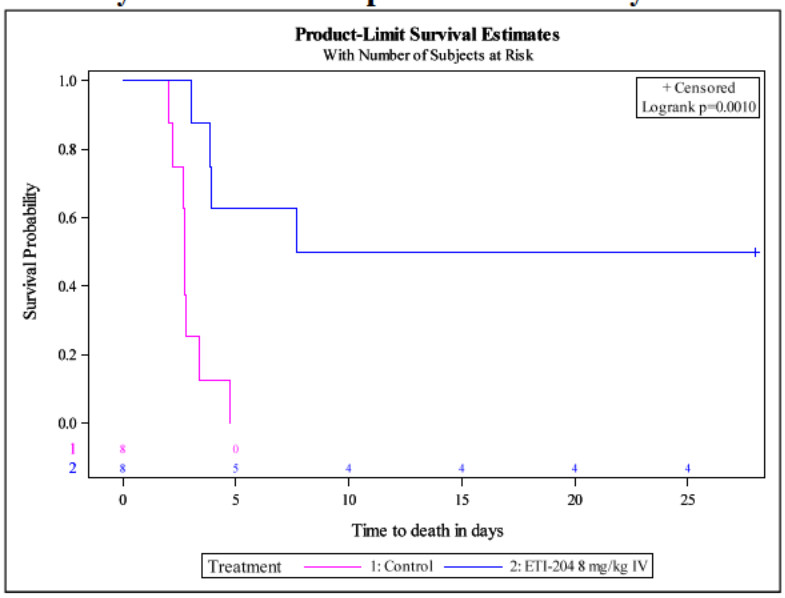
Table 44. Study NIAID 1056: Survival at Day 28 by treatment group

	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.

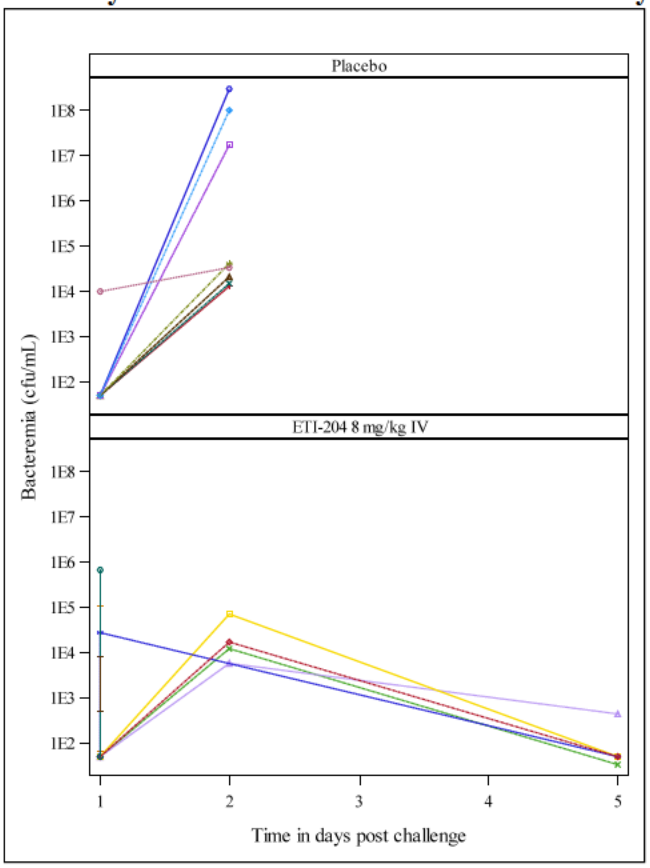
Figure 27. Study NIAID 1056: Kaplan-Meier curve by treatment group



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.

Reference ID: 3859664

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

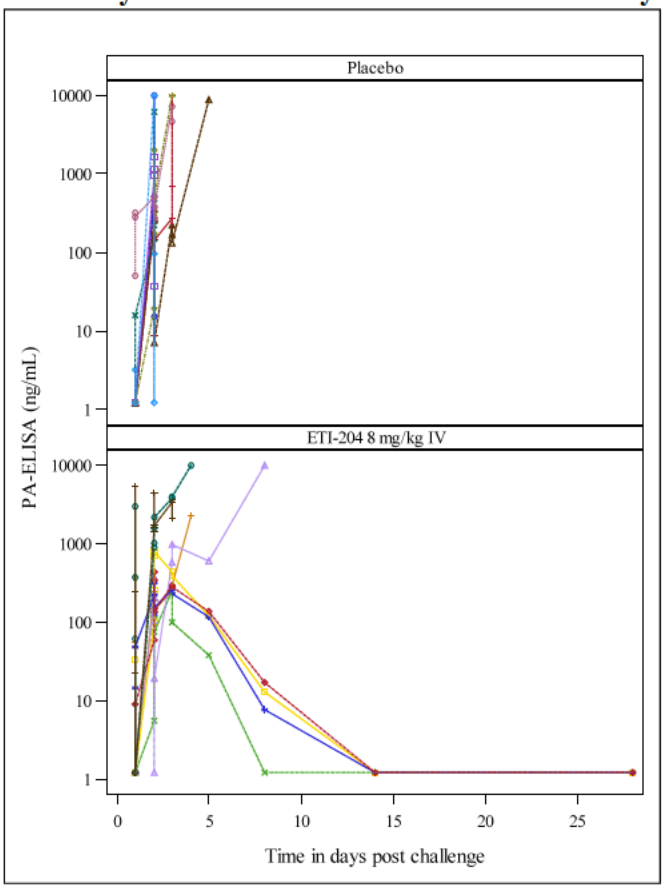


Table 45. Study NIAID1056: Survival at Day 28 by challenge dose, bacteremia, and PA-ELISA

	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.

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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.

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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

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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.

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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)

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	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

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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)

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	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

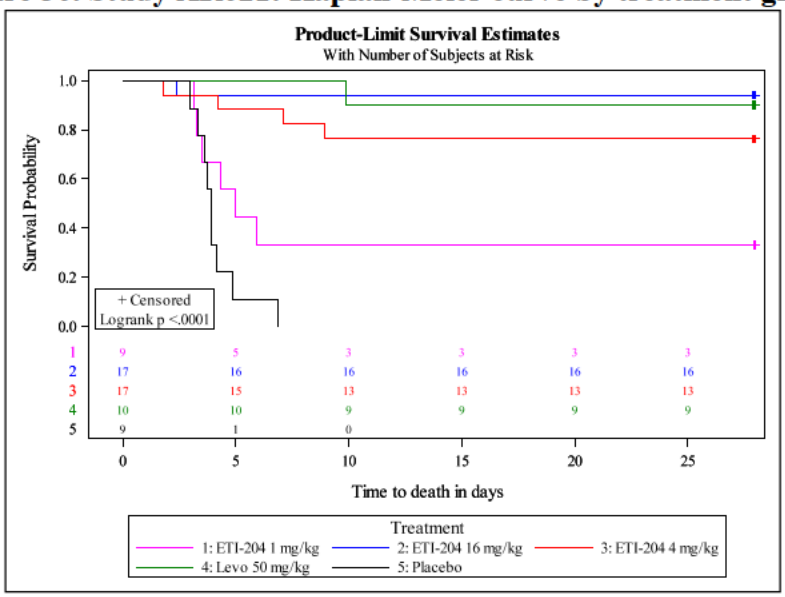
Figure 30and Figure 30 demonstrated that there was a significant difference between the 4 mg/kg dose and 16 mg/kg dose of ETI-204 groups and the placebo group, using a two-sided significance level of 0.05/3=0.0167. The levofloxacin group was also significantly different from placebo.

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.

Reference ID: 3859664

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.

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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.

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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.

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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

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*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

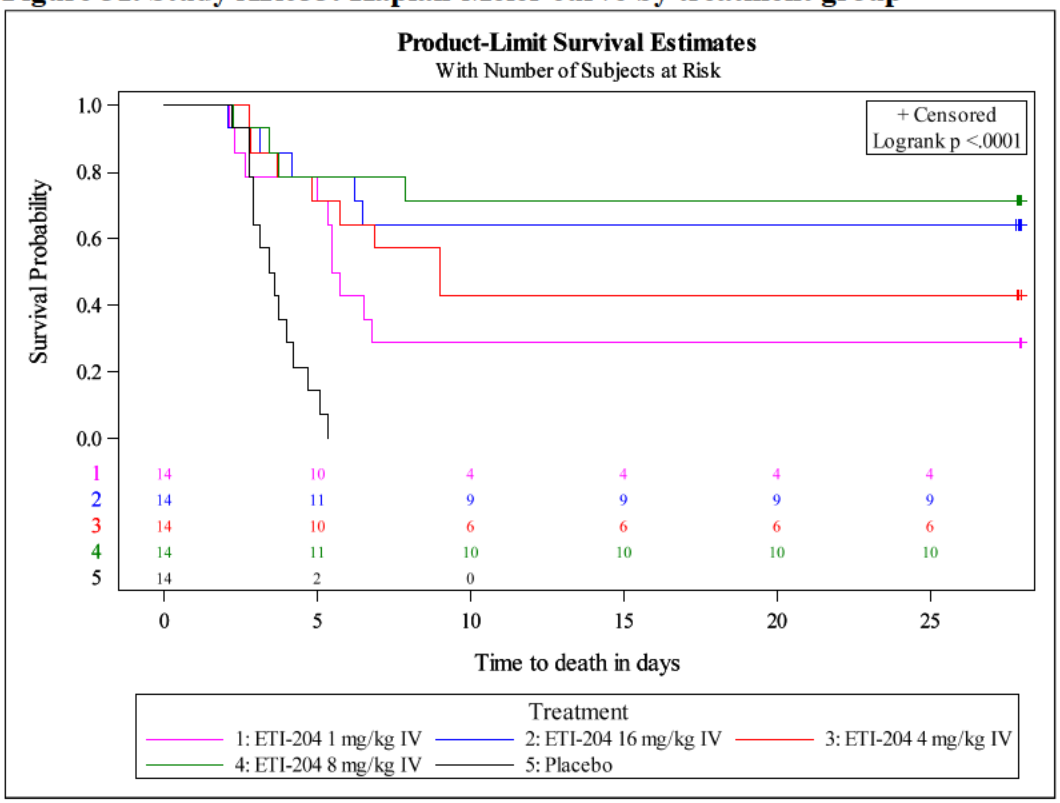


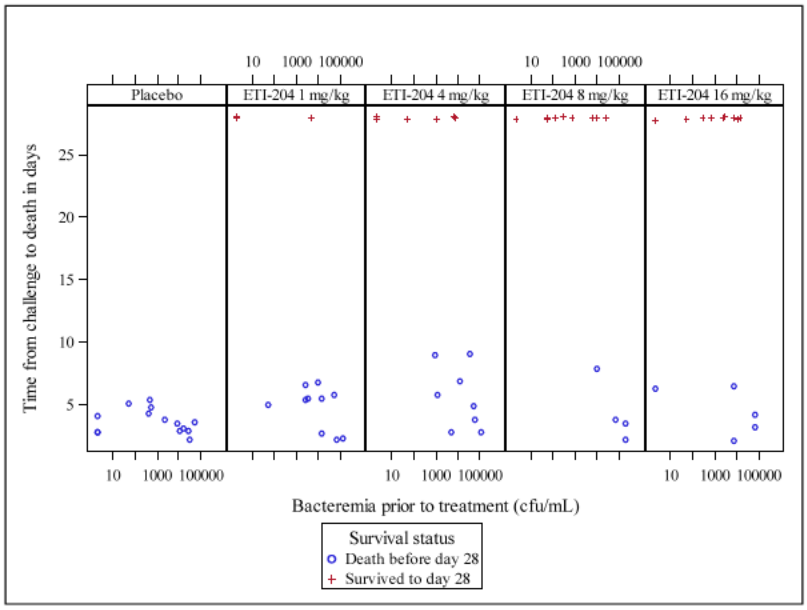
Table 55. Study AR033: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death among groups

	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

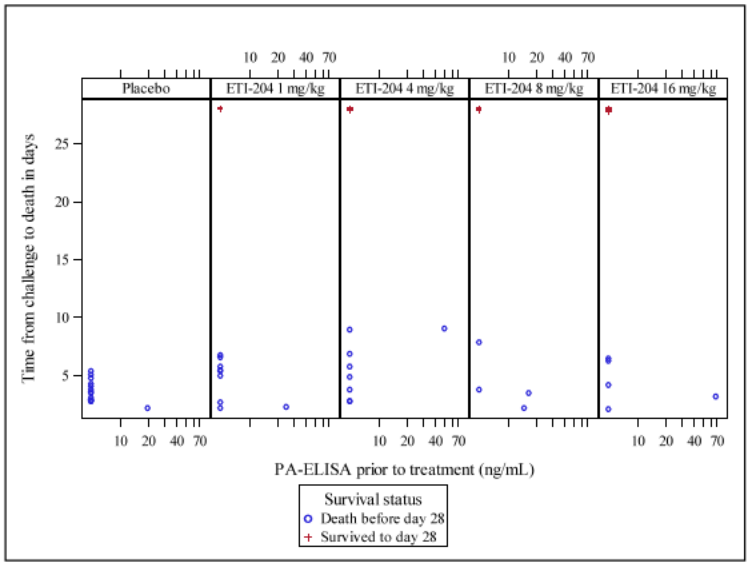
Figure 32 shows the time to death versus bacteremia prior to treatment. In this study, deaths occurred across all bacteremia levels. Therefore, it was not possible to find a cut-off point of bacteremia to separate surviving and non-surviving animals. However, there does appear to be a trend with animals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



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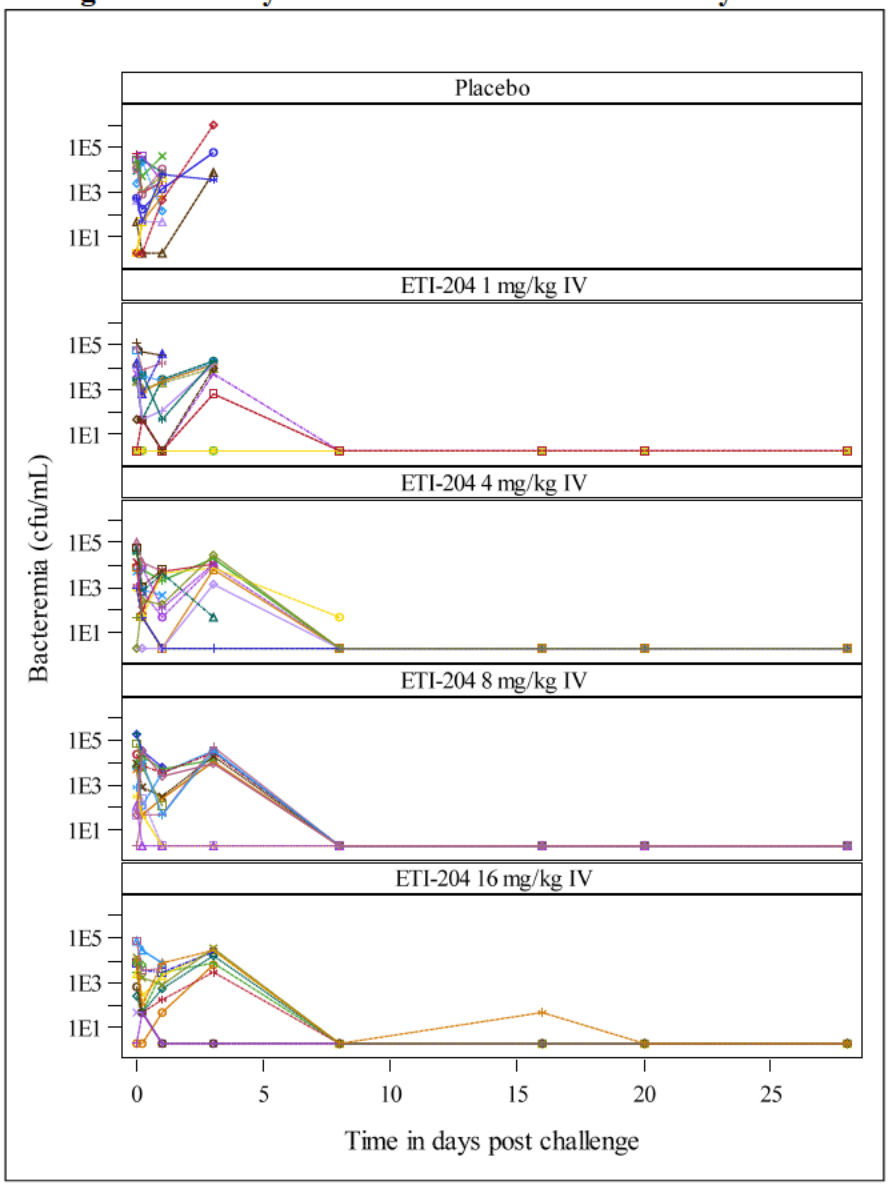
Bacteremia over time

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

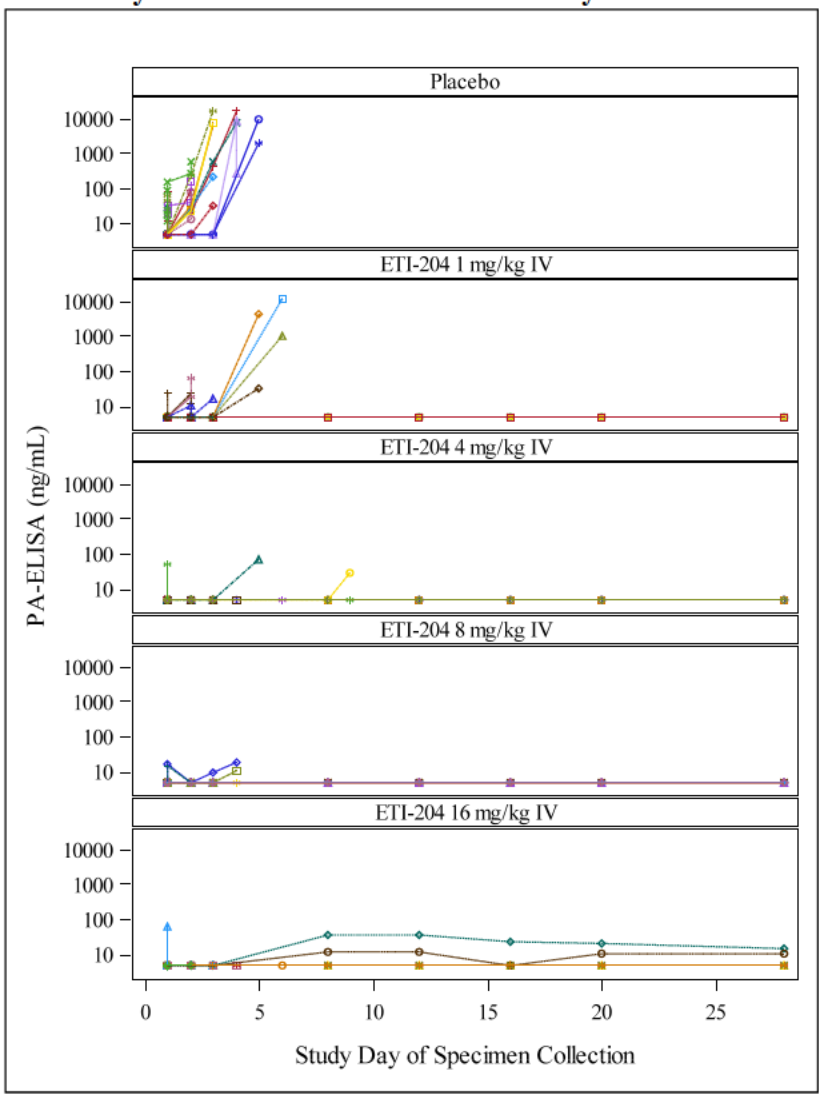


Reference ID: 3859664

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.

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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.

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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

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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.

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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.

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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

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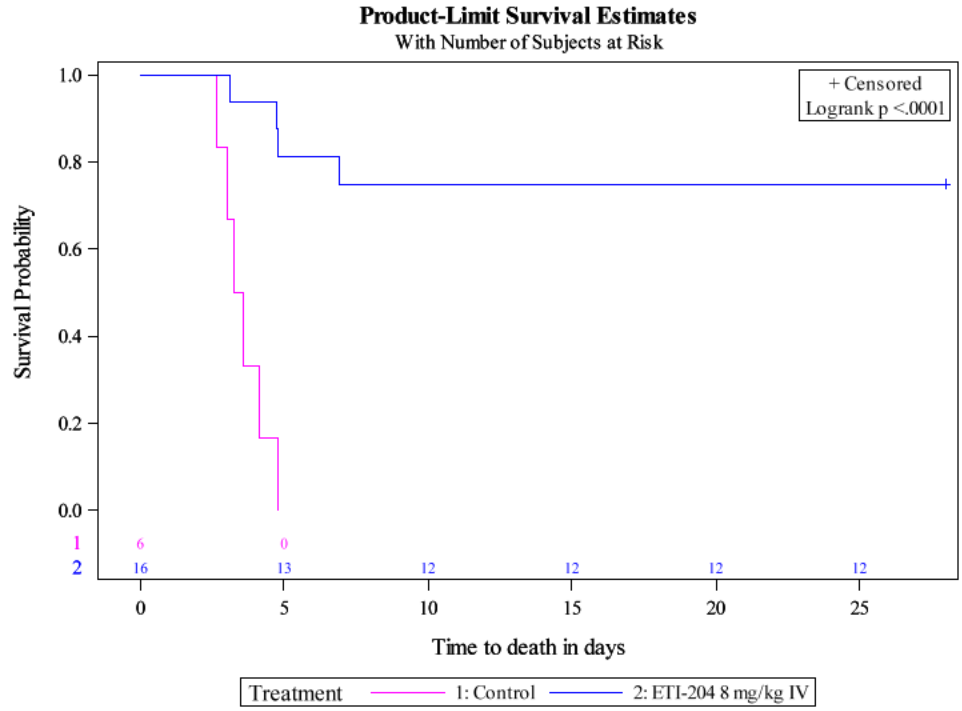
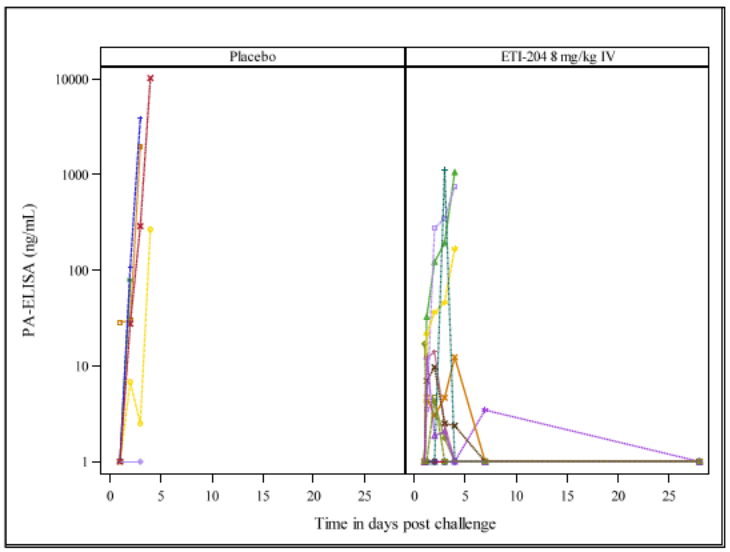
Figure 36. Study NIAID 1030: Kaplan-Meier curve by treatment group



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%.

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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

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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.

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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

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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.

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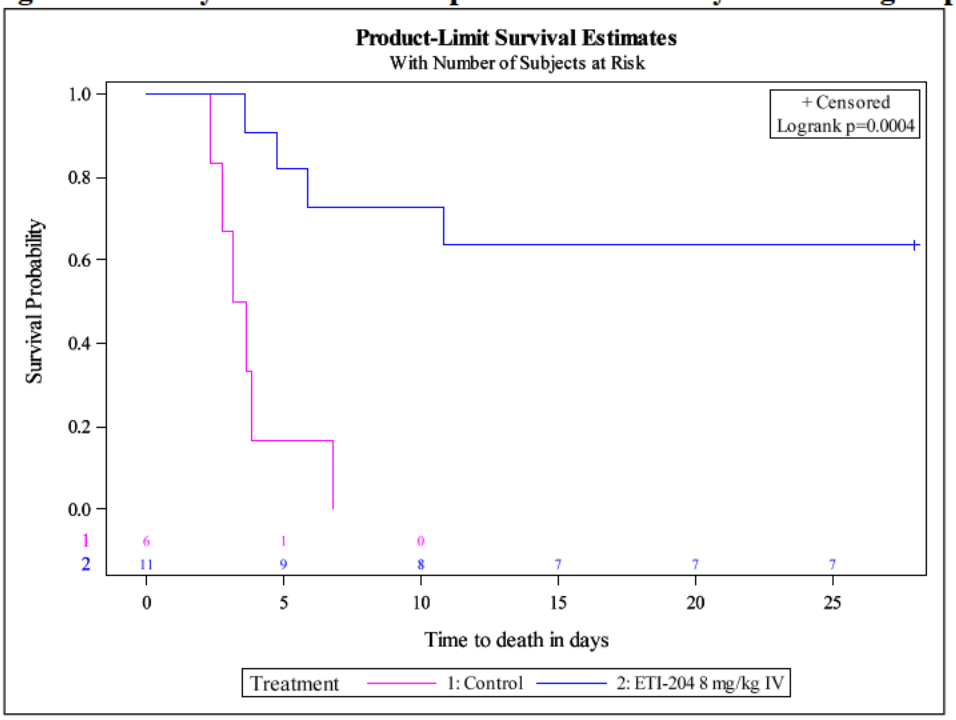
Figure 38. Study NIAID 1045: Kaplan-Meier curve by treatment group



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.

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

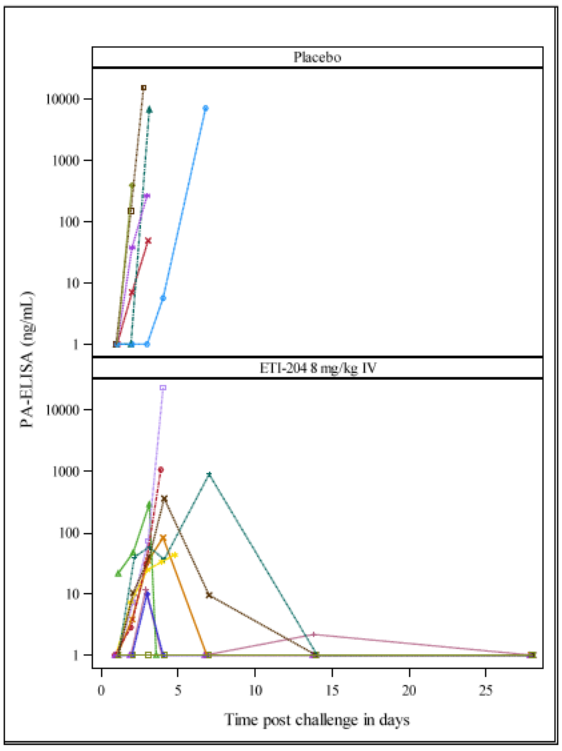

Table 64. Study NIAID1045: Survival at Day 28 by challenge dose, bacteremia, and PA-ELISA

	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.

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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.

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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.

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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 Male	3 (50.0) 3 (50.0)	5 (55.6) 4 (44.4)	4 (50.0) 4 (50.0)	4 (44.4) 5 (55.6)	5 (55.6) 4 (44.4)	21 (51.2) 20 (48.8)
Body weight (kg) Mean (SD) Range	2.4 (0.2) 2.2, 2.6	2.4 (0.2) 2.2, 2.7	2.4 (0.2) 2.1, 2.6	2.5 (0.3) 2.1, 3.1	2.6 (0.4) 2.1, 3.5	2.5 (0.3) 2.1, 3.5
Challenge dose (LD_{50}) Mean (SD) Range	324.2 (70.6) 254.0, 458.0	315.6 (83.4) 213.0, 451.0	366.0 (113.6) 198.0, 551.0	289.0 (51.8) 222.0, 351.0	288.7 (49.1) 225.0, 370.0	314.9 (78.3) 198.0, 551.0
Challenge dose (LD_{50}) (n(%)) <200 200 or higher	0 6 (100)	0 9 (100)	1 (12.5) 7 (87.5)	0 9 (100)	0 9 (100)	1 (2.4) 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 Mean (SD) Range	6 33.3 (7.9) 23.9, 48.1	9 30.2 (3.5) 24.0, 32.1	7 33.2 (5.6) 24.1, 40.2	7 29.7 (3.9) 24, 32	7 32.0 (4.6) 24.1, 40.1	36 31.6 (5.1) 23.9, 48.1

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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

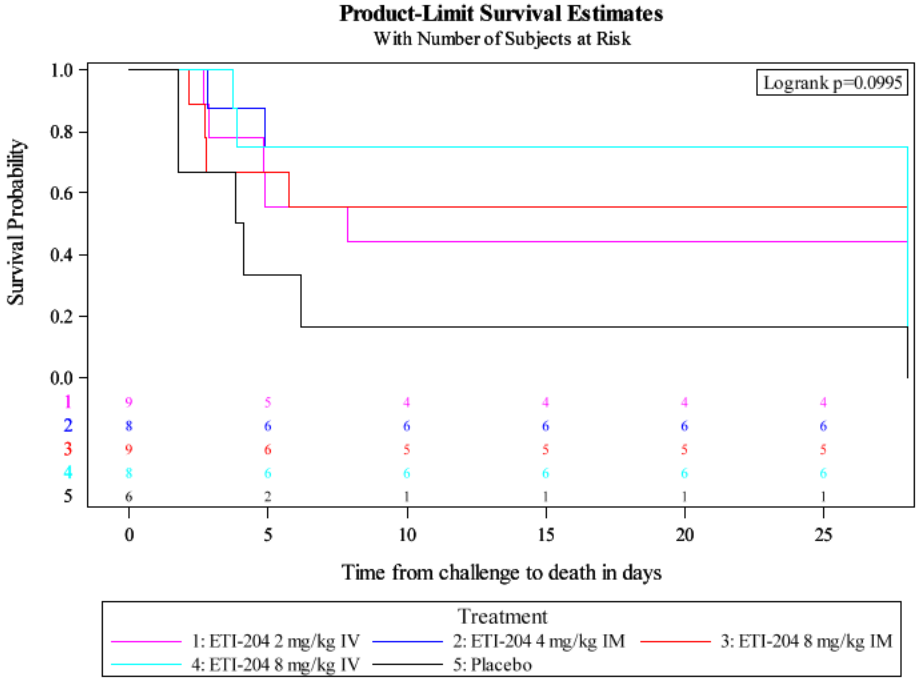

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Table 68. Study AP107: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

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.

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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).

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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.

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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.

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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.

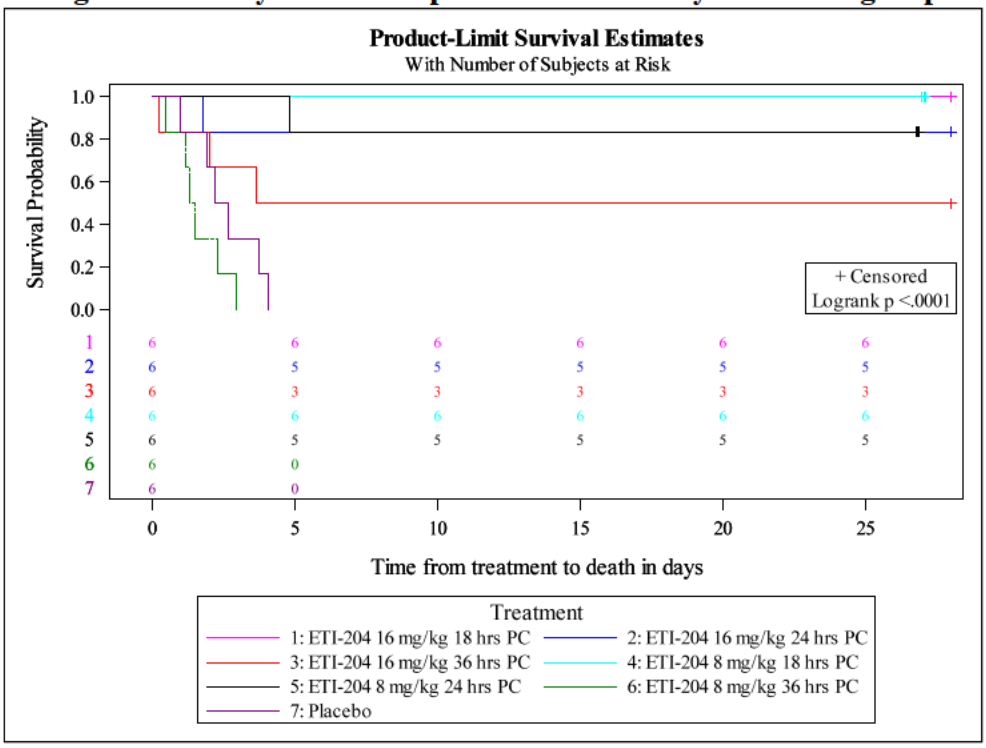
Reference ID: 3859664

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



Reference ID: 3859664

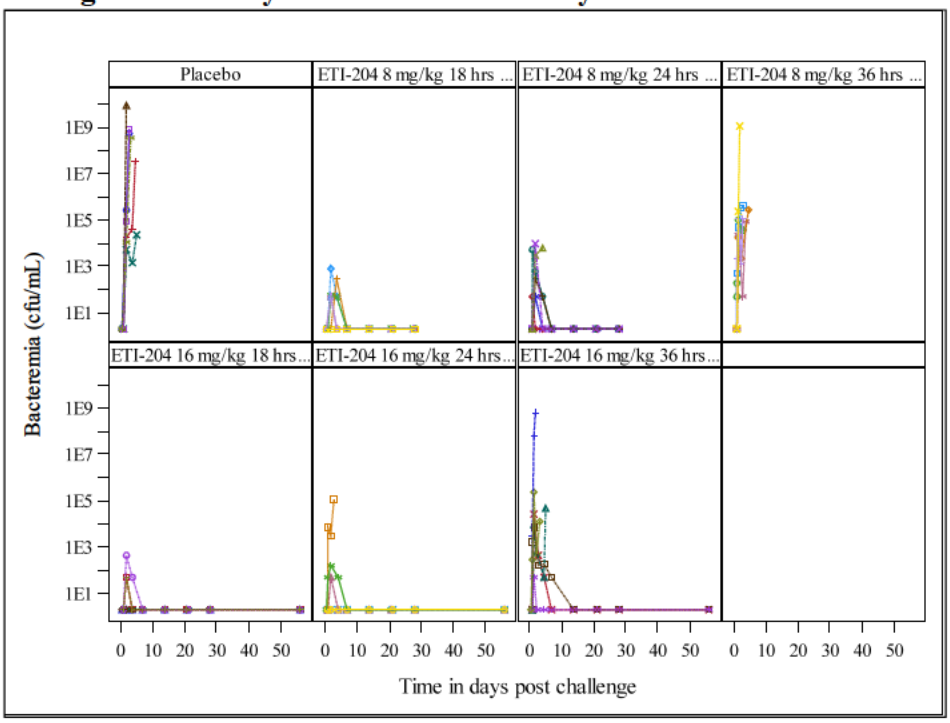
Table 73. Study AP301: Two-sided p-values of pairwise log-rank tests comparing time from treatment to death between a treatment group and the placebo group

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.

Reference ID: 3859664

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.

Reference ID: 3859664

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.

Reference ID: 3859664

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

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	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.

Reference ID: 3859664

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.

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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

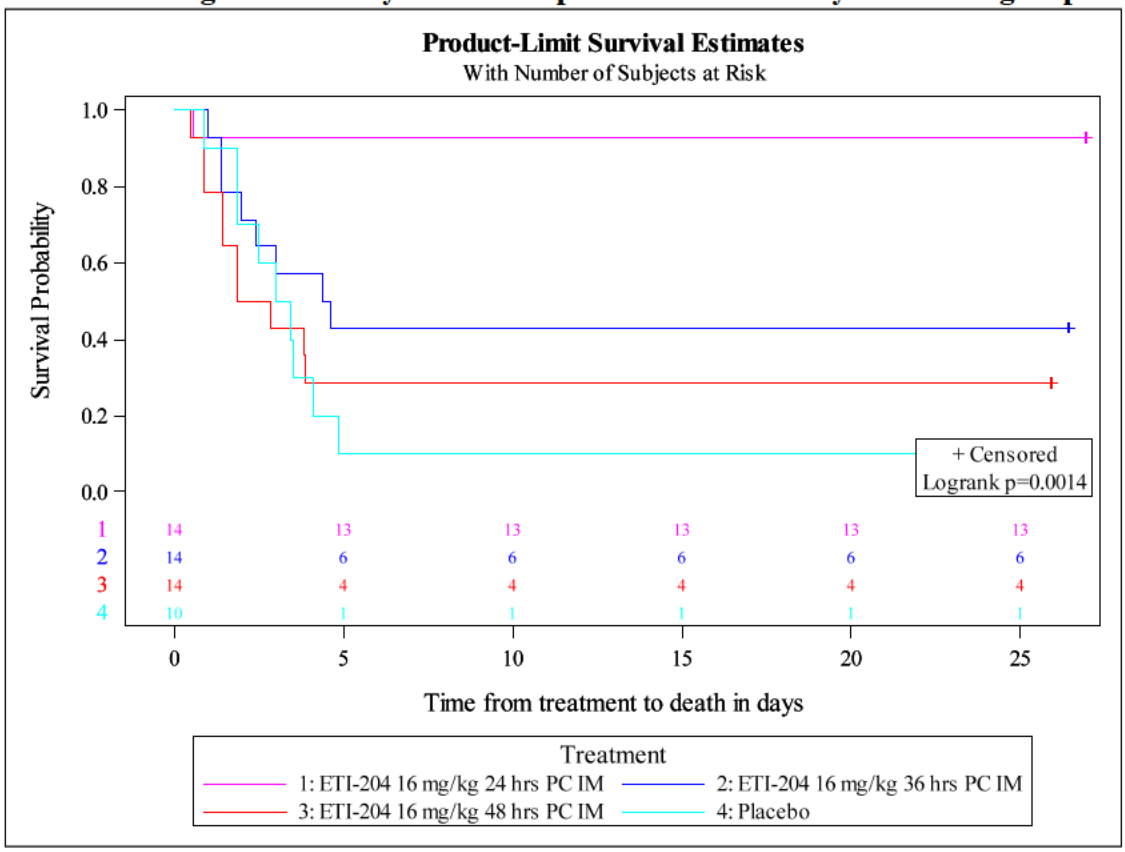

Table 78. Study AP307: Two-sided p-values of pairwise log-rank tests comparing time from treatment to death between a treatment group and the placebo group

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.

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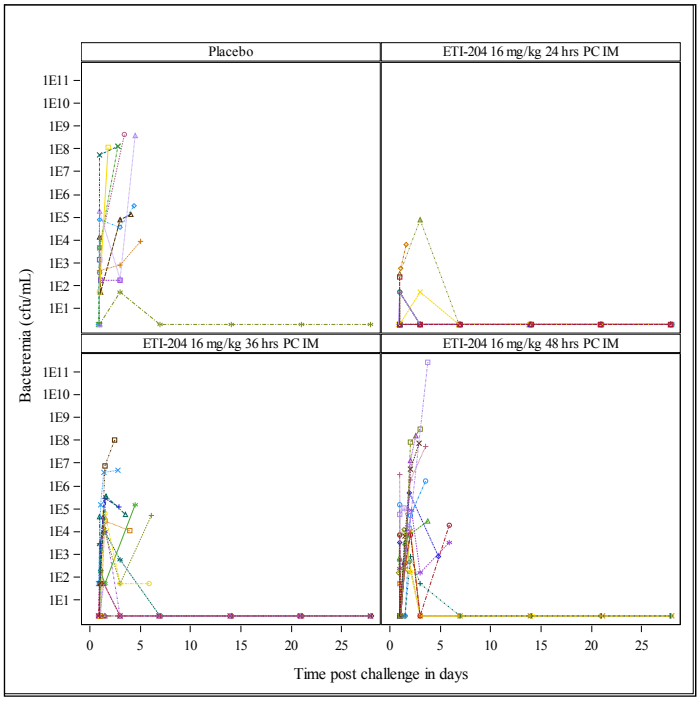
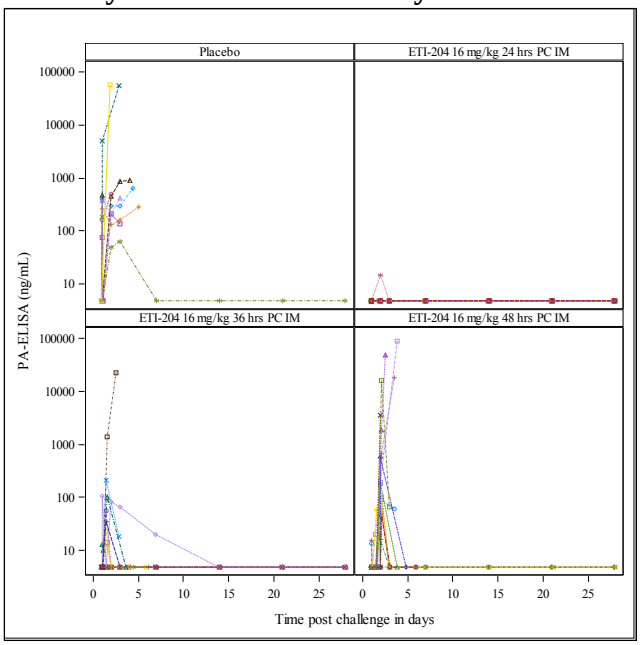
Figure 44. Study AP307: Bacteremia by treatment and animals



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



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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%)

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	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.

Reference ID: 3859664

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.

Reference ID: 3859664

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

Reference ID: 3859664

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).

Reference ID: 3859664

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

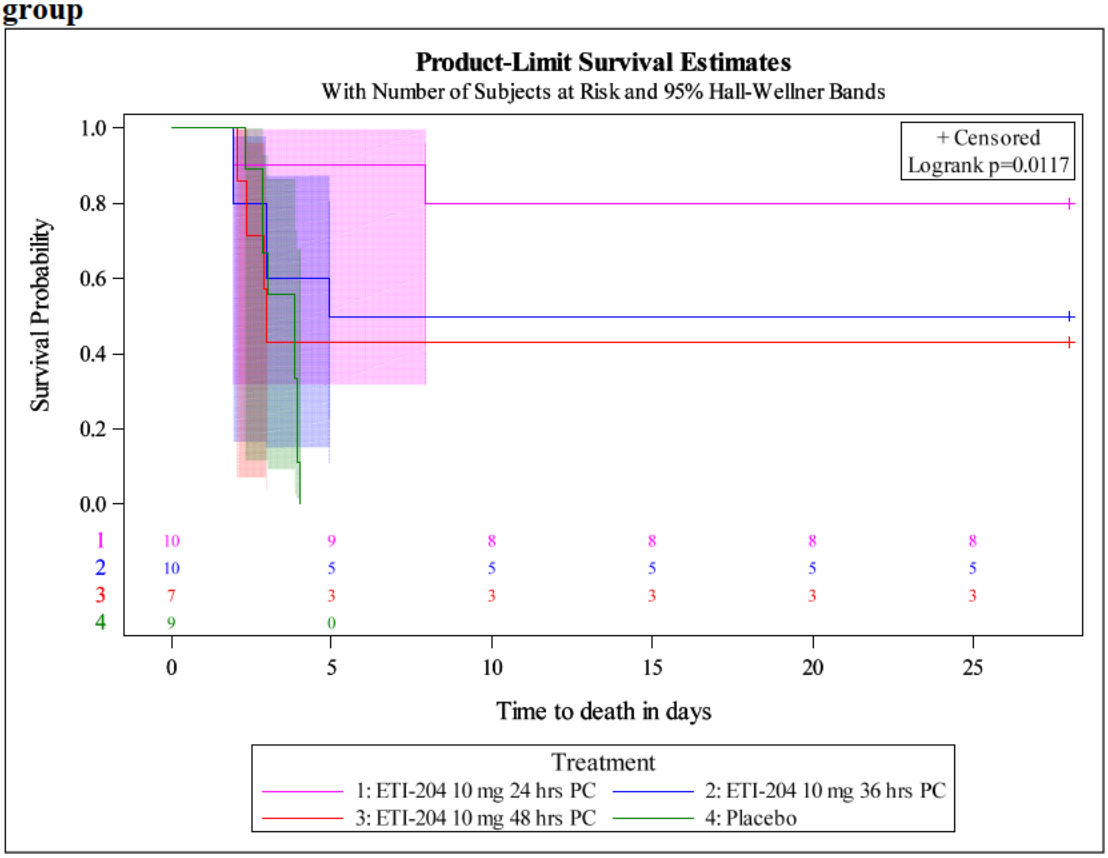


Table 83. Study AR004: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

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.

Reference ID: 3859664

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.

Reference ID: 3859664

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

Reference ID: 3859664

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.

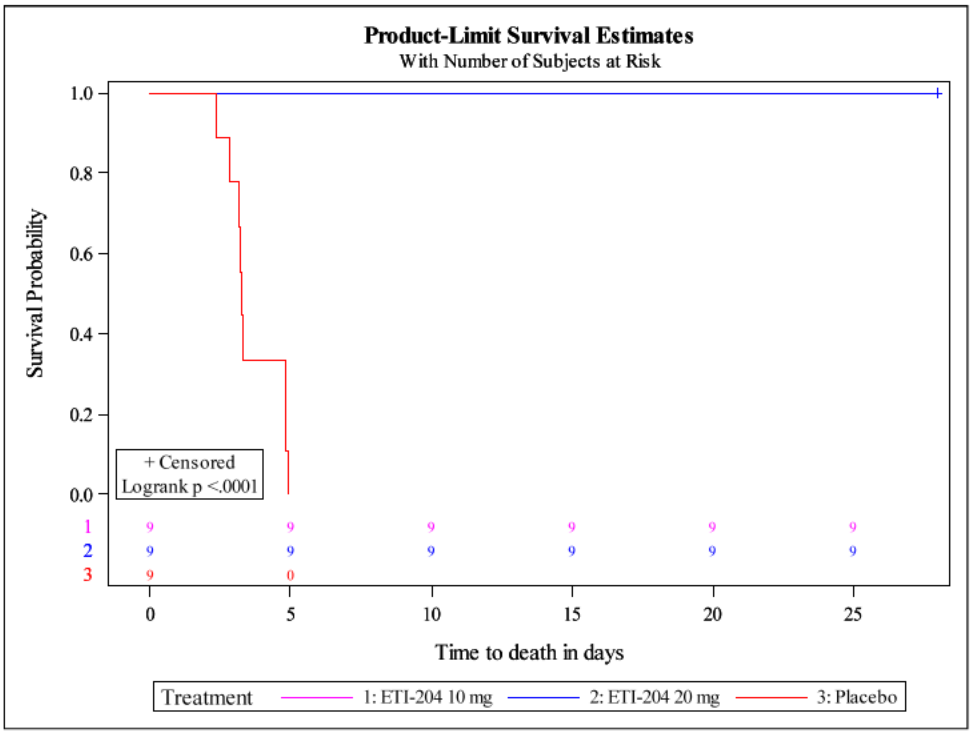
Table 86. Study AR007: Survival at Day 28 by treatment group

	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



Reference ID: 3859664

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.

Reference ID: 3859664

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

Reference ID: 3859664

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%

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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)

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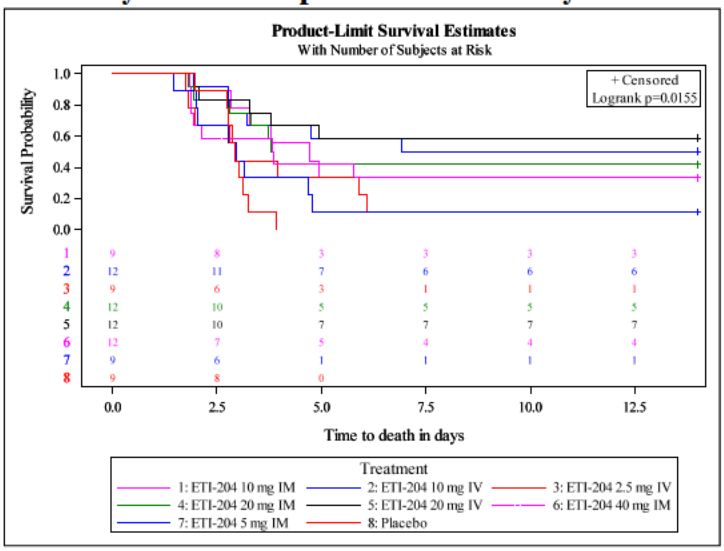
Table 89. Study AR012: Survival at Day 28 by treatment group

	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.

Reference ID: 3859664

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.

Reference ID: 3859664

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.

Reference ID: 3859664

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.

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***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

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	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

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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.

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Figure 49. Study AR034: Kaplan-Meier curves by treatment group in Phase I

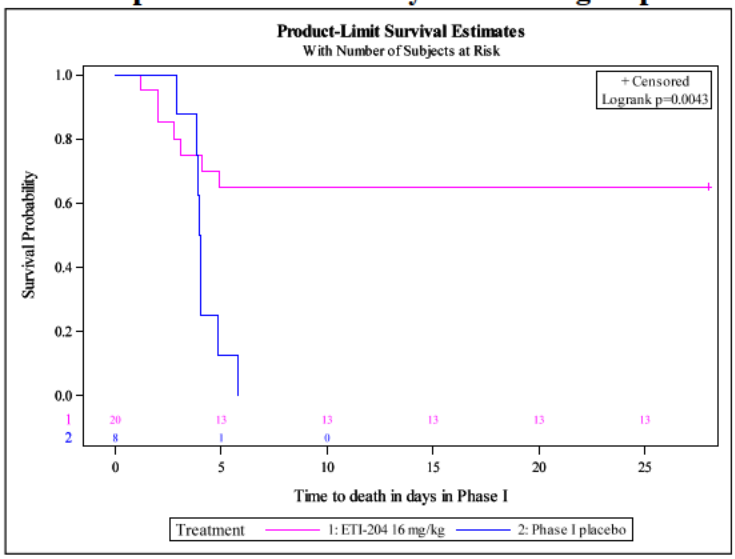
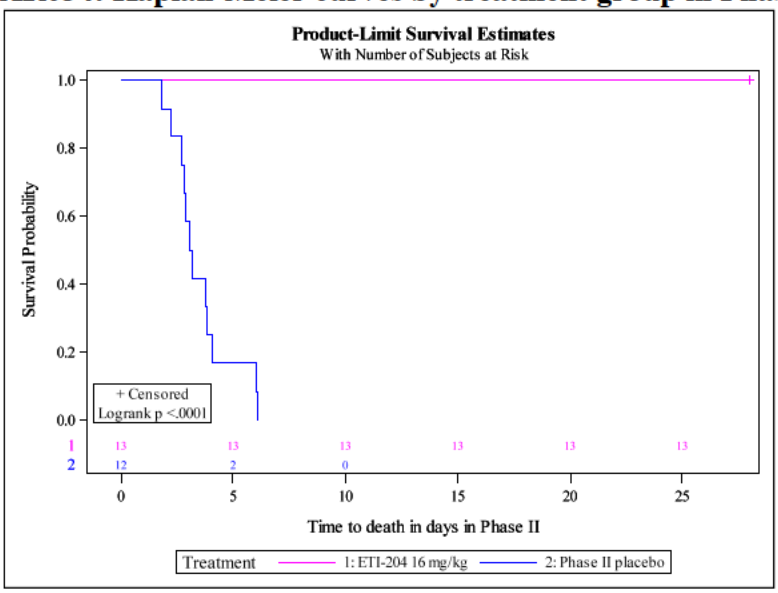


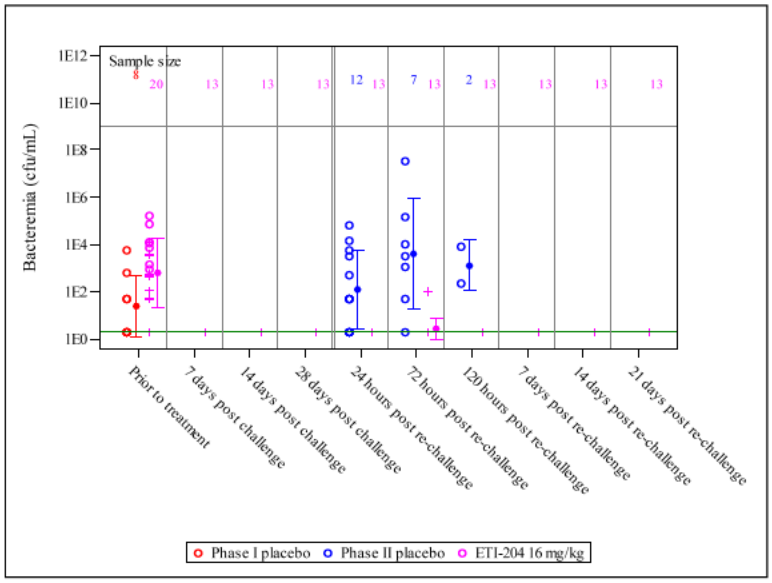
Figure 50. Study AR034: Kaplan-Meier curves by treatment group in Phase II



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.

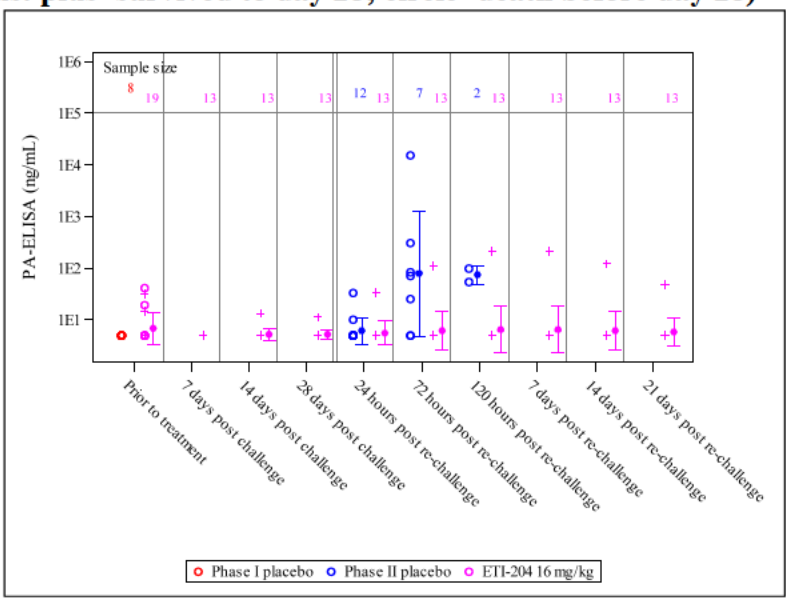
Figure 51. Study AR034: PA-ELISA by visit with geometric mean and standard deviation (by survival status: plus=survived to day 28; circle=death before day 28)



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)



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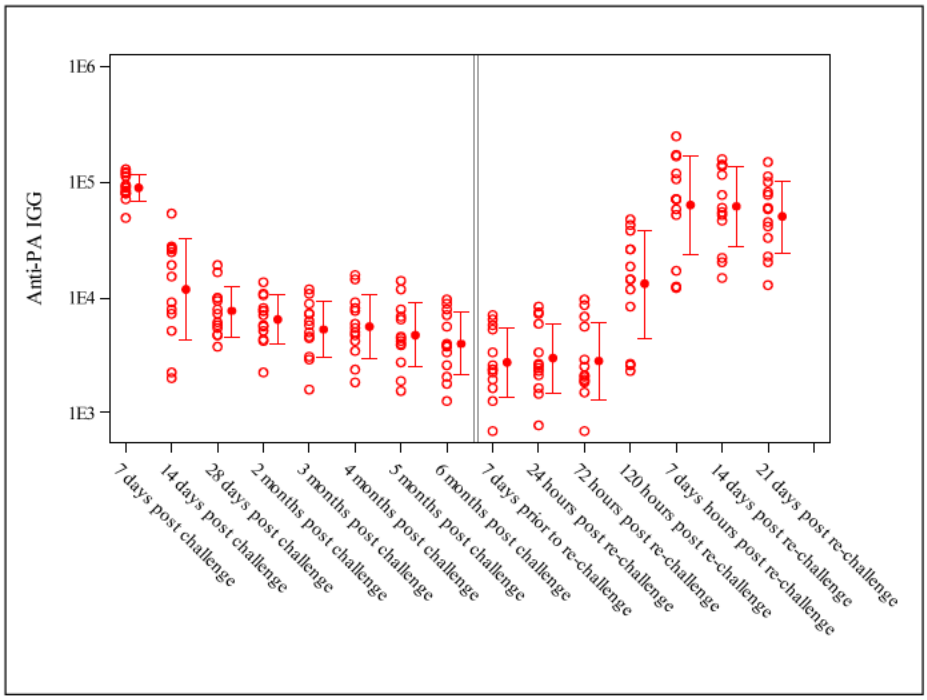
Immune response: anti-PA-IgG and TNA

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

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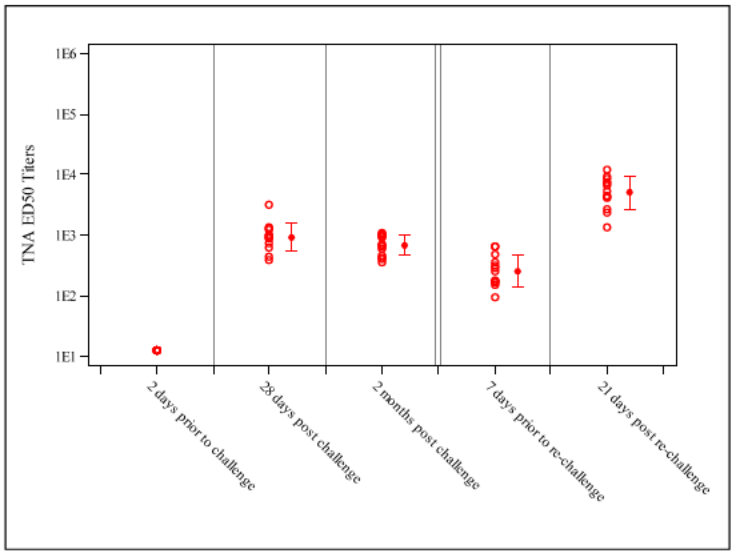
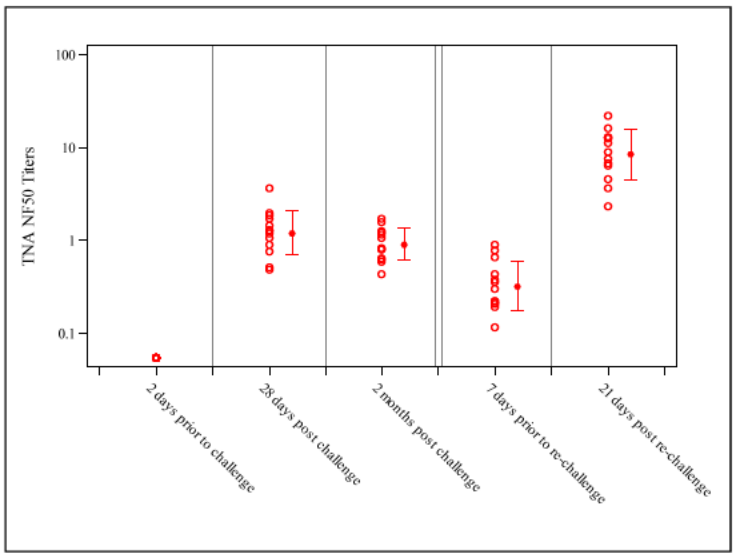
The following two figures show a summary of the geometric mean and 95% confidence intervals for the TNA ED_{50} and NF_{50} titers for 13 ETI-203 treated animals and in Phase I and Phase II. As stated before, these variables measured the functional ability of serum to neutralize *B. anthracis* lethal 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

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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

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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.

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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

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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.

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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.

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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

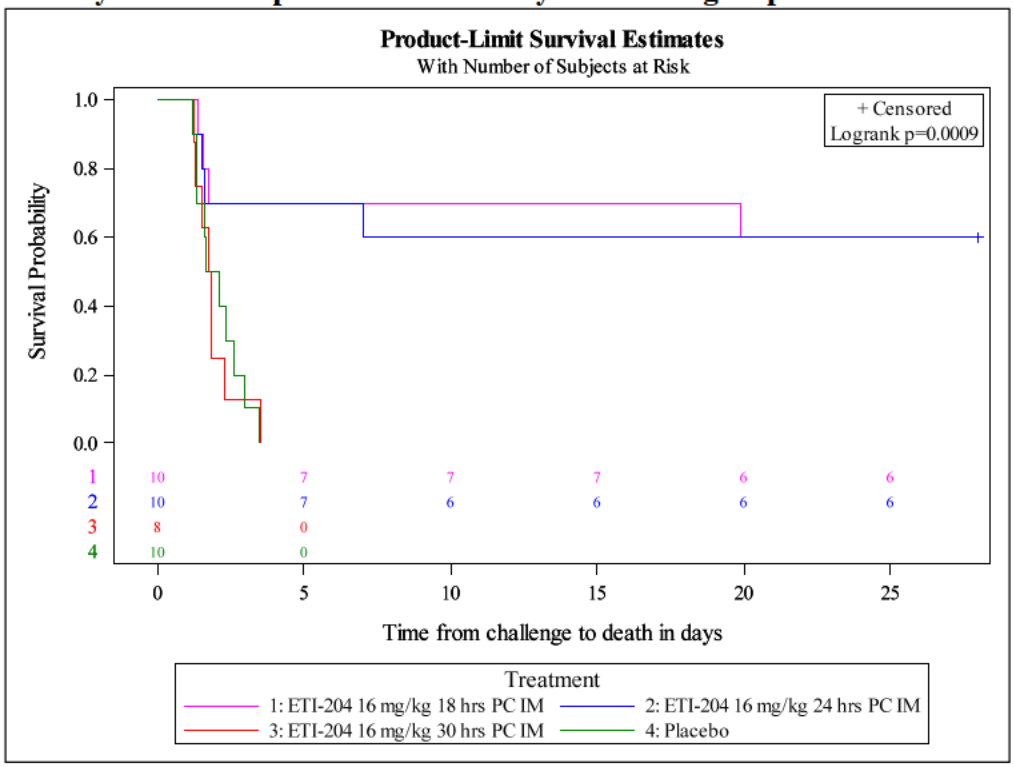


Table 99. Study AR035: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

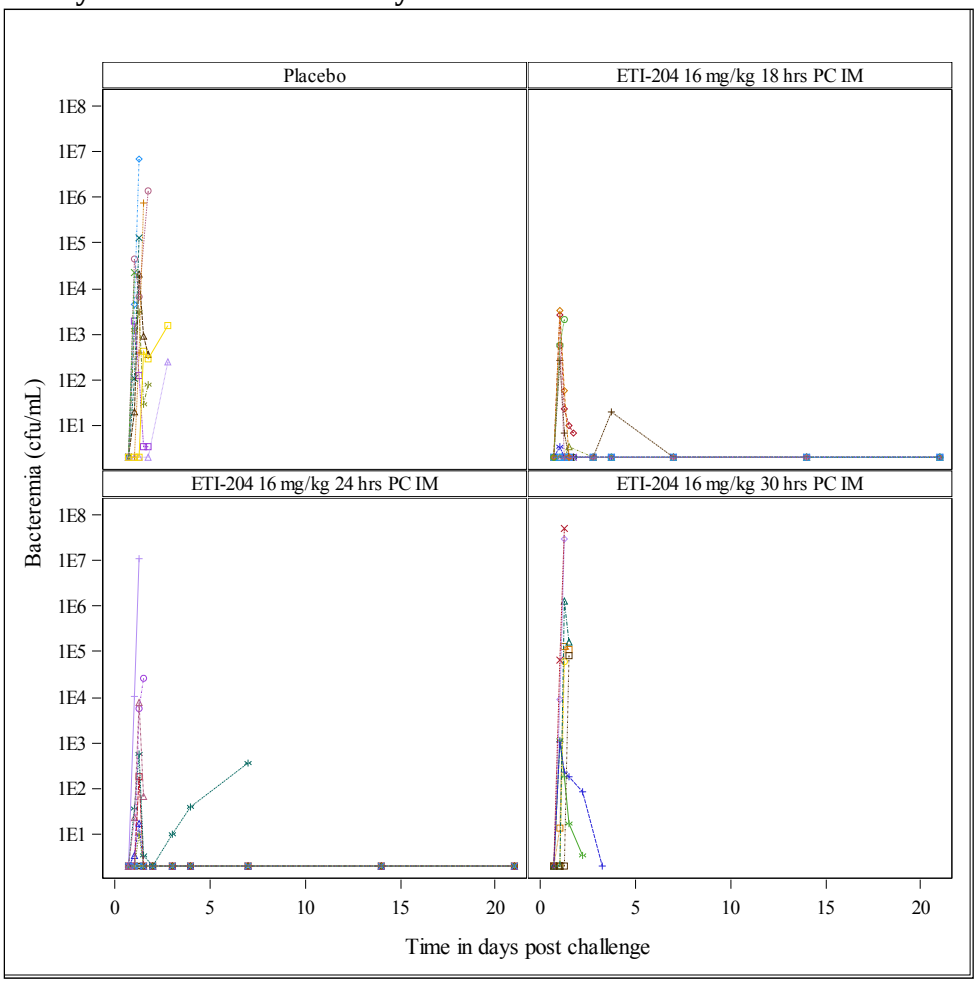
ETI-204 16 mg/kg 18 hrs PC IM (N=10)	ETI-204 16 mg/kg 24 hrs PC IM (N=10)	ETI-204 16 mg/kg 30 hrs PC IM (N=8)
0.0033*	0.0054*	0.894

*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.

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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.

Reference ID: 3859664

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

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	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

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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

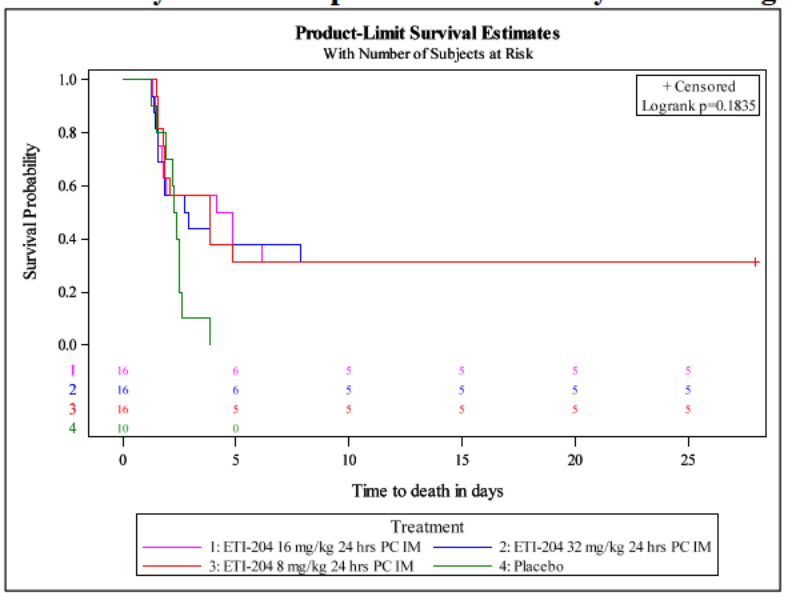

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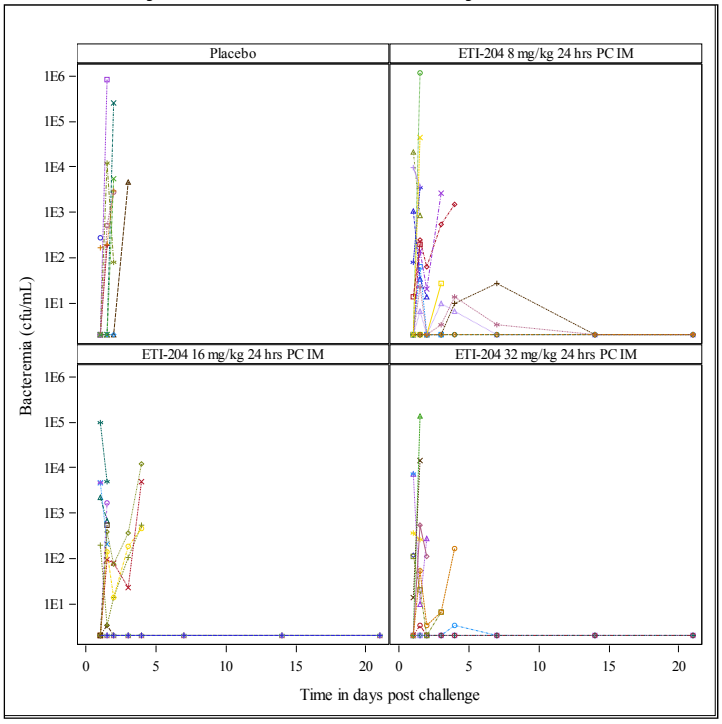
Table 104. Study AR037: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

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.

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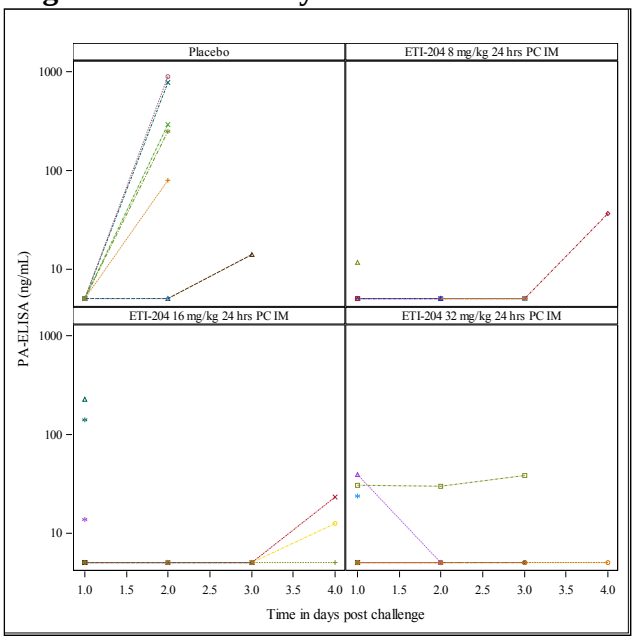
Figure 60. PA level by treatment and animal



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.

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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.

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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)

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	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.

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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

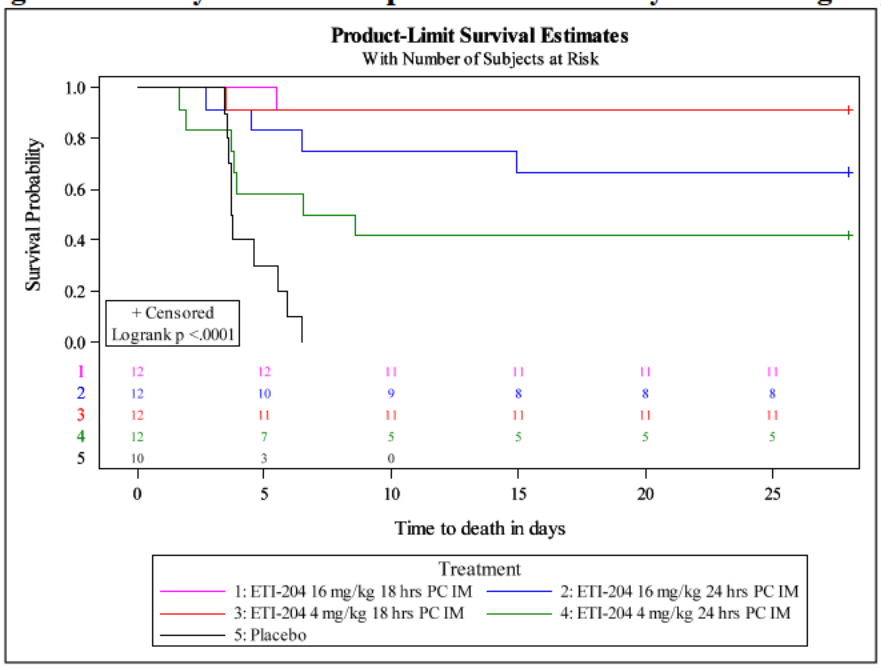

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Table 109. Study AR0315: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

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

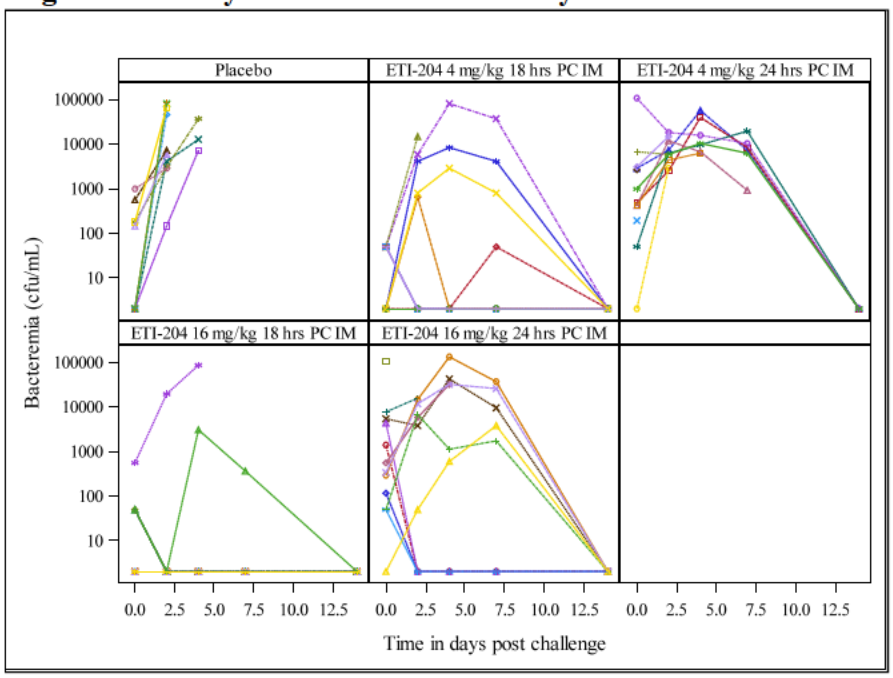
The following graph shows that the four treated groups had a reduced bacteremia level from Day 7 to Day 14 and all control animals died with a high bacteremia level.

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



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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.

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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.

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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

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(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.

Reference ID: 3859664

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.

Reference ID: 3859664

Figure 63. Study AP305: Kaplan-Meier curve by treatment group

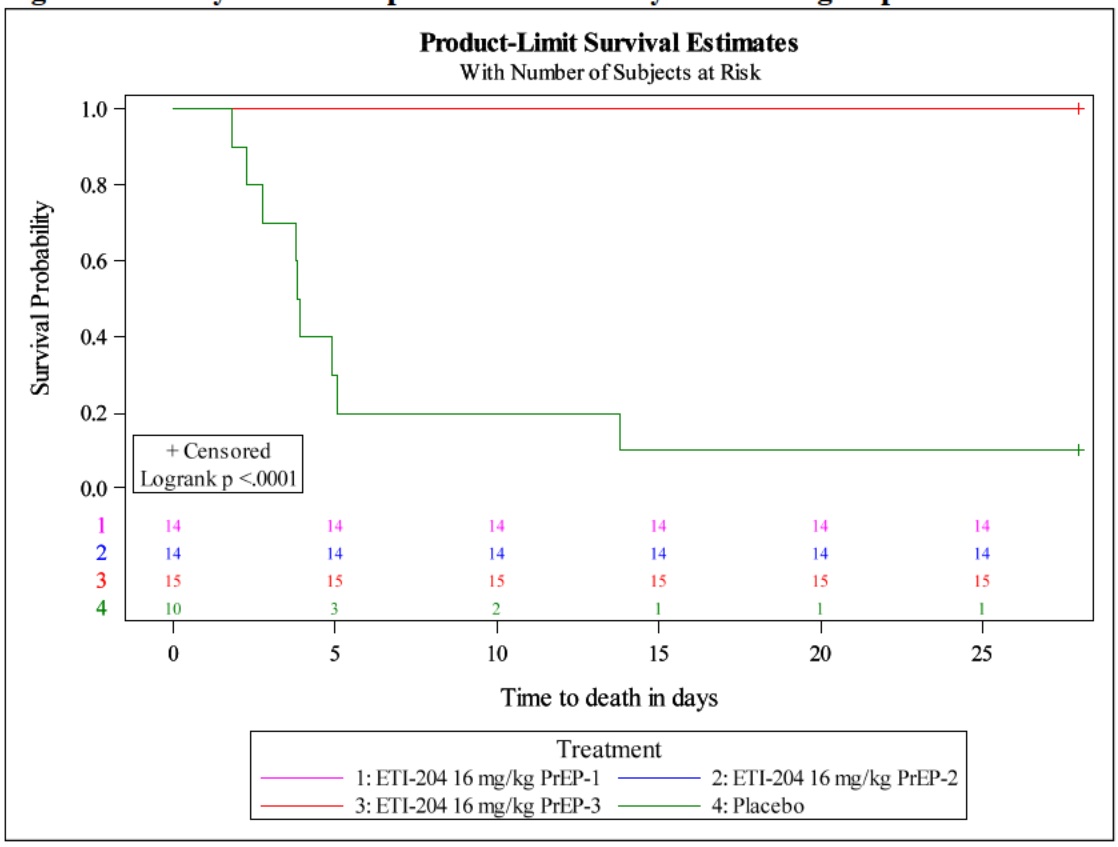


Table 114. Study AP305: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

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

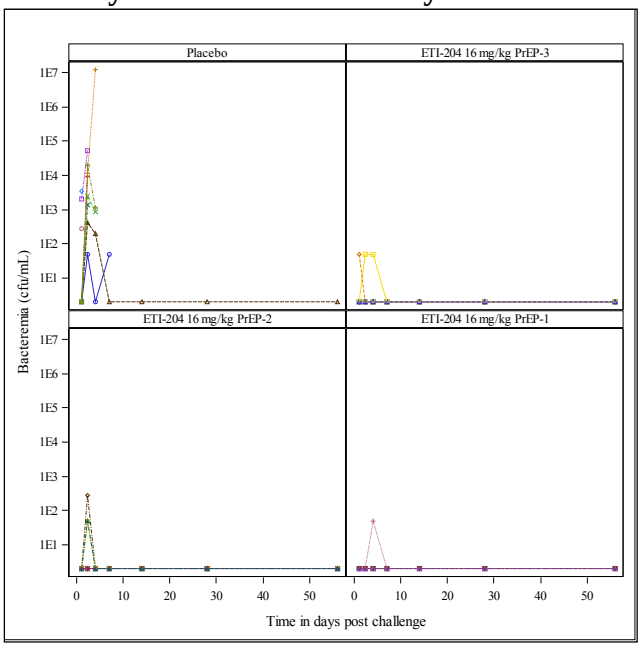
As shown in the following graph, the bacteremia levels in control group were higher at 54 hours post-challenge than at 24 hours. In contrast, all the bacteremia levels in the treatment groups remained at a lower level. From 7 days post-challenge on, all surviving animals in the treatment groups had a 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.

Reference ID: 3859664

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.

Reference ID: 3859664

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

Reference ID: 3859664

	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

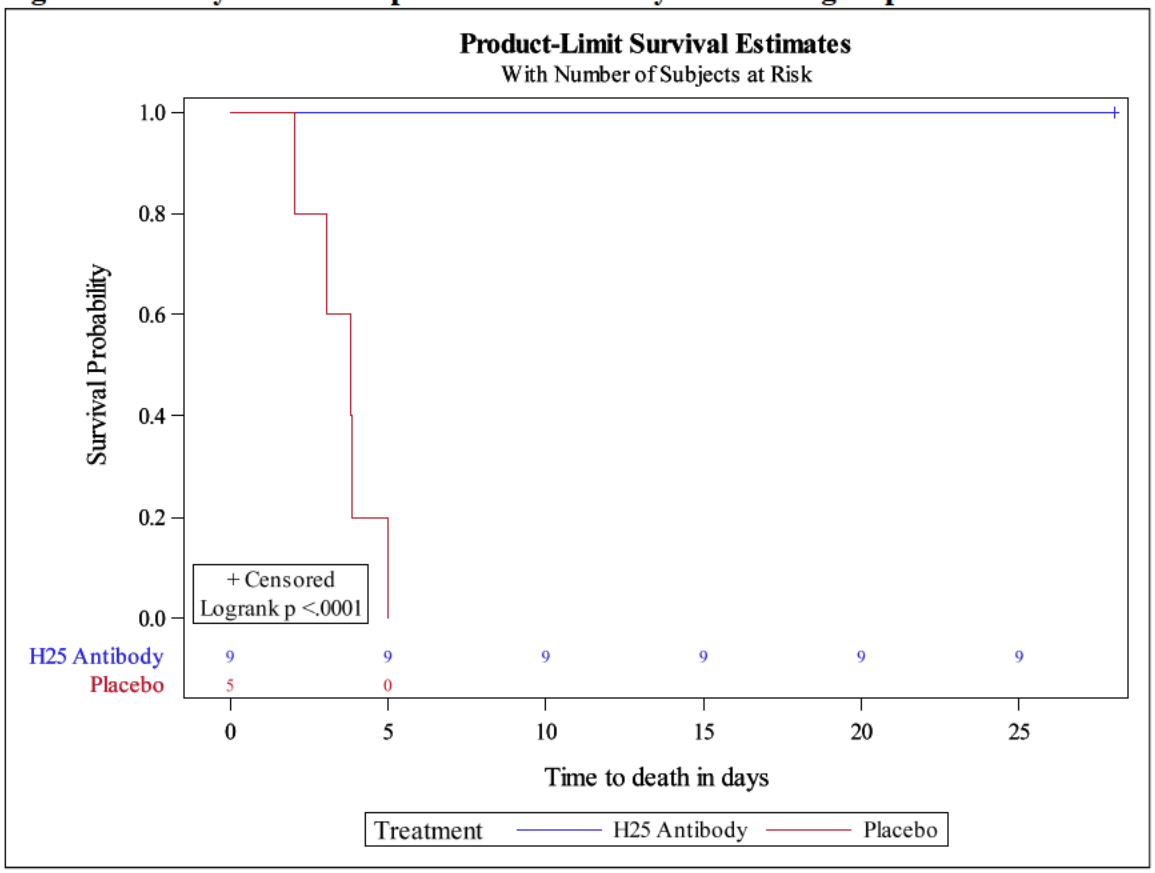
There was a statistically significant difference in survival proportions between the two groups, as the following table and graph show.

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.

Reference ID: 3859664

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

Reference ID: 3859664

	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

Reference ID: 3859664

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

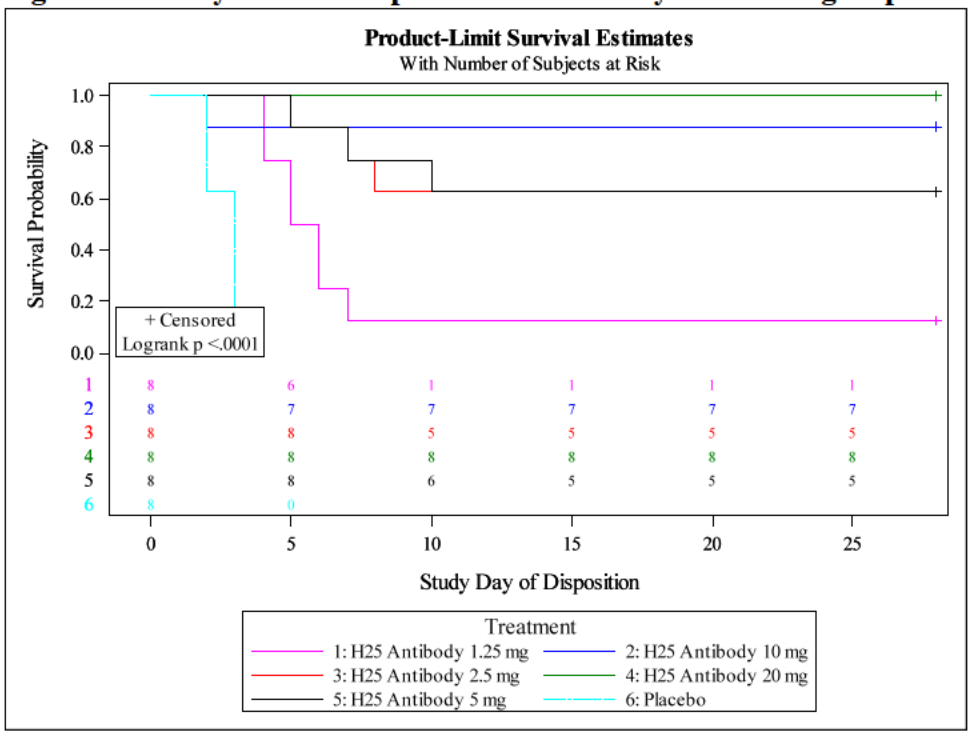


Table 123. Study AR003: Two-sided p-values of pairwise log-rank tests comparing time from challenge to death compared with the placebo group

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

Reference ID: 3859664

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]*

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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]

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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.

Reference ID: 3859664

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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

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 AND SAFETY STUDIES

BLA #:	125509
Drug Name:	Anthim (obiltoxaximab, ETI-204)
Indications:	Treatment of inhalational anthrax
Applicant:	Elusys Therapeutics, Inc.
Date(s):	Submission date: March 20, 2015
	PDUFA due date: March 18, 2016
Review Priority:	Standard
Biometrics Division:	IV
Statistical Reviewer:	Ling Lan, Ph.D.
Concurring Reviewers:	Karen Higgins, Sc.D. (Team Leader)
	Daphne Lin, Ph.D. (Deputy Division Director)
Medical Division:	Division of Anti-Infective Products (DAIP)
Clinical Team:	Elizabeth O'Shaughnessy, M.D., Medical Reviewer
	Ramya Gopinath, M.D., Medical Reviewer
	John J. Alexander, M.D. (Team Leader)
Project Manager:	Jane Dean, R.N., M.S.N.

Keywords: Animal studies, added benefit, central nervous system (CNS)

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1 EXECUTIVE SUMMARY

This Biological Licensing Application (BLA) was submitted by Elusys for Obiltoxaximab (ETI-204, Anthim), a monoclonal antibody directed against the *Bacillus anthracis* protective antigen, for the treatment of subjects with (b) (4) inhalational *B. anthracis*. Because it is not feasible or ethical to conduct controlled clinical trials in humans with inhalational *B. anthracis*, the applicant conducted efficacy studies in New Zealand White rabbits and cynomolgus monkeys. This review focuses on the effect of ETI-204 when given with antibacterial therapy.

This BLA contains eight animal studies to evaluate the added benefit and safety of a single intravenous dose of ETI-204 when given with antibacterial therapy for the treatment or prophylaxis of *B. anthracis*. Antibacterial therapy is the standard treatment of *B. anthracis* infection in humans and it is important to ensure that ETI-204 does not interfere with the efficacy of antibacterial therapy. The efficacy of antibacterial therapy in the animal models is high when the antibacterial therapy is dosed at a level that will obtain similar exposures to the dose used in humans, so in order to determine an added effect of ETI-204 over antibacterial therapy alone, the efficacy of antibacterial therapy needs to be reduced in the model. This is done by either delaying treatment or administering the antibacterial therapy at a less than human equivalent dose (HED). Most of the combination studies submitted by the applicant either delayed treatment, used a less than HED of antibacterial therapy, or both.

The eight studies had various study designs with a number of doses of ETI-204 administered at different times in relation to exposure of *B. anthracis*. This led to a wide variation in the treatment effects and survival rates. All the trials, however, compared ETI-204 given in combination with antibacterial therapy to antibacterial therapy alone, which allows for the assessment of the added contribution of ETI-204. The studies were open-label randomized trials conducted by (b) (4) except for Study AP 10-055 which was conducted by the Army and was not randomized. The proposed dose to be marketed is 16 mg/kg IV. Efficacy results seen at lower doses were seen as supportive of the efficacy of the 16 mg/kg IV dose.

Rabbit studies using levofloxacin (Levo) at HED (50 mg/kg) show that ETI-204 (IV) does not reduce the efficacy of antibacterial drugs and likely adds to the efficacy.

- Study AR007 administered the treatments before the development of symptoms (a post-exposure prophylaxis (PEP) study). Levo, though given at HED, was only given for 5 days to reduce the efficacy of the antibacterial alone group. The duration of antibacterial treatment is longer than 5 days in a PEP setting. ETI-204 (4 mg/kg) provided significant improvement in survival rates 56% (95% CI: 11%, 82%), when co-administered with Levo (89%, 8/9) compared to Levo alone (33%, 4/12).
- In Study NIAID 1030, only 28% of the animals challenged survived to treatment at 96 hours post spore exposure. The combination of ETI-204 (8 mg/kg) and Levo had numerically greater survival rate (100%, 4/4) relative to the Levo alone group (40%, 2/5) with risk difference of 60% (95%CI: -9%, 95%).

- Rabbits in Study NIAID 1045 had similar survival rates in the ETI-204 (8 mg/kg) plus Levo combination group (82%, 9/11) compared to the Levo alone group (78%, 7/9) with risk difference of 4% (95%CI: -36%, 44%) when administered treatment at 72 hours post exposure.
- Study AR034 compared 16 mg/kg ETI-204 and Levo with Levo alone. Treatments were initiated at about 30 hours post spore challenge. Similar survival rates were seen between these treatment arms (95%, 19/20, for the combination group and 100%, 20/20, for Levo alone).

Studies at a less than HED clearly demonstrate ETI-204 (IV) administered in combination with antibacterial drugs results in higher survival outcomes than antibacterial therapy alone.

- Study AR028 randomized 70% of rabbits, who survived to 72 hours post exposure, to receive the ETI-204 (16 mg/kg) and Levo (6.5 mg/kg x 3) combined treatment and the Levo monotherapy. The lower dose monotherapy led to 58% survival on the Levo alone therapy arm. There was a numerical added benefit of ETI-204 in survival when administered in combination with Levo (68%, 23/34) over Levo alone therapy (58%, 22/38).
- Rabbits in Study AP 10-055 received 8 mg/kg ETI-204 and oral doxycycline (2 mg/kg x 3) at 30 hours post exposure. The combination group (90%, 9/10) had a 40% (95%CI: -0.02, 0.72) higher probability of survival than that the doxycycline group (50%, 5/10).
- Monkeys in Study NIAID 1056 received oral Cipro (10 mg/kg, <HED) and 8 mg/kg ETI-204 at about 24 hours post first positive qualitative PA result (around 48 hours post spore challenge). Eighty one percent of the animals challenged survived to receive treatments. ETI-204 provided significant added benefit in survival when co-administered with Cipro (62%, 8/13) over Cipro alone therapy (15%, 2/13), with a risk difference of 46% (95% CI: 4%, 77%).
- Study NIAID 2469 repeated NIAID 1056 with 84% monkeys surviving to receive treatment to show a 26% (95% CI: -14%, 60%) numerical difference in survival rates for the ETI-204 and Cipro group (57%, 8/14) over Cipro alone group (31%, 4/13).

Two limitations of the studies reviewed include the schedule for randomization and the power of primary efficacy analyses. Seven out of the eight studies randomized animals to treatment prior to challenge, which resulted in animal deaths in each treatment arm occurring prior to treatment. An improved design would be to randomize animals post challenge and just prior to treatment initiation, as was done for Study AR028. Therefore this review focused on an analysis population that included animals who survived to receive study treatment assuming that the assigned randomized treatment did not affect the initiation of treatment. A problem with powering a study occurs when treatment is delayed in order to reduce the efficacy of antibacterial treatment, because many animals do not survive to treatment. This leads to a smaller sample size for analysis and a difficultly in adequately powering the trial.

Despite the limitations, ETI-204 administered in combination with antibacterial drugs did not interfere with the efficacy of antibacterial drugs and resulted in higher survival outcomes than

antibacterial therapy alone. The stratified meta-analyses confirmed the improvement in survival rates regardless of the animal species and doses of antibacterial used.

In conclusion, the eight combination studies adequately address the added survival benefit of ETI-204 when administered in combination with antibacterial drugs for the treatment of systemic inhalational anthrax disease, compared to the antibacterial therapy alone.

2 INTRODUCTION

2.1 Overview

Obiltoxaximab (ETI-204, Anthim) is a monoclonal antibody directed against the *Bacillus anthracis* protective antigen and developed by Elusys for the treatment of subjects with (b) (4) inhalational *B. anthracis*. The proposed dosage of ETI-204 is a single intravenous (IV) 16 mg/kg infusion over 90 minutes. ETI-204 will likely be administered in combination with antimicrobials.

The BLA was submitted to the FDA on 20 March 2015. On August 2, 2005, the applicant submitted IND 12285 for ETI-204. Due to the lethality of the anthrax infection, clinical trials in humans are not ethically feasible, so the applicant is relying on the use of the Animal Efficacy Rule (21 CFR 314.600-650). This application contained 26 studies conducted in cynomolgus monkeys and New Zealand White rabbits, including 8 studies that assessed the combination of ETI-204 with antibacterial therapy. These included 1 post-exposure prophylaxis (PEP) study and 7 treatment studies, one of which was also a re-challenge study. A PEP study initiates treatment after exposure to *B. anthracis* spores, but before the development of clinical signs and/or symptoms. A treatment study initiates treatment after exposure to *B. anthracis* and after the development of clinical signs and/or symptoms.

Antibacterial therapy, the standard treatment of *B. anthracis,* is not 100% effective in humans and it is possible that a strain of *B. anthracis* could be resistant to antimicrobial therapy. ETI-204 could provide additional efficacy in combination with antibacterial therapy over the antibacterial therapy alone. However, it is important to ensure that the efficacy of the antibacterial therapy is not diminished by the administration of ETI-204. For this reason this review evaluated the added survival benefit of ETI-204 administered in combination with antibacterial therapy in the 8 relevant studies listed in Table 1. (b) (4)

Dr. Xianbin Li's review of this BLA covers the efficacy of ETI-204 alone and addresses the efficacy of the product from different manufacturing facilities.

The efficacy of antibacterial therapy is high in the animal models in the treatment of *B. anthracis*, so in order to determine an added effect of ETI-204 over antibacterial therapy alone, the efficacy of antibacterial therapy needs to be reduced. This is done by either delaying treatment or administering the antibacterial therapy at a less than human equivalent dose (HED). Most of the combination studies submitted by the applicant either delayed treatment, used a less than human equivalent dose (HED) of antibacterial therapy, or both. Table 1 shows the list of studies related to the evaluation of added benefit in ETI-204 co-administered with antibacterial therapy reviewed for survival outcomes. The eight studies were categorized by animal species and dose of antibacterial into three clusters, four rabbit studies that administered antibacterial at a HED, two rabbit studies that used a less than HED antibacterial therapy and two monkey studies that gave antibacterial at a less than HED. Note that efficacy results of lower doses were seen as supportive of the efficacy of the 16 mg/kg IV dose.

Table 1 List of combination studies reviewed for survival outcomes

Study (ETI-204 Product)	No. of animals for groups reviewed (dose in mg/kg)	Treatment initiation post exposure in hours	Follow-up Period
Rabbits studies with antibacterial in human equivalent dose (HED)			
AR007 ((b) (4))	Levofloxacin (50): 12 Levo + ETI-204 (4 IV): 9 Levo + ETI-204 (8 IM): 9	9 ± 3 (post-exposure prophylaxis)	Day 28 post exposure (PE)
1030 (Baxter)	Levo (50): 16 Levo + ETI-204 (8 IV): 16	96±1	Day 28 PE
1045 (Baxter)	Levo (50): 16 Levo + ETI-204 (8 IV): 16	72±1	Day 28 PE
AR034 (Phase I) (Lonza)	Levo (50): 20 Levo + ETI-204 (16 IV): 20	30 (re-challenge)	Month 9 PE
Rabbits studies with < HED antibacterial			
AR028 (Baxter)	Levo (6.5): 38 Levo + ETI-204 (16 IV): 34	72±4	Day 28 PE
AP-10-055 (Baxter)	Doxy (2):10 Doxcycycline + ETI-204 (8 IV): 10	ECL Positive by 30	Day 28 PE
Monkeys studies with < HED antibacterial			
1056 (Baxter)	Ciprofloxacin (10 mg/kg): 16 Cipro + ETI-204 (8 mg/kg IV): 16	ECL Positive + 24±12	Day 28 PE
2469 (Baxter)	Cipro (10 mg/kg): 16 Cipro + ETI-204 (8 mg/kg IV): 16	ECL Positive + 24±12	Day 28 PE

ECL: protective antigen (PA) result determined by electrochemiluminescence (ECL).
ETI-204 was manufactured by (b) (4), Baxter and Lonza.

2.2 Data Sources

Data sets for 8 combination studies (Table 1) were submitted electronically, except for Study AP 10-055 where the reviewer extracted some of the data directly from the study report. The full electronic path according to the CDER EDR naming convention is as follows: \\CDSESUB1\evsprod\BLA125509

The electronic data sets (ADSL) generally represented the data described in the study report.

3 STATISTICAL EVALUATION

3.1 Data and Analysis Quality

Most of the submitted data followed FDA guidance and were ready to be reviewed, except Study AP 10-055 which had no electronic datasets. Information requests were issued to obtain or validate information relevant to the review when it was missing from or inconsistent with the ADSL data. The applicant provided a weight variable in Study NIAID 1045, corrected challenge

time for some animals in Study AR028, and provided a challenge dose for Study NIAID 1056 after information requests.

3.2 Evaluation of Efficacy

Eight combination studies, of which seven were treatment studies (one had a re-challenge component) and one was a post-exposure prophylaxis study, were conducted to support the added benefit of ETI-204 when administered in combination with antibacterial in animals post anthrax exposure. Different study designs were used for the combination studies which all contained at least four treatment groups. Some of these studies contained monotherapy ETI-204 and untreated controls. For a review of these treatment arms refer to the review by Dr. Xianbin Li. This section presents and discusses the details of the ETI-204 and antibacterial combination group and the antibacterial group in the eight studies. Table 2 summarizes the characteristics and survival outcomes of the studies reviewed in the population of animals who received treatment as planned.

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Table 2 Survival results for treated animals in combination studies

Study	ETI-204 Dose IV (mg/kg)	PA-ELISA (ng/ml) GM[1](95%CI)	Treatment Initiation Pose Exposure (hour)	Survival % (# survived / # treated)		Difference and 95%CI (ETI-204&Anti – Anti)
				ETI-204 + Antibacterial	Antibacterial	
Rabbits (antibacterial HED[2]: levofloxacin 50 mg/kg)						
AR007 (b) (4)[3]	4	N/A	9 ± 3	89% (8/9)	33% (4/12)	56% (11, 82)
1030 **Baxter**	8	84.8 (24, 303)	96±1	100% (4/4)	40% (2/5)	60% (-9, 95)
1045 **Baxter**	8	7.9 (4, 17)	72±1	82% (9/11)	78% (7/9)	4% (-36, 44)
AR034 (Phase I) **Lonza**	16	5.9 (5, 7)	30	95% (19/20)	100% (20/20)	-5% (-26, 11)
Rabbits (antibacterial < HED: Levo 6.5 mg/kg (AR028) , doxycycline 2 mg/kg (AP 10-055))						
AR028 **Baxter**	16	31.7 (20, 71)	72±4	68% (23/34)	58% (22/38)	10% (-12, 32)
AP-10-055[4] **Baxter**	8	N/A	ECL[5] Positive by 30	90% (9/10)	50% (5/10)	40% (-2, 72)
Monkeys (antibacterial < HED: ciprofloxacin10 mg/kg)						
1056 **Baxter**	8	235.3 (160, 345)	ECL Positive + 24±12	62% (8/13)	15% (2/13)	46% (4, 77)
2469 **Baxter**	8	310.1 (193.6, 496.6)	ECL Positive + 24±12	57% (8/14)	31% (4/13)	26% (-14, 60)

1. GM: geometric mean for quantitative PA by PA-ELISA prior to treatment
2. HED: human equivalent antibacterial dose.
3 (b) (4) Baxter and Lonza were the manufactures of ETI-204.
4. Study AP 10-055: Treatment was initiated at first positive PA or by 30 hours post exposure. No electronic data was provided (USAMRIID).
5. ECL: protective antigen (PA) result determined by electrochemiluminescence (ECL)

Reference ID: 3857343

3.2.1 Study NIAID 1056

Study NIAID 1056: Efficacy of a Monoclonal Antibody Given in Combination with Ciprofloxacin in the Cynomolgus Macaques Therapeutic Model of Inhalational Anthrax

3.2.1.1 Study Design

Study NIAID 1056 was a parallel-group, open-label, randomized, controlled study in healthy male and female cynomolgus monkeys to evaluate the added benefit of combination therapy of ETI-204 and ciprofloxacin (Cipro) compared with Cipro alone. Table 3 provides details of the study design.

Table 3 Study 1056: Study design

Group	Treatment	ETI-204 Dose	Ciprofloxacin Dose	Treatment Initiation post challenge	Therapy Duration	No. of Animals
1	ETI-204	8 mg/kg	N/A	ECL Positive	Single dose (IV)	8
2	Ciprofloxacin	N/A	10 mg/kg	ECL Positive + 24±12 hours	4 days (oral)	16
3	ETI-204+ Ciprofloxacin	8 mg/kg	10 mg/kg	ECL Positive + 24±12 hours	Single dose (ETI-204; IV) + 4 days (Cipro; oral)	16
4	Control	N/A	N/A	N/A	N/A	8

ECL positive = positive protective antigen (PA) result determined by electrochemiluminescence (ECL).
IV = intravenous
N/A = not applicable

The proposed primary objective was to assess the efficacy in survival rate of ETI-204 (8 mg/kg) in combination with Cipro at a less than HED (10 mg/kg) compared to Cipro at the same dose, when administered at a delayed time, 24±12 hours after the first positive protective antigen (PA) result by electrochemiluminescence (ECL), following inhalational exposure to *B. anthracis* in cynomolgus monkeys.

A total of 48 monkeys were randomized pre-exposure by weight into two groups of size eight (group 1 and 4) and two groups of size 16 (group 2 and 3) with each group consisting of 50% male and 50% female animals. Animals in each group were then randomized to three challenge days (challenge day A, B and C) with a randomized challenge order each day.

Comment: Randomization should be conducted just prior to treatment initiation. The study performed randomization prior to spore challenge instead, which led to possible inclusion of events (death) occurring post challenge but prior to treatment initiation.

This review will focus on the comparison of group 2 to group 3 to assess the added benefit of ETI-204 when given with Cipro. For discussion of the results of Group 1 and 4 see statistical review by Xianbin Li.

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Animals randomized were aerosol exposed to 200 x LD_{50} of *B. anthracis* spores on Day 0 (challenge day) at the (b) (4) Monkeys that were alive at 24 hours (±12 hours) after obtaining the first positive PA-ECL result received study treatment. Animals randomized to group 2 and 3 received a first dose of Cipro (10 mg/kg; oral gavage) at 24 hours (±12 hours) after obtaining a positive PA-ECL and three subsequent Cipro doses (10 mg/kg) by oral gavage 24 hours (±3 hours) following the previous dose. Animals randomized to group 3 also received antibody (8 mg/kg; IV bolus) once at 24 hours (±12 hours) after obtaining a positive PA-ECL result. Animals were monitored until Day 28 post challenge and then euthanized.

The primary efficacy endpoint of this study was 28-day survival, defined as the proportion of animals that survive to Day 28 post spore challenge. The primary efficacy analysis was the comparison of survival rates in the four study groups using one sided Fisher's exact test at an overall alpha level of 0.05. The protocol did not mention method to account for multiple comparisons. The study report stated that Bonferroni–Holm adjustment was conducted to adjust for multiple comparisons. The Bonferroni–Holm method is a stepwise method used to control the overall (familywise) error rate at a specific alpha level when multiple comparisons are performed. The analysis was performed in all animals randomized and challenged. This analysis was based on the planned treatment group rather than the actual treatment group received and monkeys that died between challenge and the first dose of study agent would be analyzed as an event (death).

Comment: This trial in essence contained two studies in one, each with its own test and control arm. A comparison of group 1 with group 4 can determine the effect of ETI-204 as a monotherapy. A comparison of group 2 with group 3 can determine the effect of ETI-204 when administered with ciprofloxacin. Therefore, in our focus of group 2 versus group 3 we will consider a one-sided 0.025 type I error using a Fisher's exact test and not adjust for multiple comparisons. The primary population for this review was animals who survived to receive at least one treatment.

As an exploratory efficacy analysis, the log-rank test was used to compare survival time from initiation of spore challenge between Cipro only group and the ETI-204 plus Cipro group. The analysis was performed in treated animals. In addition, survival time from treatment initiation was compared between the combination group and the Cipro group using the same method (Section 3.2.1.3.2.1).

The applicant proposed sample size of 16 animals in each group based on the assumptions that the probability of survival was 87% and 40% in the ETI-204 & Cipro group and Cipro group, respectively, using one-sided Fisher's exact test with a 0.05 alpha level.

Comment: If a one-sided Fisher's test was used for sample size calculation, an alpha level of 0.025 would have resulted in a power of 70% with 16 animals per group under the same assumptions on survival rates.

Reference ID: 3857343

3.2.1.2 Animal Disposition, Demographic and Baseline Characteristics

Table 4 provides a summary of animal disposition in Study NIAID 1056. Among the 32 monkeys that were randomized to treatment groups 2 and 3 and challenged with *B. anthracis* spores, 26 (81%) monkeys survived to 24±12 hours after the first positive PA-ECL post exposure. There then remained 13 monkeys each in the Cipro group and the ETI-204 & Cipro group, that received randomized treatment.

Table 4 Study 1056: Animal disposition before treatment initiation

	Cipro	ETI-204 & Cipro	Total
Animals challenged	16	16	32
Animals who died before treatment	3	3	6/32 (19%)
Animals who survived to be treated	13	13	26/32 (81%)
Analysis population			
Animals randomized and challenged	16	16	32
Toxemic at or before treatment initiation	13	13	26
Bacteremic at or before treatment initiation	13	13	26
Animals survived to treatment	13	13	26

As shown in Table 5, animals treated in the Cipro and ETI-204 & Cipro groups were comparable in sex, age, weight at baseline, exposure at challenge, PA-ELISA and quantitative bacteremia prior to treatment (PTT). None of the animals were positive for anti-PA IgG ELISA and *Klebsiella* prior to challenge. No significant differences were observed between the 2 treatment groups for any of the demographic or baseline characteristics. The results were consistent in treated animals and animals challenged and randomized (results not shown).

Table 5 Study 1056: Demographics and characteristics at baseline and prior to treatment

	Cipro n = 13	ETI-204&Cipro n = 13	P-value*
Sex [n(%)]			
Male	6 (46.2)	7 (53.8)	
Female	7 (53.8)	6 (46.2)	1.00
Weight (kg) pre-exposure			
Mean ± SD	2.9±0.3	2.9±0.4	
Median	2.9	2.9	
(Min, Max)	(2.2, 3.5)	(2.3, 4.1)	0.92
Age (years)			
Mean ± SD	3.1±0.3	2.9±0.3	
Median	3.0	3.0	
(Min, Max)	(3.0, 4.0)	(2.0, 3.0)	0.17
Challenge dose ($\times 10^7$ cfu)			
Mean ± SD	1.2±0.4	1.1±0.4	
Median	1	1	
(Min, Max)	(0.8, 1.9)	(0.7, 2.3)	0.34

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	Cipro n = 13	ETI-204&Cipro n = 13	P-value*
LD_{50} groups [n(%)]			
< 200	12 (92.3)	8 (61.5)	
200 or higher	1 (7.7)	5 (38.5)	0.16
Bacteremia ($\times 10^4$ cfu/mL) PTT			
Geometric Mean	5.7	1.8	
95% CI	(1.1, 29.7)	(0.3, 10.7)	0.37
PA-ELISA (ng/ml) PTT*			
Geometric Mean	225.6	433.9	
95% CI	(103.6, 491)	(205.8, 914.6)	0.08

* P-value was based on Fisher's exact test for categorical data and 1-way ANOVA for continuous data

During the course of this study, all monkeys treated became toxemic (detectable PA-ELISA) before treatment initiation. Table 6 shows that the average time to toxemia is 37.9 hours in the Cipro group and 35 hours in the ETI-204 & Cipro combination group. All monkeys treated became bacteremic PTT, with the same mean time to bacteremia being 23.7 hours in the Cipro group and the combination group. The trigger to treatment was the first positive PA-ECL, therefore time to first positive PA-ECL was identical to time to trigger to treatment. The mean time to first positive PA-ECL was 34.2 hours in Cipro group and 33.1 hours in the combination group. There was no significant difference in the time to treatment initiation between the 2 treatment groups based on log-rank test. The average time to treatment initiation was 48.2 hours and 48.8 hours in the Cipro group and the combination group, respectively.

Table 6 Study 1056: Time to detectable PA, bacteremia and treatment initiation in hours

	Cipro n = 13	ETI-204 & Cipro n = 13	P-value*
Time to first positive PA-ECL			
Mean ± SD	34.2±1.9	33.1±6.6	
Median	34.3	35.2	
(Min, Max)	(30.8, 37.5)	(21.2, 44)	0.44
Time to first positive PA-ELISA			
Mean ± SD	37.9±3.8	35±5.3	
Median	36.9	36	
(Min, Max)	(32.8, 46.9)	(21.2, 43.1)	0.21
Time to bacteremia			
Mean ± SD	23.7±1.6	23.7±1.8	
Median	24.2	22.9	
(Min, Max)	(21.2, 25.7)	(21.9, 27.4)	0.72
Time to treatment initiation			
Mean ± SD	48.7±1.6	49.3±3.3	
Median	48.2	48.8	
(Min, Max)	(46.6, 51.7)	(45.4, 56.3)	0.58

*P-value was based on log-rank test.

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Figure 1 illustrates the changes in quantitative PA from 24 hours post exposure to Day 28 in animals surviving to receive treatment in the combination group and the Cipro alone group. Animals died around PA peaks and the PA-ELISA level decreased to undetectable for those who survived.

Note the reported quantitative bacteremia were measured PTT for animals treated and at 48 hours post challenge for controls. There were no more than two bacteremia values per group at 24 hours and 5 days post challenge, therefore bacteremia over time was not plotted.

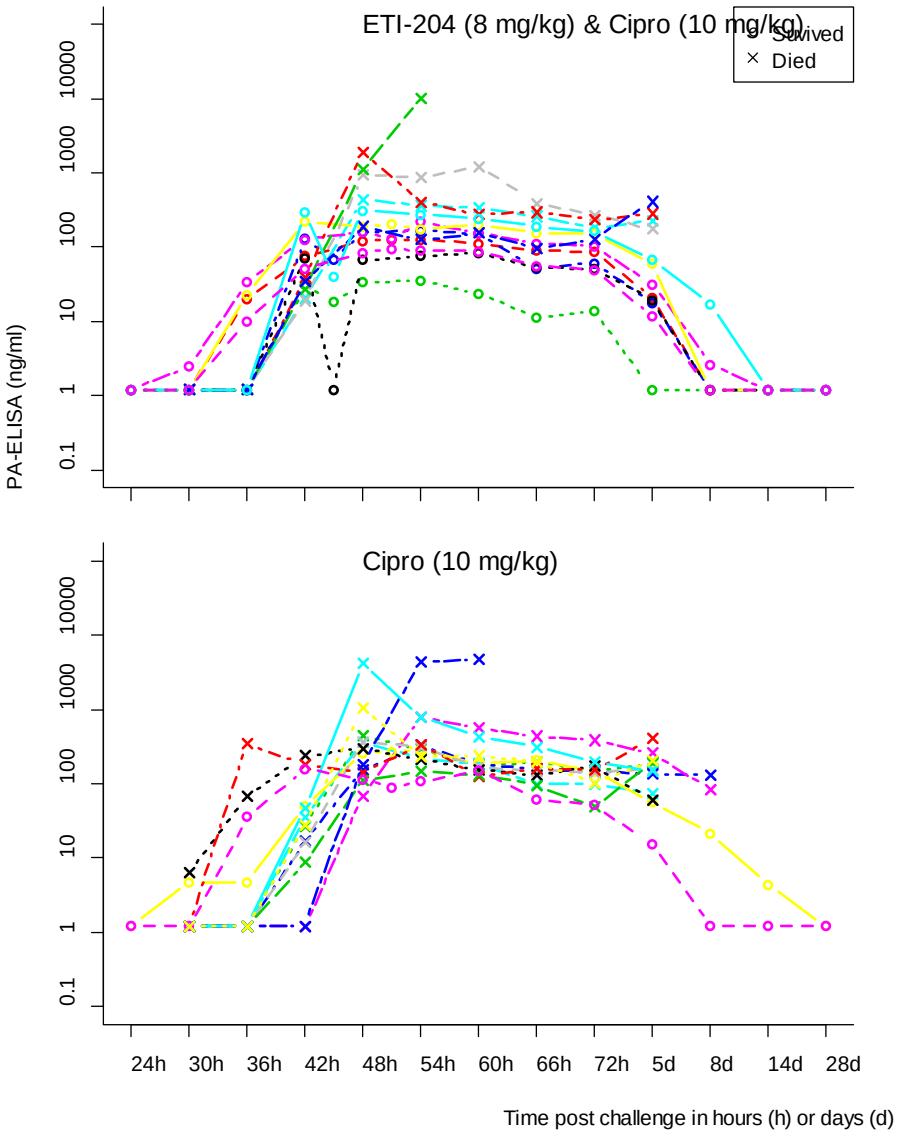

Figure 1 Study 1056: PA-ELISA over time for animals treated

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3.2.1.3 Results and Conclusions

Study NIAID 1056 followed the protocol and demonstrated that ETI-204 provided significant added benefit in survival rates when administered in combination with Cipro (61.5%, 8/13) over Cipro alone therapy (15.4%, 2/13) on the treatment of Anthrax in cynomolgus monkeys (One-sided Fisher's exact test, p-value = 0.021) when Cipro was doses at less than HED. This section provides detailed results from primary and exploratory efficacy analyses.

3.2.1.3.1 Primary Efficacy Analysis

Table 7 presents the primary efficacy results in Study NIAID 1056. Sixteen out of the 27 animals that received study agent died, 11 in the Cipro group and 5 in the combination group. The survival rate in the ETI 204 & Cipro combination group (61.5%) was significantly higher than the Cipro group (15.4%), with a difference of 46.1% (95% CI: 4%, 77%), p-value of 0.021 from one-sided Fisher's exact test. An additional analysis was conducted that looked at survival in all challenged and randomized animals. In this analysis, animals that died before treatment were counted as deaths in the analyses. These results are supportive. The lower bounds of 95% confidence interval (CI) for the survival rate difference were above zero in animals treated and animals challenged and randomized, which supported the added benefit of ETI-204 in combination therapy.

Following spore challenge, all animals became toxemic and bacteremic PTT so the analysis for the animals treated was the same as that for toxemic treated animals and bacteremic treated animals.

Table 7 Study 1056: Survival rates at Day 28 post exposure

Animals	Cipro	ETI-204& Cipro	Difference (ETI-204&Cipro – Cipro) 95% CI**	P-value*
Challenged and randomized	2/16 (12.5%)	8/16 (50%)	0.38 (0.05, 0.66)	0.027
Survived to be treated	2/13 (15.4%)	8/13 (61.5%)	0.46 (0.04, 0.77)	0.021

*P-value based on a 1-sided Fisher's exact test compared to 0.025.
**Difference in % survivors with 95% exact confidence interval (CI).

Comment: The significant 46% difference in survival rates between the 2 treatment groups supports the added benefit of ETI-204 when co-administered with Cipro at a less than HED. The applicant has submitted results of Study NIAID 2469 with a similar study design to the current study. An exploratory meta-analysis of these two studies was performed to investigate the added benefit of ETI-204 in combination with Cipro (< HED) in monkeys (Section 5.2.1).

3.2.1.3.2 Exploratory Efficacy Analysis

3.2.1.3.2.1 Survival Time from Spore Challenge to Treatment Initiation

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As an exploratory efficacy analysis, time from the initiation of spore challenge to death was compared between the ETI-204 & Cipro combination group and the Cipro group. For animals that died on study, most deaths occurred within 8 days of spore challenge. The 11 deaths in the Cipro group happened between 2.17 days and 7.31 days following spore challenge while the 5 deaths in the ETI-204 & Cipro group happened between 1.96 days and 4.2 days following spore challenge. The average time from spore challenge to death is 4.21 days in the Cipro group and 3.41 days in the combination group. No animals died beyond 3 days post the last dose of Cipro. According to the log-rank test, the probability of survival was significantly greater in the ETI-204 & Cipro combination group than in the Cipro group (Figure 2, two-sided P-value = 0.044). For the sake of completeness, Figure 3 illustrated survival times from challenge in all animals challenged and randomized by group.

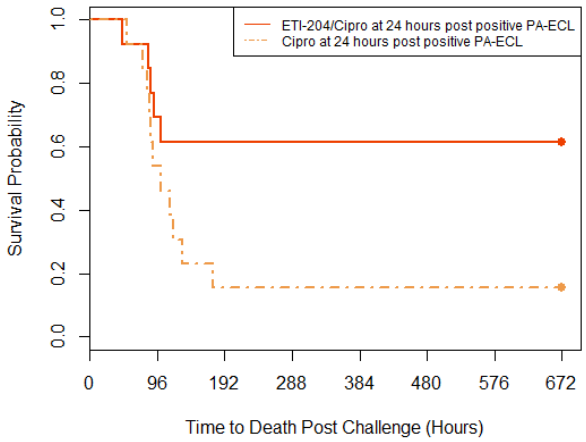

Figure 2 Study 1056: Survival time for animals treated with ciprofloxacin

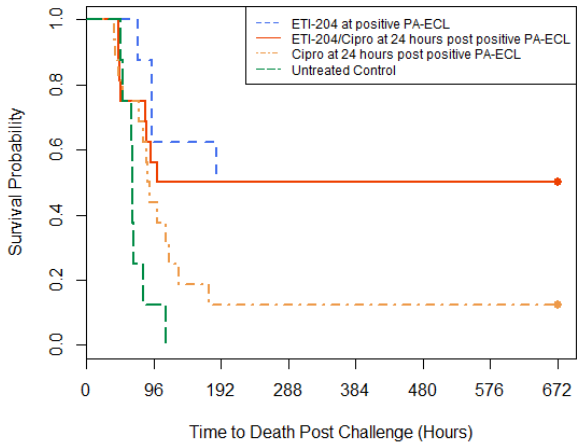

Figure 3 Study 1056: Survival time for all animals randomized and challenged

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Likewise, the survival time from the treatment initiation was compared between the combination group and the Cipro group. The probability of survival was statistically greater in the ETI-204 & Cipro group than in the Cipro group (P-value = 0.047). Both analyses were performed in the animals survived to treatment and similar results were obtained in animals challenged and randomized.

3.2.1.3.2.2 Analyses by Spore Challenge, PA-ELISA and Bacteremia

Two-sided Spearmen's rank correlation test was conducted to examine the association between the exposure (challenge dose), severity of anthrax infection (PA-ELISA and quantitative bacteremia) and time to death. Spearmen's correlation coefficient, r_s, is a measure of non-parametric correlation between two variables using a monotonic function, giving a value between +1 and −1 inclusive, where 1 is total positive correlation, 0 is no correlation, and −1 is total negative correlation. As PA-ELISA PTT increases, time to death decreases ($r_s = -0.70$, p-value < 0.001), and level of bacteremia PTT increases ($r_s = 0.59$, p-value = 0.002). The challenge dose (LD_{50}) was not significantly associated with PA-ELISA ($r_s = 0.16$), bacteremia ($r_s = 0.03$) and time to death ($r_s = 0.16$). Quantitative bacteremia was not significantly associated with time to death ($r_s = -0.36$, p-value = 0.09).

Table 8 summarized survival rates in the Cipro group and ETI-204 & Cipro group by exposure, PA-ELISA and bacteremia. These factors were categorized by targeted challenge dose 200 x LD_{50} and median (PA-ELISA and quantitative bacteremia). As predicted, lower PA-ELISA level was associated with significantly greater survival rate (7/8 versus 1/5) in combination group (Fisher's exact test p-value = 0.03). The correlation between time to death and PA-ELISA could be driven by the animals treated in ETI-204 & Cipro group with lower PA-ELISA PTT. Given the limited sample size for each PA-ELISA group by treatment, regression analysis was not conducted to further explore the association between PA-ELISA and survival rate.

Table 8 Study 1056: Survival rates by challenge dose, PA-ELISA and bacteremia

	Cipro (# survived/n) n = 13	**ETI-204 & Cipro (# survived/n) n = 13**	**Total (# survived/n) n = 26**
Challenge dose (LD_{50})			
< 200	2/12 (17%)	5/8 (63%)	7/20 (35%)
200 or higher	0/1 (0%)	3/5 (60%)	3/6 (50%)
PA-ELISA			
< 240	1/5 (20%)	7/8 (88%)*	8/13 (62%)**
240 or above	1/8 (13%)	1/5 (25%)	2/13 (15%)
Quantitative bacteremia			
< 11000	0/4 (0%)	6/9 (67%)	6/13 (46%)
11000 or higher	2/9 (22%)	2/4 (50%)	4/13 (31%)

*Two-sided Fisher's exact test p-value = 0.03.
**Two-sided Fisher's exact test p-value = 0.04.

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3.2.2 Study NIAID 2469

Study NIAID 2469: Efficacy of Ciprofloxacin in an Aerosol Challenge Model of *B. anthracis* (Ames strain) With and Without Adjunctive Therapy in Cynomolgus Macaques: Study 2

3.2.2.1 Study Design

Study NIAID 2469 was the second combination study in monkeys and used a similar study design to Study NIAID 1056. Study NIAID 2469 was a parallel-group, open-label, randomized, controlled study in healthy male and female cynomolgus monkeys to evaluate the added benefit of combination therapy of ETI-204 and ciprofloxacin (Cipro) compared with Cipro alone. Table 9 illustrates the details of the study design, which is the same as Study NIAID 1056 except replacing the ETI-204 alone group with a Cipro (26 mg/kg, HED) group.

Table 9 Study 2469: Study design

Group	Treatment	ETI-204 Dose	Ciprofloxacin Dose	Treatment Initiation	Therapy Duration	No. of Animals
1	ETI-204+ Ciprofloxacin	8 mg/kg	10 mg/kg	ECL Positive + 24±12 hours	Single dose (ETI-204; IV) + 4 days (Cipro; oral)	16
2	Ciprofloxacin	N/A	10 mg/kg	ECL Positive + 24±12 hours	4 days (oral)	16
3	Ciprofloxacin	N/A	26 mg/kg	ECL Positive + 24±12 hours	4 days (oral)	16
4	Control	N/A	N/A	N/A	N/A	8

ECL positive = positive protective antigen (PA) result determined by electrochemiluminescence (ECL).
N/A = not applicable

The proposed primary objective was to assess the efficacy of ETI-204 (8 mg/kg) in combination with Cipro at 10 mg/kg, a less than HED, compared to Cipro at the same dose in survival rate, when administered at a delayed time, 24±12 hours after the first positive PA-ECL result, following inhalational exposure to *B. anthracis* in cynomolgus monkeys.

A total of 56 monkeys were randomized prior to challenge for each sex by weight, *Klebsiella* status, and anti-PA IgG ELISA status into three groups of size 16 (Group 1, 2 and 3) and one groups of size eight (Group 4). Animals in each group were then randomized to four challenge days (challenge day A, B, C and D) with a challenge order each day.

Comment: Randomization should be conducted just prior to treatment initiation. The study performed randomization prior to spore challenge instead, which led to possible inclusion of events (death) occurring post challenge but prior to treatment initiation.

This review will focus on the comparison of group 1 to group 2 to assess the added benefit of ETI-204 when given with the Cipro.

Animals randomized were aerosol exposed to 200 x LD_{50} of *B. anthracis* spores on Day 0 (challenge day) at the (b) (4) Monkeys that were alive at 24 ±12 hours after obtaining the first

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positive PA-ECL result received treatment. Animals randomized to group 1 and 2 received a dose of Cipro (10 mg/kg; oral gavage) at 24 ±12 hours after obtaining the first positive PA-ECL and three subsequent Cipro doses (10 mg/kg) by oral gavage 24 ± 3 hours following the previous dose. Animals randomized to group 1 also received antibody (8 mg/kg; IV bolus) at 24 ±12 hours after obtaining the first positive PA-ECL. Animals were monitored until Day 28 post challenge and then euthanized.

The primary efficacy endpoint of this study was 28-day survival, defined as the proportion of animals that survive to Day 28 post challenge. The primary efficacy analysis was the comparison of survival rates between the Cipro (10 mg/kg) group and the combination group using one sided Fisher's exact test at an alpha level of 0.05. The analysis was performed in animals who survived to receive at least one treatment dose. Animals randomized and challenged, and treated animals with bacteremia PTT were used for sensitivity analyses.

Comments: This review compared the survival rates in animals treated in the ETI-204 & Cipro group to that in the Cipro group at the same dose using a one-sided 0.025 level Fisher's exact test, to evaluate the added benefit of ETI-204 when administered in combination with Cipro.

As an exploratory efficacy analysis, the log-rank test was used to compare survival time from initiation of spore challenge between Cipro only group and the ETI-204 plus Cipro group. The analysis was performed in treated animals and in all challenged and randomized animals. In addition, survival time from treatment initiation was compared between the combination group and the Cipro group using the same method (Section 3.2.2.3.2.1).

Analogous to Study NIAID 1056, the applicant proposed sample size of 16 animals in each group based on the assumptions that the probability of survival was 87% and 40% in the ETI-204 & Cipro group and Cipro group, respectively, using one-sided Fisher's exact test with a 0.05 alpha level.

Comment: If one-sided Fisher's test was used for sample size calculation, an alpha level of 0.025 should be used instead which would have resulted in a power of 70% with 16 animals per group under the same assumptions on survival rates.

3.2.2.2 Animal Disposition, Demographic and Baseline Characteristics

Table 10 provides a summary of animal disposition in Study NIAID 2469. Among the 32 monkeys that were randomized to the ETI-204 & Cipro and Cipro 10 mg/kg treatment groups (groups 1 and 2) and challenged with *B. anthracis* spores, 27 (84%) monkeys survived to 24±12 hours after the first positive PA-ECL result post exposure. There were 13 and 14 monkeys in Cipro 10 mg/kg group and ETI-204 & Cipro group, respectively, that received randomized treatment.

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Table 10 Study 2469: Animal disposition before treatment initiation

	ETI-204& Cipro	Cipro (10 mg/kg)	Total
Animals challenged	16	16	32
Animals who died before treatment	2	3	5/32 (16%)
Animals who survived to be treated	14	13	27/32 (84%)
Analysis population			
Animals challenged and randomized	16	16	32
PA-ECL at or before treatment initiation	14	13	27
PA-ELISA at or before treatment initiation	14	11*	25
Bacteremic at or before treatment initiation	14	13	27
Animals survived to treatment	14	13	27

*Two animals in Cipro (10 mg/kg) reported missing value for PA-ELISA. A12240 had no value due to either no sample or an insufficient volume of the sample was available for initial analysis, A10768's titration curve was not within specifications- the test sample was not parallel to the reference standard or departed from monotonicity.

As shown in Table 11, animals treated in the Cipro group and the ETI-204 & Cipro combination group were comparable in sex, age and weight at baseline; and *Klebsiella* and anti-PA IgG status pre-exposure. For the two treatment groups compared, 11% (3/27) of animals received treatment were positive for *Klebsiella* prior to shipment and 22% (6/27) of those had positive anti-PA IgG pre-screen results prior to randomization. No significant differences were observed between the 2 treatment groups for any of the demographic or baseline characteristics. The results were consistent in animals treated and animals challenged and randomized.

Comment: Sensitivity analyses in survival rates were conducted for the following populations 1) animals treated excluding Klebsiella positive animals, 2) animals treated excluding pre-exposure anti-PA IgG ELISA positive animals, 3) animals treated excluding those were positive for Klebsiella or anti-PA IgG ELISA.

Table 11 Study 2469: Demographics and baseline characteristics

	ETI-204&Cipro n = 14	Cipro (10 mg/kg) n = 13	P-value*
Sex [n (%)]			
Male	6 (48.1)	7 (53.8)	
Female	8 (57.1)	6 (46.2)	0.71
Weight (kg) pre-exposure			
Mean ± SD	3.6±0.8	3.8±0.8	
Median	3.4	3.5	
(Min, Max)	(2.7, 5.4)	(2.8, 5.7)	0.68
Age (month)			
Mean ± SD	47.1±2.7	49.1±4.6	
Median	46.5	49.0	
(Min, Max)	(43, 51)	(45, 63)	0.19
***Klebsiella* pre-exposure [n (%)]**			
Negative	12 (85.7)	12 (92.3)	
Positive	2 (14.3)	1 (7.7)	1.00
Anti-PA IgG pre-exposure [n (%)]			
Negative	10 (71.4)	11 (84.6)	
Positive	4 (28.6)	2 (15.4)	0.65

* P-value was based on Fisher's exact test for categorical data and 1-way ANOVA for continuous data

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Animals treated in the Cipro group received marginally more challenge dose (LD_{50}) ≥ 200 than those in ETI-204 & Cipro group (11/13 versus 6/14; Fisher's exact test p-value = 0.05) (Table 12). The rates of higher LD_{50} in animals challenged and randomized were comparable in the Cipro group (75%, 12/16) and the ETI-204 and Cipro group (43.8%, 7/16) with p-value of 0.12. There is not much difference in LD_{50} across challenge days for animals randomized in either group or for animals that died before randomization (results not shown).

PA-ELISA and quantitative bacteremia levels were similar for animals treated in the two treatment groups. The quantitative bacteremia for animal A11973 is greater than the ULOD (24000 ng/mL), replaced with 24000 ng/mL for the statistical analysis.

Table 12 Study 2469: Extent of Anthrax exposure, toxemia and bacteremia pre-treatment

	ETI-204 & Cipro n = 14	**Cipro (10 mg/kg) n = 13**	**P-value***
Challenge dose (×10^7 cfu)			
Mean ± SD	1.4±0.4	1.4±0.2	
Median	1.4	1.3	
(Min, Max)	(1, 2.3)	(1.1, 1.7)	0.81
Challenge dose (LD_{50}) [n(%)]			
< 200	8 (57.1)	2 (15.4)	
200 or higher	6 (42.9)	11 (84.6)	0.05
LD_{50} by Challenge Day (N) Mean ± SD			
Day A	(3) 216±28	(4) 233±27	0.51
Day B	(4) 203±66	(4) 215±23	0.26
Day C	(4) 200±16	(2) 205±34	0.16
Day D	(3) 206±49	(3) 247±12	0.58
Bacteremia PTT (× 10^4 cfu/mL)			
Geometric Mean	0.8	8.8	
95% CI	(0.1, 5.7)	(1.0, 77.4)	0.15
PA-ELISA PTT (ng/ml)		n = 11	
Geometric Mean	350.7	393.6	
95% CI	(200.8, 612.4)	(122.2, 1268.3)	0.13

* P-value was based on Fisher's exact test for categorical data and 1-way ANOVA for continuous data. Comparison for LD50 by challenge day used two-sided t-test.

During the course of this study, all monkeys treated became toxemic (detectable PA-ECL or PA-ELISA) before treatment initiation. Table 13 shows that the average time to toxemia is, 1) for PA-ECL, 36.9 hours in the Cipro group and 34.7 hours in the ETI-204 & Cipro combination group; 2) for PA-ELISA, 35.6 hours in the Cipro group and 32.2 hours in the ETI-204 & Cipro combination group. Note the time to first positive PA-ECL coincides with time to trigger to treatment. All monkeys treated became bacteremic PTT, with the mean time to bacteremia being 36 hours in the Cipro group and 31.5 hours for the combination group. Note animal A11047 in Cipro (10 mg/kg) group was included as bacteremia in the analysis although it had only one

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detectable quantitative bacteremia PTT but positive PA levels over time. There was no difference in the time to treatment initiation between the 2 treatment groups based on log-rank test. The average time to treatment initiation was 53.4 hours and 50.4 hours in the Cipro group and the combination group, respectively.

Table 13 Study 2469: Time to toxemia, bacteremia and treatment initiation in hours

	ETI-204 & Cipro n = 14	Cipro (10 mg/kg) n = 13	P-value
Time to first positive PA-ECL			
Mean ± SD	34.7±4	36.9±7.6	
Median	35	35.1	
(Min, Max)	(24.5, 42.7)	(24.1, 54.4)	0.28
Time to first positive PA-ELISA			
Mean ± SD	32.2±4.7	35.6±9.2	
Median	30.5	34.9	
(Min, Max)	(24.5, 42.7)	(23.6, 60.6)	0.21
Time to bacteremia			
Mean ± SD	31.5±5.1	36±12.4	
Median	30.5	34.6	
(Min, Max)	(23.7, 38.2)	(25.1, 73)	0.13
Time to treatment initiation			
Mean ± SD	50.4±6.7	53.4±10.4	
Median	49.1	49	
(Min, Max)	(46, 73.1)	(45.2, 73)	0.96

Figure 4 demonstrates changes in PA-ELISA over time in animals who received the ETI-204 and Cipro combination therapy and the Cipro monotherapy. Animals died at a relatively high level of quantitative PA and for those who survived, the PA decreased to undetectable by Day 28. One monkey, A11436, that survived in the Cipro alone group had relatively low PA during this study and was negative on *Klebsiella* and Anti-PA IgG prior to challenge. However the patterns mentioned above was not as clear as those observed in Study 1056 and the cause remains unclear.

Of note, the reported quantitative bacteremia was measured PTT and 24 hours for animals treated and at 48 hours post challenge for controls. There were no more than two bacteremia values per group at 24 hours post challenge, therefore bacteremia over time was not plotted.

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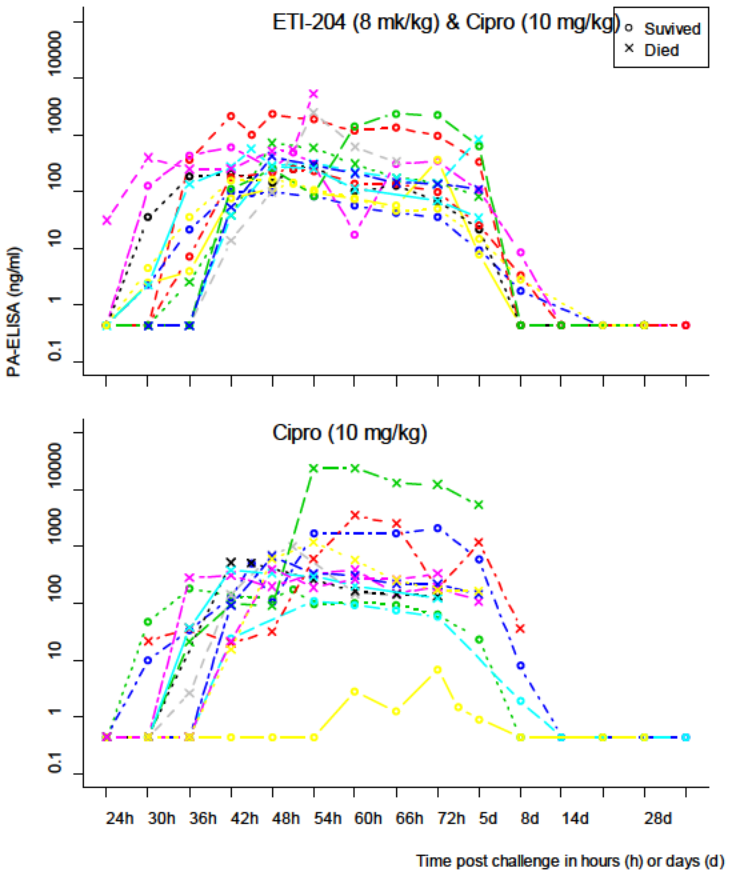

Figure 4 Study 2469: PA-ELISA over time for animals treated

3.2.2.3 Results and Conclusions

Overall, Study NIAID 2469 followed the study protocol and indicated that ETI-204 had some added benefit in survival rates when administered in combination with Cipro (57.1%, 8/14) over Cipro alone therapy (30.8%, 4/13) on the treatment of anthrax in cynomolgus monkeys (p-value = 0.16). This section provides detailed results from primary and exploratory efficacy analyses.

3.2.2.3.1 Primary Efficacy Analysis

Table 14 presents the primary efficacy results in Study NIAID 2469. Fifteen out of the 27 animals that received randomized treatment died, 9 in the Cipro group and 6 in the combination group. The survival rate in the ETI 204 & Cipro combination group (57.1%) was numerically higher than the Cipro group (30.8%), with a difference of 26% (95% CI: -14%, 60%), p-value of 0.16 from a one-sided Fisher's exact test. Following spore challenge, all animals became positive for bacteremia or toxemia PTT so the analysis for bacteremic or toxemic animals is the same as that for the animals treated.

Various sensitivity analyses were performed and the results showed consistent survival benefits of ETI-204 & Cipro therapy over Cipro treatment alone. The sensitivity analyses were conducted in five populations: all animals challenged and randomized, treated animals only, treated animals without positive Anti-PA IgG prior to spore challenge, treated animals negative on *Klebsiella* status, and treated animals that were negative on positive Anti-PA IgG and *Klebsiella* prior to spore challenge (Table 14). For treated animals with negative *Klebsiella* and anti-PA ELISA results prior to challenge, ETI-204 & Cipro group had similar survival rates (33.3%) compared to Cipro group (30%) with survival rates difference (3%, 95%CI: -40%, 46%). In addition, the combination groups still had numerically higher survival rates than the Cipro only groups in treated animals that were positive for Anti-PA IgG or *Klebsiella* before challenge.

Table 14 Study 2469: Survival rates at Day 28 post exposure

Animals	ETI-204 & Cipro	Cipro	Difference (ETI-204 & Cipro – Cipro) 95% CI**	P-value*
Challenged and randomized	8/16 (50%)	4/16 (25%)	0.25 (-0.10, 0.56)	0.14
Treated	8/14 (57.1%)	4/13 (30.8%)	0.26 (-0.14, 0.60)	0.16
Treated with PA negative pre-exposure	4/10 (40%)	3/11 (27.3%)	0.13 (-0.30, 0.54)	0.65
Treated with negative *Klebsiella* pre-challenge	6/12 (50%)	4/12 (33.3%)	0.17 (-0.27, 0.56)	0.34
Treated with PA neg & *Klebsiella neg* pre-exposure	3/9 (33.3%)	3/10 (30.0%)	0.03 (-0.40, 0.46)	0.63

*P-value based on a 1-sided Fisher's exact test compared to 0.025.
**Difference in % survivors with 95% exact confidence interval (CI).

Comment: The 26% difference in survival rates between the 2 treatment groups did not reach statistical significance (p = 0.16). However, this study was not adequately powered to detect a difference of 26%. It is difficult to adequately power added-benefit trials. Eighty percent power to detect a difference seen in the current study would require 65 animals per group to be treated (130 animals). With 84.4% of the animals estimated to be alive by treatment initiation, the total sample size of spore-challenged animals would need to be approximately 155. A trial of this size would not be ethical or feasible. An earlier study, NIAID 1056, had a similar study design to the current study. An exploratory meta-analysis of these two studies was conducted to investigate the added benefit of ETI-204 in combination with Cipro (< HED) in monkeys (Section 5.2.1).

3.2.2.3.2 Exploratory Efficacy Analysis

3.2.2.3.2.1 Survival Time from Spore Challenge to Treatment Initiation

As an exploratory efficacy analysis, time from the initiation of spore challenge to death was compared between the ETI-204 & Cipro combination group and the Cipro group. For animals that died on study, most deaths occurred within 6 days of spore challenge. The 6 deaths in the ETI-204 & Cipro group happened between 2.5 days and 5.2 days following spore challenge while the 9 deaths in the Cipro group happened between 3.1 days and 5.8 days following spore challenge. The average time from spore challenge to death is 4.1 days in the combination group

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and 4.0 days in the Cipro group. No animals died beyond 2 days post the last dose of Cipro. According to the log-rank test, the probability of survival was numerically greater in the ETI-204 & Cipro combination group than in the Cipro group (Figure 5, P-value = 0.13). For the sake of completeness, Figure 6 illustrated survival times from challenge in all animals challenged and randomized by group.

Likewise, the survival time from the treatment initiation was compared between the combination group and the Cipro group. The probability of survival was numerically greater in the ETI-204 & Cipro group than in the Cipro group (P-value = 0.42). Both analyses were performed in the animals survived to treatment and similar results were obtained in animals challenged and randomized.

There was a difference in p-values of 0.13 to 0.42 when using time to death post challenge or time to death post treatment, respectively. This difference seems to be due to the fact that the mean time to treatment was slightly longer in the Cipro alone group, 53.4 hours, than 50.4 hours in the combination group. Therefore the survival analysis is more appropriate to use time from treatment initiation to death than time from exposure to death to evaluate treatment effect in treated animals given that they had to be alive to start treatment.

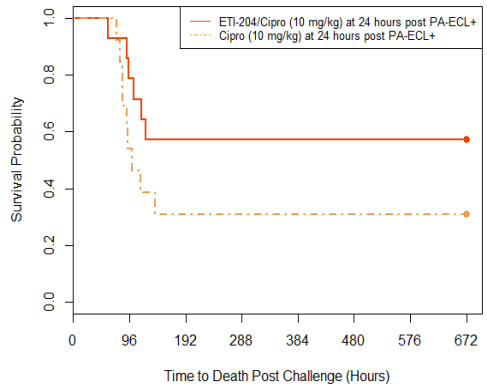

Figure 5 Study 2469: Survival time for animals treated with ciprofloxacin

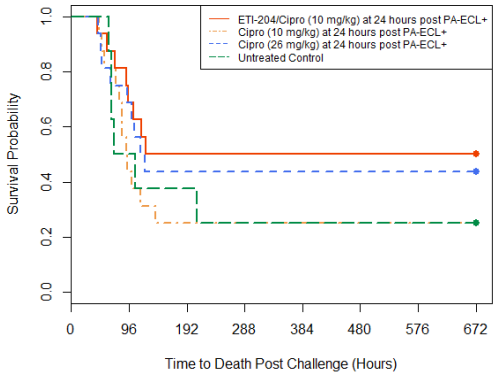

Figure 6 Study 2469: Survival time for animals randomized and challenged

Reference ID: 3857343

3.2.2.3.2.2 Analyses by Spore Challenge, PA-ELISA and Bacteremia

The reviewer conducted two-sided Spearmen's rank correlation test to examine the association between the exposure (challenge dose), severity of anthrax infection (PA-ELISA and quantitative bacteremia) and time to death. As PA-ELISA PTT or quantitative bacteremia increases, time to death decreases ($r_s = -0.50$, p-value = 0.01; $r_s = -0.74$, p-value < 0.001, respectively). Quantitative bacteremia PTT was significantly associated with PA-ELISA PTT ($r_s = 0.71$, p-value < 0.001). The challenge dose (LD_{50}) was not significantly associated with PA-ELISA (Spearmen's correlation coefficient, $r_s = -0.23$), bacteremia ($r_s = -0.09$) and time to death ($r_s = 0.16$) with p-value > 0.05.

Table 15 summarized survival rates in Cipro and ETI-204 & Cipro groups by exposure, PA-ELISA and bacteremia. Challenge dose, PA-ELISA and quantitative bacteremia were categorized based on targeted challenge dose 200 x LD_{50} and median (PA-ELISA and quantitative bacteremia). Lower PA-ELISA level was associated with significantly greater survival rates in Cipro group (75%, 3/4) and all treated animals (64%, 9/13) (Fisher's exact test p-value = 0.02). Lower quantitative bacteremia level was associated with significantly greater survival rates in ETI-204 & Cipro group (89%, 8/9) and all treated animals (83%, 10/13) (Fisher's exact test p-value < 0.01). The association between PA-ELISA or quantitative bacteremia and survival rate could be driven by the animals with lower levels of PA and bacteremia. Note the regression analyses was not conducted in this review because the sample size for animals treated would not power further regression analyses to quantify the relationship between PA or bacteremia and the survival outcome.

Table 15 Study 2469: Survival rates by challenge dose, PA-ELISA and quantitative bacteremia

	ETI-204 & Cipro (# survived/n) n = 14	**Cipro (# survived/n) n = 13**	**Total (# survived/n) n = 27**
Challenge dose (LD_{50})			
< 200	4/8 (50%)	0/2 (0%)	4/10 (60%)
200 or higher	4/6 (67%)	4/11 (36%)	8/17 (67%)
PA-ELISA		n = 11	
< 271	6/9 (67%)	3/4 (75%)*	9/13 (64%)*
271 or above	2/5 (40%)	0/7 (0%)	2/12 (36%)
Quantitative bacteremia			
< 15300	8/9 (89%)**	2/4 (50%)	10/13 (83%)***
15300 or higher	0/5 (0%)	2/9 (22%)	2/14 (17%)

Two-sided Fisher's exact test *p-value = 0.02, **p-value = 0.003, ***p-value = 0.004.

Reference ID: 3857343

3.2.3 Study AR007

Study AR007: Test of ETI-204 in Rabbit Spore Challenge Model Post-Exposure with/without Levofloxacin

3.2.3.1 Study Design

Study AR007 was a post-exposure prophylaxis (PEP) study with treatment initiation after exposure to *B. anthracis* spores, but before the development of clinical signs and/or symptoms. The study was a parallel-group, open-label, randomized, controlled study in healthy male and female New Zealand White (NZW) rabbits to evaluate the added benefit of combination therapy of ETI-204 (IM or IV) and levofloxacin (Levo) compared with the Levo alone group. Table 16 provides details of the study design. The proposed primary objective was to assess the efficacy in survival rate of ETI-204 (10 mg, about 4 mg/kg, IV) in combination with a humanized dose of Levo (50 mg/kg, HED) compared to Levo at the same dose, when administered at a fixed time 9 hours (±3 hours) after inhalational exposure to *B. anthracis* in NZW rabbits.

The secondary objective was to demonstrate that intramuscular (IM) ETI-204 (20 mg, about 8 mg/kg) and Levo increased survival rate compared to Levo alone at HED in the same setup as the primary objective. Of note, this review also reported the analyses for the secondary objective because this was the only study including combination groups for ETI-204 IM.

Table 16 Study AR007: Study design

Group	Treatment	ETI-204 Dose (mg/kg)	Levo Dose (mg/kg)	Treatment Initiation Post Exposure	Therapy Duration	No. of Animals
1	Control (PBS*)	N/A**	N/A	9±3 hours	Single dose (IV)	9
2	Levo	N/A	50	9±3 hours	5 days (oral)	12
3	ETI-204 IV	4 (10 mg)	N/A	9±3 hours	Single dose (IV)	9
4	ETI-204 IV + Levo	4 (10 mg)	50	9±3 hours	Single dose (ETI-204; IV) + 5 days (Levo; oral)	9
5	ETI-204 IM	8 (20 mg)	N/A	9±3 hours	(IM)	9
6	ETI-204 IM + Levo	8 (20 mg)	50	9±3 hours	dose (ETI-204; IM) + 5 days (Levo; oral)	9

* Placebo used phosphate buffered saline (PBS) IV
** N/A – Not applicable

A total of 57 rabbits were randomized pre-challenge by sex and weight into five groups of size nine (group 1, 3, 4, 5 and 6) and one groups of size 12 (group 2) with each group consisting of 50% male and 50% female animals. Equal number of animals from each group was challenged each day of three challenge days (challenge day A, B and C) at a randomized challenge order.

Reference ID: 3857343

This review will focus on the comparison of group 4 to group 2 and group 6 to group 2 to assess the added benefit of ETI-204 when given with Levo. For discussion of the results of ETI-204 monotherapy compared to untreated controls see statistical review by Xianbin Li.

Animals randomized were aerosol exposed to 200 x LD_{50} of *B. anthracis* spores on Day 0 (challenge day) at the [redacted] (b) (4) ([redacted] (b) (4) Rabbits in the Levo & placebo (IV) group received a dose of phosphate buffered saline (PBS) IV and a dose of Levo (50 mg/kg; oral gavage) at 9 hours (±3 hours) post anthrax exposure and four subsequent oral Levo doses (50 mg/kg) 24 hours (±3 hours) following the previous dose. Animals in the ETI-204 (IV) & Levo combination group received 10 mg antibody (about 4 mg/kg; IV bolus) and a dose of Levo (50 mg/kg; oral) at 9 hours (±3 hours) post anthrax exposure and four subsequent oral Levo doses (50 mg/kg) 24 hours (±3 hours) following the previous dose. Animals in the ETI-204 (IM) & Levo combination group received 20 mg antibody (about 8 mg/kg; IV) and a dose of Levo (50 mg/kg; oral) at 9 hours (±3 hours) post anthrax exposure and four subsequent oral Levo doses (50 mg/kg) 24 hours (±3 hours) following the previous dose. Animals were monitored until Day 30 post challenge and then euthanized.

The primary efficacy endpoint of this study was 30-day survival, defined as the proportion of animals that survive to Day 30 post spore challenge. The primary efficacy analysis was the comparison of survival rates in each antibody group versus the control, or each antibody group versus the Levo group using one sided Fisher's exact test at an alpha level of 0.05 without adjustment for multiple comparisons. The analysis was performed in all animals randomized and challenged, which were the same as animals treated because none of the rabbits died before the treatment initiation.

As a secondary efficacy analysis, the log-rank test was used to compare survival time from initiation of spore challenge between the Levo group and the ETI-204 (IV) plus Levo group or the ETI-204 (IM) & Levo group. The analysis was performed in all animals randomized and treated. In addition, survival time from treatment initiation was compared between the combination group and the Levo group using the same method (Section 3.2.3.3.1).

Comments: For the added benefit objective, a one-sided 0.025 level Fisher's exact test should be used instead of a one-sided alpha level of 0.05. This review compares the survival proportion of animals in each ETI-204 & Levo group to that in the Levo group using a one-sided 0.025 level Fisher's exact test. There is no comment on the power analysis because the sample size calculation was not based on the comparison between the combination groups and the Levo group. We did not adjust for two comparisons because the comparison of the IV formulation was considered primary and comparison of the IM was secondary.

3.2.3.2 Animal Disposition, Demographic and Baseline Characteristics

Under this current PEP study design, all animals challenged and randomized survived to receive treatment as planned. As shown in Table 17, animals treated in the Levo group and the ETI-204 (IV) & Levo group were comparable in sex, weight at baseline, exposure at challenge and time

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from spore challenge to treatment initiation. All animals were aged 4 months. Note there was no data on anti-PA IgG ELISA prior to challenge. No significant differences (p-value > 0.05) were observed between the 2 treatment groups for any of the demographic or baseline characteristics based on Fisher's exact test for categorical data, log-rank test for time to treatment and t-test for continuous data where normality assumption held. Similar results were identified for the comparison between the Levo group and ETI-204 (IM) & Levo group in baseline characteristics and time to treatment. Of note, the mean challenge dose was higher in this study, 270 LD_{50}, than the rest of the study reviewed with 200 LD_{50}.

Table 17 Study AR007: Demographics and baseline characteristics

	ETI-204 (IV) & Levo n = 9	**ETI-204 (IM) & Levo n = 9**	**Levo n = 12**
Sex [n(%)]			
male	4 (44.4)	5 (55.6)	6 (50.0)
female	5 (55.6)	4 (44.4)	6 (50.0)
Weight (kg)			
Mean ± SD	2.5±0.2	2.5±0.1	2.5±0.1
Median	2.6	2.4	2.5
(Min, Max)	(2.2, 2.7)	(2.3, 2.7)	(2.4, 2.6)
Challenge dose ($\times 10^7$ cfu)			
Mean ± SD	2.8±0.4	2.7±0.4	3.1±0.6
Median	2.8	2.8	3.0
(Min, Max)	(2.0, 3.4)	(2.0, 3.1)	(2.3, 4.2)
Challenge dose (LD_{50}) [n(%)]			
< 200	1 (11.1)	2 (22.2)	0 (0.0)
200 or higher	8 (88.9)	7 (77.8)	12 (100.0)
Time to treatment initiation (hour)			
Mean ± SD	8.2±0.7	9.1±1.1	8.7±1
Median	8.6	9.2	8.9
(Min, Max)	(7.2, 9.1)	(7, 10.3)	(7.3, 10.2)

There was no toxemia or quantitative bacteremia data in this study under the PEP setting with early initiation of treatment at about 9 hours post exposure. Qualitative bacteremia was conducted in serum and tissue (spleen) samples at unscheduled terminal (died prior to terminal) or terminal sacrifice in all animals (Table 18). As compared to the ETI-204 (IV) & Levo group, the Levo group had significantly higher rate of bacteremia by death (66.7% versus 11.1%, 8/12 versus 1/9; p-value = 0.02). Spleen tissue bacterial load rate was numerically higher in the Levo group (7/12) than in the ETI-204 (IV) & Levo group (1/9) with p-value of 0.07. Similar pattern of tissue and serum bacterial load were observed in the Levo group as compared to the ETI-204 (IM) & Levo group. As expected, animals died before Day 28 were positive for bacteremia and those survived to the end of this study were negative.

Reference ID: 3857343

Table 18 Study AR007: Serum and tissue culture at death/sacrifice for all animals

		ETI-204 (IV) & Levo n = 9	ETI-204 (IM) & Levo n = 9	Levo n = 12
Bacteremia [n(%)]				
	No	8 (88.9)	9 (100.0)	4 (33.3)
	Yes	1 (11.1)	0 (0.0)	8 (66.7)
Spleen [n(%)]				
	No	8 (88.9)	9 (100.0)	5 (41.7)
	Yes	1 (11.1)	0 (0.0)	7 (58.3)

3.2.3.3 Results and Conclusions

Overall, Study AR 007 followed the protocol and demonstrated that ETI-204 (IV and IM) had significant added benefit in survival when administered concomitantly with Levo over the Levo therapy on the treatment of anthrax in NZW rabbits. Of note, the survival rate in the Levo group, 33%, was low but expected in this PEP setting, where antibacterial treatment early after spore challenge has been observed to lead to persistence of spores and disease development following antibacterial cessation, when treatment is not extended well past the 5 days given in this study. CDC recommends that humans exposed to anthrax take antibacterial drugs up to 60 days to ensure protection[1]. This section provides detailed results from primary and secondary efficacy analyses.

3.2.3.3.1 Primary Efficacy Analysis

Table 19 presents the primary efficacy results in Study AR 007. Nine out of the 21 animals that received study agent died by Day 30, 1 in the ETI-204 (IV) & Levo group and 8 in the Levo group. The survival rate in the ETI 204 (IV) & Levo group (89%) was significantly higher than the Levo group (33%), with a difference of 56% (95% CI: 11%, 82%), p-value of 0.02 from one-sided Fisher's exact test. The lower boundary of 95% CI for the survival rate difference was above zero, which supported the added benefit of ETI-204 (IV) administered in combination with Levo over the Levo therapy.

Compared to the Levo group, the ETI-204 (IM) & Levo group had a significantly higher survival rate (100% versus 33.3%) with a difference of 66.7% (95% CI: 27%, 90%), p-value of 0.002 Fisher's exact test. The lower boundaries of 95% CI for the survival rate differences were above zero, which supported the added benefit of ETI-204 (IM) administered in combination with Levo over the Levo therapy.

All animals randomized and challenged survived to receive treatment so the analysis for the animals treated was the same as that for animals randomized and challenged.

[1] http://www.cdc.gov/anthrax/medical-care/prevention.html

Table 19 Study AR007: Survival rates at Day 30

Animals	Levo & Placebo (IV)	ETI-204 (IV) & Levo	ETI-204 (IM) & Levo	Difference (ETI-204 & Levo – Levo & Placebo) 95% CI**	P-value*
Challenged, randomized and treated	4/12 (33%)	8/9 (89%)		0.56 (0.11, 0.82)	0.02
	4/12 (33%)		9/9 (100%)	0.67 (0.27, 0. 90)	0.002

*P-value based on a 1-sided Fisher's exact test compared to 0.025.
**Difference in % survivors with 95% exact confidence interval (CI).

Comment: The significant 56% difference in survival rates between the combination group and levo alone treatment group supports the added benefit of ETI-204 (IV) when administered in combination with Levo. A significant difference in survival rates was also seen with the IM dosing.

3.2.3.3.2 Secondary Efficacy Analysis

A secondary efficacy analysis of survival time from spore challenge to death showed that animals receiving the ETI-204 (IV) & Levo combined therapy survived significantly longer than animals in the Levo group (Figure 7). For animals that died on study, the 8 deaths of Levo group occurred between 11.9 days and 20.9 days of spore challenge while the 1 death in the ETI-204 (IV) & Levo group happened 16.8 day following spore challenge. The mean time from challenge to death of the Levo group was 14.3 days versus 16.8 days in the ETI-204 (IV) & Levo group (p-value = 0.012). In addition, a log-rank test confirmed the significant difference in survival time for Levo versus ETI-204 (IM) & Levo (p-value = 0.003). For demonstration purpose, Figure 8 included K-M curves for all animals studied.

This review did not conduct a survival analysis using time from treatment initiation to death because all animals randomized and challenged were alive prior to treatment initiation in this PEP setting.

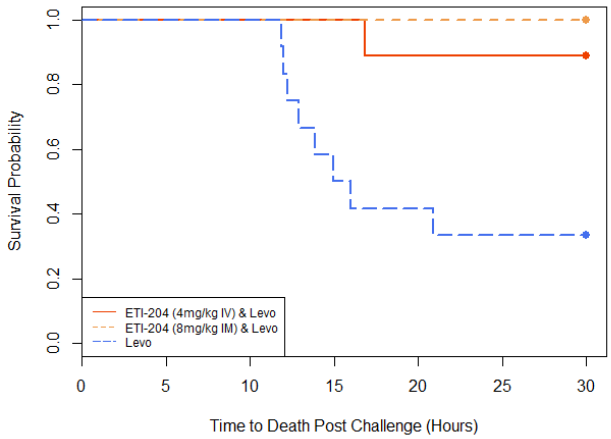

Figure 7 Study AR007: Survival time for animals treated with levofloxacin

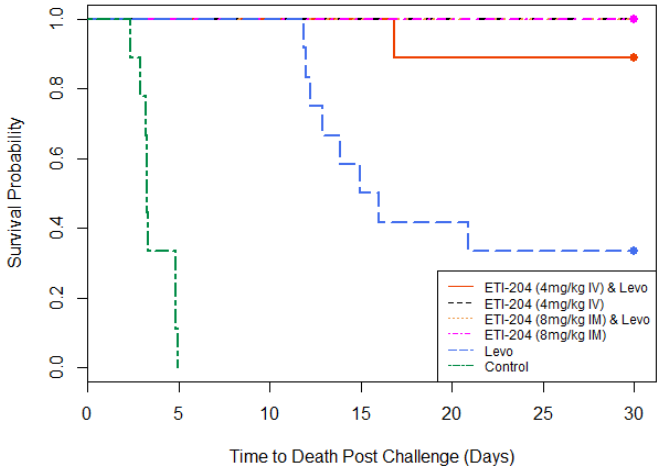

Figure 8 Study AR007: Survival time for all animals randomized and challenged

3.2.3.3.3 Exploratory Efficacy Analysis

An exploratory analysis was performed to examine the association between spore challenge dose and the time to death and the Spearman's correlation test reported a non-significant association of 0.38 with p-value 0.31. The limited sample size in study groups prevented further regression analyses.

3.2.4 Study NIAID 1030

Study NIAID 1030: 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

3.2.4.1 Study Design

Study NIAID 1030 was a parallel-group, open-label, randomized, controlled study in healthy male and female NZW rabbits to evaluate the added benefit of combination therapy of ETI-204 and levofloxacin (Levo) compared with Levo alone. Table 20 provides details of the study design.

The proposed primary objectives were to 1) assess the efficacy of ETI-204 (8 mg/kg, IV) in survival rate, when administered following a significant increase in body temperature (SIBT) in NZW rabbits exposed to *B. anthracis*, 2) assess the efficacy in survival rate of ETI-204 (8 mg/kg, IV) in combination with humanized Levo (50 mg/kg) compared to Levo, when administered at a delayed time, 96±1 hours following inhalational anthrax exposure in NZW rabbits.

Reference ID: 3857343

Comment: This review will focus on the second primary objective, directly related to the evaluation of the added benefit of ETI-204 in combination therapy, with no comments on the first primary objective.

Table 20 Study 1030: Study design

Group	Treatment	ETI-204 Dose	Levo Dose	Treatment Initiation Post Exposure	Therapy Duration	No. of Animals
1	ETI-204	8 mg/kg	N/A	SIBT	Single dose (IV)	16
2	Levofloxacin	N/A	50 mg/kg	96±1 hours	3 days (oral)	16
3	ETI-204+ Levo	8 mg/kg	50 mg/kg	96±1 hours	Single dose (ETI-204; IV) + 3 days (Levo; oral)	16
4	Control	N/A	N/A	N/A	N/A	6

N/A – Not applicable

A total of 54 rabbits were randomized pre-challenge into three groups of size 16 (Group 1, 2 and 3) and one group of size 6 (Group 4) with each group consisting of 50% male and 50% female animals. Animals in each group were then randomized to two challenge days with a randomized challenge order per day.

Comment: Randomization should be conducted just prior to treatment initiation. The study performed randomization prior to spore challenge instead, which led to possible inclusion of events (death) occurring post challenge but prior to treatment initiation.

This review will focus on the comparison of group 3 to group 2 to assess the added benefit of ETI-204 when given with Levo. For discussion of the results of ETI-204 monotherapy compared to untreated controls see statistical review by Xianbin Li.

Animals randomized were aerosol exposed to 200 x LD_{50} of *B. anthracis* spores on Day 0 (challenge day) at the (b) (4) ((b) (4) Rabbits in the Levo and combination group (groups 1 and 3) that were alive at 96 hours (±1 hours) post median challenge time received treatment with oral Levo (50 mg/kg; oral gavage) with two subsequent oral Levo doses (50 mg/kg) once daily following the previous dose. Animals in the combination group also received antibody (8 mg/kg; IV bolus) once at 96 hours (±1 hours) post median challenge time. Animals were monitored until Day 28 post challenge and then euthanized.

The primary efficacy endpoint of this study was 28-day survival, defined as the proportion of animals that survive to Day 28 post spore challenge. The primary efficacy analysis was the comparison of survival rates in four study groups using one sided Fisher's exact test at an overall alpha level of 0.05. The study protocol did not specify method for multiple comparison adjustment. The study report stated the primary comparisons were adjusted with Bonferroni-Holm method, previously explained in Section 3.2.1.1. The analysis was performed in animals surviving to receive treatment and bacteremic. The study also conducted analyses in all animals randomized and challenged.

Comment: This review compared the proportion surviving in the ETI-204 & Levo group to that in the Levo group using a one-sided 0.025 level Fisher's exact test, to evaluate the added benefit of ETI-204 when administered in combination with Levo. The primary population for this review was animals surviving to receive treatment regardless of the bacteremia status. Sensitivity analyses of survival rate were performed in animals randomized and challenged and in animals treated who were bacteremic (Section 3.2.4.3.1).

As an exploratory efficacy analysis, the log-rank test was used to compare survival time from initiation of spore challenge between the Levo only group and the ETI-204 plus Levo group. The analysis was performed in treated animals with bacteremia. In this review, survival time from treatment initiation was compared between the combination group and the Levo group using the same method (Section 3.2.4.3.2.1).

3.2.4.2 Animal Disposition, Demographic and Baseline Characteristics

Table 21 provides a summary of animal disposition in Study NIAID 1030. Among the 32 rabbits that were randomized to the combination or Levo alone groups and challenged with *B. anthracis* spores, 9 (28%) rabbits survived to 96±1 hours post exposure. There were 5 rabbits each in the Levo group and 4 rabbits in the ETI & Levo group that received treatment planned. Among animals treated, two rabbits did not become bacteremic prior to treatment (PTT), rabbit L23016 in the Levo group and rabbit L23040 in ETI-204 & Levo group.

Table 21 Study 1030: Animal disposition before treatment initiation

	Levo	**ETI-204 & Levo**	**Total**
Animals challenged	16	16	32
Animals who died before treatment	11	12	23/32 (72%)
Animals who survived to be treated	5	4	9/32 (28%)
Analysis population			
Animals randomized and challenged	16	16	32
PA-ECL at or prior to treatment (PTT)	5	4	9
PA-ELISA at or PTT	5	4	9
Bacteremic at or PTT	4	3	7
Animals treated	5	4	9
Animals treated and bacteremic PTT	4	3	7

As shown in Table 22, animals treated in the Levo group and the ETI-204 & the Levo group were comparable in sex, weight at baseline, exposure at challenge, bacteremia status and PA-ELISA before treatment. All animals were aged 4 months pre-challenge. None of the animals were positive for anti-PA IgG ELISA prior to challenge. The animals in the ETI-204 & Levo group received marginally higher dose of exposure than those in the Levo alone group (P-value = 0.05). No significant differences were observed between the 2 treatment groups for the rest of the demographic or baseline characteristics. Of note, quantitative bacteremia was not measured in this study. The results were consistent in animals treated, animals treated and bacteremic and animals challenged and randomized (results not shown).

Reference ID: 3857343

Table 22 Study 1030: Demographics and baseline characteristics

	Levo n = 5	ETI-204 & Levo n = 4	P-value*
Sex [n(%)]			
Male	2 (40)	1 (25)	
Female	3 (60)	3 (75)	1.00
Weight (kg)			
Mean ± SD	2.6±0.1	2.5±0.1	
Median	2.6	2.6	
(Min, Max)	(2.5, 2.7)	(2.2, 2.7)	0.20
Challenge dose ($\times 10^7$ cfu)			
Mean ± SD	2±0.4	1.5±0.2	
Median	1.9	1.5	
(Min, Max)	(1.5, 2.5)	(1.3, 1.7)	0.05
Challenge dose (LD_{50}) [n(%)]			
< 200	3 (60)	4 (100)	
200 or higher	2 (40)	0 (0)	1.00
PA-ELISA PTT (ng/ml)			
Geometric mean	108.8	62.1	
95% CI	(30.2, 392.1)	(4.6, 835.6)	0.14
Bacteremia PTT			
Positive	4 (80)	3 (75)	
Negative	1 (20)	1 (25)	1.00

* P-value was based on Fisher's exact test for categorical data and t-test for continuous data. Challenge dose and PA-ELISA were log transformed with base 10.

During the course of this study, all rabbits treated became toxemic (detectable PA-ECL) before treatment initiation. Table 23 shows that the average time to toxemia is 44.4 hours in the Levo group and 55 hours in the ETI-204 & Levo combination group. The mean time to first detectable PA-ELISA was 95.9 hours in Levo group or in the combination group. All rabbits treated became bacteremic PTT, with the mean time to bacteremia being 53.8 hours in the Levo group and 72.9 in the combination group. There was no difference in the time to treatment initiation between the 2 treatment groups based on log-rank test. The average time to treatment initiation was 95 hours and 94.9 hours in the Levo group and the combination group, respectively.

The time between challenge and first positive PA-ECL, PA-ELISA and bacteremia, and time to treatment initiation was summarized in Table 23. There were no differences in these times between Levo and ETI-204 & Levo groups (p-value > 0.05).

Reference ID: 3857343

Table 23 Study 1030: Time to toxemia, bacteremia and treatment initiation in hours

	Levo n = 5	ETI-204 & Levo n = 4	P-value
Time to first positive PA-ECL			
Mean ± SD	44.4±10.9	55±10.1	
Median	48.6	50.5	
(Min, Max)	(25.1, 50.8)	(49, 70.1)	0.13
Time to first positive PA-ELISA		n = 3	
Mean ± SD	95.9±1.3	95.9±2	
Median	95.5	96.3	
(Min, Max)	(94.2, 97.7)	(93, 97.8)	0.35
Time to bacteremia	n = 4	n = 3	
Mean ± SD	53.8±22.9	72.9±24.5	
Median	59	72.1	
(Min, Max)	(24.1, 73.2)	(48.8, 97.8)	0.32
Time to treatment initiation			
Mean ± SD	95±1.4	94.9±2	
Median	94.6	95.3	
(Min, Max)	(93.2, 96.7)	(92, 96.8)	0.63

*P-value was based on log-rank test.

Figure 9 shows the PA-ELISA changes in animals who received ETI-204 and Levo combination treatment or Levo monotherapy from 24 hours post exposure to Day 28 of the study. The levels of PA-ELISA peaked between 4-7 days post challenge with treatment initiated around 96 hours. For animals survived, the PA level reduced to undetectable by Day 28. Two out of the three animals died at peaks of PA-ELISA and one died after the PA peak at an undetectable level.

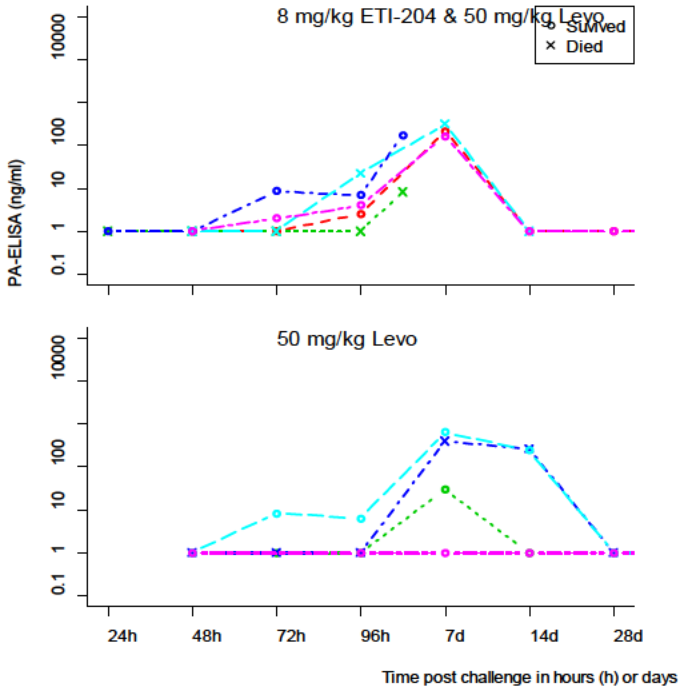

Figure 9 Study 1030: PA-ELISA over time for animals treated with levofloxacin

Reference ID: 3857343

3.2.4.3 Results and Conclusions

Study NIAID 1030 followed the protocol and showed that ETI-204 provided a numerical added benefit in survival rates when administered in combination with Levo (100%, 4/4) over Levo alone therapy (40%, 2/5) for the treatment of anthrax in NZW rabbits. This section provides detailed results from primary and exploratory efficacy analyses.

3.2.4.3.1 Primary Efficacy Analysis

Table 5 presents the primary efficacy results in Study NIAID 1030. Three out of the 9 animals that received study agent died, 3 in the Levo group and none in the combination group. The survival rate in the ETI 204 & Levo combination group (100%) was numerically higher than the Levo group (40%), with a difference of 60% (95% CI: -9%, 95%), p-value of 0.17 from one-sided Fisher's exact test.

Various sensitivity analyses were performed and the results showed consistent survival benefits of ETI-204 & Levo therapy over Levo treatment alone. The sensitivity analyses were conducted in three populations: all animals challenged and treated, treated animals only, treated animals with positive bacterial load (Table 24). For treated animals with bacteremia, the ETI-204 & Levo group had numerically greater survival rates (100%) than the Levo group (50%) with survival rates difference of 50% (95%CI: -30%, 93%; p-value = 0.43).

Table 24 Study 1030: Survival rates at Day 28

Animals	Levo	ETI-204 & Levo	Difference (ETI-204 & Levo – Levo) 95% CI**	P-value*
Randomized and challenged	2/16 (12.5%)	4/16 (25%)	0.13 (-0.17, 0.41)	0.65
Treated	2/5 (40%)	4/4 (100%)	0.60 (-0.09, 0.95)	0.17
Treated with bacteremia	2/4 (50%)	3/3 (100%)	0.50 (-0.30, 0.93)	0.43

*P-value based on a 1-sided Fisher's exact test compared to 0.025.
**Difference in % survivors with 95% exact confidence interval (CI).

Comment: The 60% difference in survival rates between the 2 treatment groups did not reach statistical significance (p = 0.17). This study had an excess pre-treatment mortality rate making the resulting sample size too small to be able to detect a difference if one were there. A trial with 80% power to detect a statistically significant result with the 60% difference seen in the current study would require 10 animals per group to be treated (20 animals). With 28% (9/32) of the animals estimated to be alive by treatment initiation, the total sample size of spore-challenged animals would be at least 72. A trial of this size is likely not ethical or feasible.

3.2.4.3.2 Exploratory Efficacy Analysis

3.2.4.3.2.1 Survival Time from Spore Challenge or Treatment Initiation

Reference ID: 3857343

As an exploratory efficacy analysis, time from the initiation of spore challenge to death was compared between the ETI-204 & Levo combination group and the Levo group. For animals that died on study, most deaths occurred within 11 days of spore challenge. The 3 deaths in the Levo group occurred between 5.9 days and 10.8 days following spore challenge. The average time from spore challenge to death is 7.6 days in the Levo group. According to the log-rank test, the probability of survival was numerically greater in the ETI-204 & Levo combination group than in the Levo group (Figure 10, P-value = 0.08). For the sake of completeness, Figure 11 illustrated survival times from challenge in all animals challenged and randomized by group.

Likewise, the survival time from the treatment initiation was compared between the combination group and the Levo group. The probability of survival was statistically greater in the ETI-204 & Levo group than in the levofloxacin group (P-value = 0.08). Both analyses were performed in the animals survived to treatment and similar results were obtained in animals challenged and randomized, and animals treated and bacteremia.

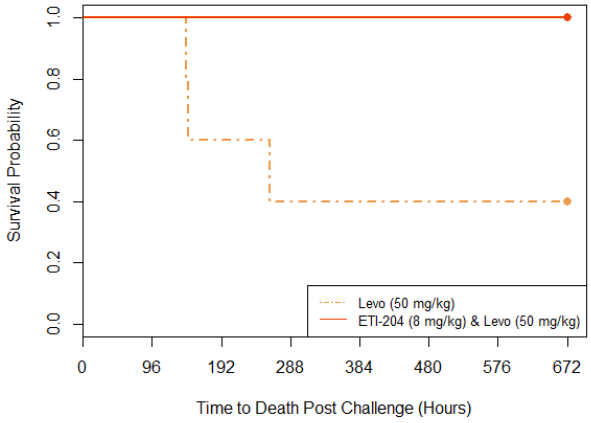

Figure 10 Study 1030: Survival time for animals treated with levofloxacin

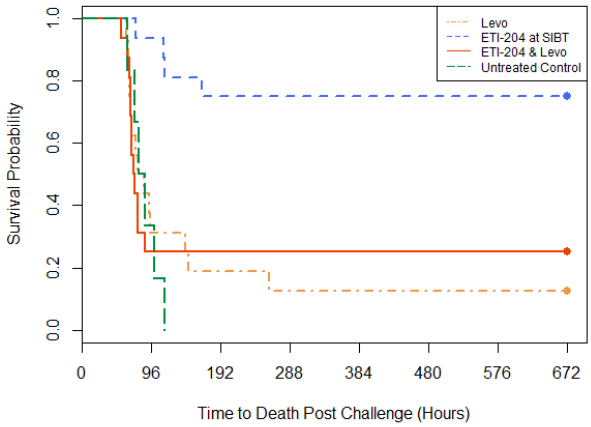

Figure 11 Study 1030: Survival time for all animals randomized and challenged

Reference ID: 3857343

3.2.4.3.2.2 Analyses by Spore Challenge, PA-ELISA and Bacteremia

Two-sided Spearmen's rank correlation test was performed to examine the association between the exposure (challenge dose), severity of anthrax infection (PA-ELISA PTT) and time to death.

As PA-ELISA PTT increases, time to death increases (r_s = 0.33, p-value = 0.43).The challenge dose (LD_{50}) was associated with PA-ELISA (Spearmen's correlation coefficient: r_s = -0.67, p-value =0.06), and time to death (r_s = -0.43, p-value=0.25). None of the associations were significant, given the small sample size.

3.2.5 Study NIAID 1045

Study NIAID 1045: 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

3.2.5.1 Study Design

Study NIAID 1045 was a parallel-group, open-label, randomized, controlled study in healthy male and female NZW rabbits to evaluate the added benefit of combination therapy of ETI-204 and levofloxacin (Levo) compared with Levo alone. Table 25 provides details of the study design.

The proposed primary objective was to assess the efficacy in survival rate of ETI-204 (8 mg/kg) in combination with Levo at a HED (50 mg/kg) compared to Levo at the same dose, when administered at a delayed time, 72±1 following inhalational exposure to *B. anthracis* in NZW rabbits.

Table 25 Study 1045: Study design

Group	Treatment	ETI-204 Dose	Levofloxacin Dose	Treatment Initiation Post Exposure*	Therapy Duration	No. of Animals
1	Levofloxacin	N/A	50 mg/kg	72±1 hours	3 days (oral)	16
2	ETI-204+ Levofloxacin	8 mg/kg	50 mg/kg	72±1 hours	Single dose (ETI-204; IV) + 3 days (Levo; oral)	16
3	ETI-204	8 mg/kg	N/A**	72±1 hours	Single dose (IV)	16
4	Control	N/A	N/A	N/A	N/A	6

* 72±1 hours post median challenge
** N/A – Not applicable

A total of 54 rabbits were randomized pre-challenge into three groups of size 16 (Group 1, 2 and 3) and one group of size 6 (Group 4) with each group consisting of 50% male and 50% female animals. Animals in each group were then randomized to two challenge days (challenge day A and B) with a randomized challenge order each day.

Reference ID: 3857343

Comment: Randomization should be conducted prior to treatment initiation. The study performed randomization prior to spore challenge instead, which led to possible inclusion of events (death) occurring post challenge but prior to treatment initiation.

This review will focus on the comparison of group 2 to group 1 to assess the added benefit of ETI-204 when given with Levo. For discussion of the results of ETI-204 monotherapy compared to untreated controls see statistical review by Xianbin Li.

Animals randomized were aerosol exposed to 200 x LD50 of *B. anthracis* spores on Day 0 (challenge day) at the (b) (4) ((b) (4) Rabbits in the combination group and the Levo group that were alive at 72 hours (±1 hour) post median challenge time received a dose of Levo (50 mg/kg; oral gavage) followed by two subsequent oral Levo doses ((50 mg/kg) once daily following the previous dose. Animals in the combination group also received antibody (8 mg/kg; IV bolus) once at 72 hours (±1 hour) post median challenge time. Animals were monitored until Day 28 post challenge and then euthanized.

The primary efficacy endpoint of this study was 28-day survival, defined as the proportion of animals that survived to Day 28 post spore challenge. The primary efficacy analysis was the comparison of survival rates in the Levo group and the ETI-204 & Levo group using a one sided Fisher's exact test at an alpha level of 0.05. The analysis was performed in animals randomized and challenged. This analysis was based on the planned treatment group rather than the actual treatment group received and rabbits that died between challenge and the first dose of study agent would be analyzed as an event (death). The protocol included a secondary analysis in only animals that survived to receive treatment (Section 3.2.5.3.1).

Comment: This review compared the proportion of animals surviving in the ETI-204 & Levo group to that in the Levo group using a one-sided 0.025 level Fisher's exact test, to evaluate the added benefit of ETI-204 when administered in combination with Levo. The primary population for this review was animals survived to receive at least one treatment.

As an exploratory efficacy analysis, the log-rank test was used to compare survival time from initiation of spore challenge between the Levo only group and the ETI-204 plus Levo group. The analysis was performed in both animals treated and animals randomized and challenged. In this review, survival time from treatment initiation was compared between the combination group and the Levo group using the same method (Section 3.2.5.3.2.1).

3.2.5.2 Animal Disposition, Demographic and Baseline Characteristics

Table 26 provides a summary of animal disposition in Study NIAID 1045. Among the 32 rabbits that were randomized to the Levo group or the ETI & Levo group and challenged with *B. anthracis* spores, 20 (63%) rabbits survived to receive treatment. There were 9 rabbits in the Levo group and 11 rabbits in the ETI & Levo group. Animals treated were positive for bacterial load prior to treatment (PTT).

Table 26 Study 1045: Animal disposition before treatment initiation

	Levo	ETI-204 & Levo	Total
Animals challenged	16	16	32
Animals who died before treatment	7	5	12/32 (38%)
Animals who survived to be treated	9	11	20/32 (63%)
Analysis populations			
Randomized and challenged	16	16	32
PA-ECL at or PTT	9	11	20
PA-ELISA at or PTT	5	10	15
Bacteremic at or PTT	9	11	20
Treated	9	11	20

As shown in Table 27, animals treated in the Levo and ETI-204 & Levo groups were comparable in sex, weight at baseline, exposure at challenge and PA-ELISA prior to treatment (PTT). Animals treated were bacteremia PTT. All animals were 5 months of age pre-exposure, except for animal L20615 in the ETI-204 & Levo group, which was one month of age at baseline and terminal sacrificed at day 28. None of the animals were positive for anti-PA IgG ELISA prior to challenge. Quantitative bacteremia was not measured in this study. No significant differences were observed between the 2 treatment groups for any of the demographic or baseline characteristics. The results were consistent in treated animals and animals challenged and randomized (results not shown).

Table 27 Study 1045: Demographics and baseline characteristics

	Levo n = 9	ETI-204 & Levo n = 11	P-value*
Sex [n(%)]			
Male	4 (44.4)	6 (54.5)	
Female	5 (55.6)	5 (45.5)	1.0
Weight (kg)			
Mean ± SD	2.7±0.2	2.8±0.1	
Median	2.7	2.7	
(Min, Max)	(2.5, 3.1)	(2.6, 2.9)	0.70
Challenge dose ($\times 10^7$ cfu)			
Mean ± SD	1.9±0.3	2.2±0.5	
Median	1.9	2.1	
(Min, Max)	(1.6, 2.4)	(1.6, 3)	0.11
Challenge dose (LD_{50}) [n(%)]			
< 200	6 (66.7)	3 (27.3)	
200 or higher	3 (33.3)	8 (72.7)	0.17
PA (ng/ml) PTT			
Geometric mean	18.1	44	
95% CI	(3.3, 100.2)	(16, 121.4)	0.33

* P-value was based on Fisher's exact test for categorical data and t-test for continuous data. Challenge dose and PA-ELISA were log transformed with base 10.

Reference ID: 3857343

During the course of this study, all rabbits treated became toxemic (detectable PA-ECL) before treatment initiation. Table 28 shows that the average time to toxemia is 38.2 hours in the Levo group and 43.7 hours in the ETI-204 & Levo combination group. The mean time to first positive PA-ELISA was 39.4 hours in Levo group and 54.8 hours in the combination group. All rabbits treated became bacteremic PTT, with the mean time to bacteremia being 40.8 hours in the Levo group and 43.7 hours in the combination group. There was no difference in the time to treatment initiation between the 2 treatment groups based on log-rank test. The average time to treatment initiation was 73.2 hours and 72.2 hours in the Levo group and the combination group, respectively.

Table 28 Study 1045: Time to toxemia, bacteremia and treatment initiation in hours

	Levo n = 9	**ETI-204 & Levo n = 11**	**P-value***
Time to first positive PA-ECL			
Mean ± SD	38.2±11.7	43.7±9.8	
Median	45.1	46.5	
(Min, Max)	(24.6, 50.1)	(23.9, 50.5)	0.48
Time to first positive PA-ELISA	n = 5	n = 10	
Mean ± SD	39.4±12.7	54.8±19	
Median	45.9	58.7	
(Min, Max)	(24.6, 50.9)	(25.7, 74.3)	0.11
Time to bacteremia			
Mean ± SD	40.8±19.6	43.7±15	
Median	27.2	46.5	
(Min, Max)	(24.6, 73.6)	(23.6, 74.3)	0.70
Time to treatment initiation			
Mean ± SD	73.2±2.1	72.2±1.8	
Median	73.7	72.2	
(Min, Max)	(69.5, 75.3)	(69.8, 74.8)	0.17

*P-value was based on log-rank test.

Figure 12 illustrates that most of the quantitative PA started to decrease after peaking at the time around treatment initiation (72 hours post challenge) for animals treated in the ETI-204 and Levo combination group or the Levo alone group. Animals died at peak PA or a detectable PA level. For those survived, the PA reduced to undetectable.

Reference ID: 3857343

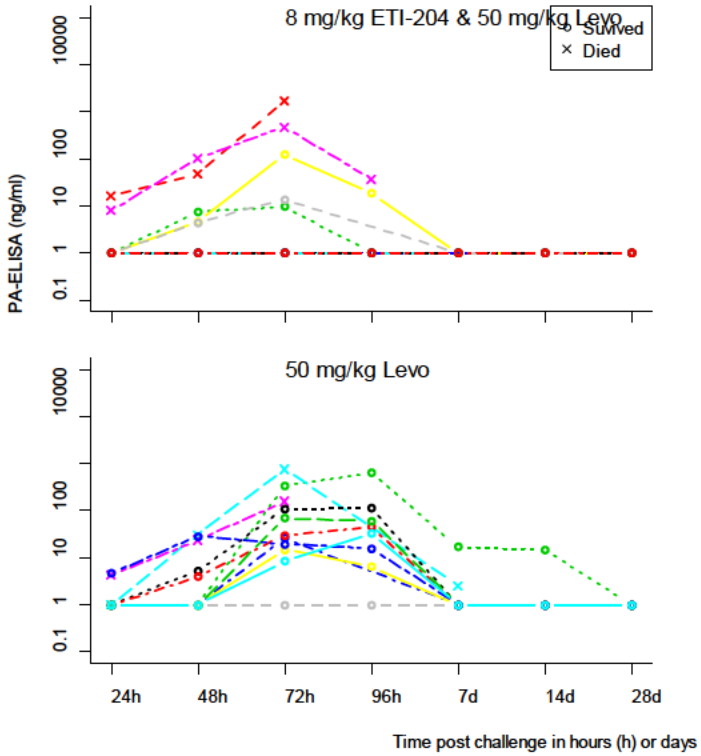

Figure 12 Study 1045: PA-ELISA over time for animals treated with levofloxacin

3.2.5.3 Results and Conclusions

Study NIAID 1045 followed the protocol and showed a similar survival rates of ETI-204 in combination with Levo (82%, 9/11) compared to Levo alone therapy (78%, 7/9) in the treatment of anthrax in NZW rabbits. This section provides detailed results from primary and exploratory efficacy analyses.

3.2.5.3.1 Primary Efficacy Analysis

Table 29 presents the primary efficacy results in Study NIAID 1045. Four out of the 20 animals that received randomized therapy died, 2 in the Levo group and 2 in the combination group. The survival rate in the ETI 204 & Levo combination group (82%) was not significantly higher than the Levo group (78%), with a difference of 4% (95% CI: -36%, 44%), p-value of 1.0 from one-sided Fisher's exact test.

Compared to animals randomized and challenged in the Levo group, animals in the ETI-204 & Levo group had numerically greater survival rates (56%) with survival rate difference of 12% (95% CI: -22%, 47%), p-value of 0.72 from one-sided Fisher's exact test. Following spore challenge, all animals became bacteremic PTT so the analysis for the animals treated was the same as that for bacteremic animals.

Reference ID: 3857343

Table 29 Study 1045: Survival rates at Day 28

Animals	Levo	ETI-204 & Levo	Difference (ETI-204 & Levo – Levo) 95% CI**	P-value*
Randomized and challenged	7/16 (44%)	9/16 (56%)	0.12 (-0.22, 0.47)	0.72
Treated (and bacteremia)	7/9 (78%)	9/11 (82%)	0.04 (-0.36, 0.44)	1.00

*P-value based on a 1-sided Fisher's exact test compared to 0.025.
**Difference in % survivors with 95% exact confidence interval (CI).

Comments: The 4% difference in survival rates between the 2 treatment groups did not reach statistical significance (p = 1.0). This study delayed treatment to 72 hours after challenge compared to the previous study which delayed treatment to 96 hours. The rate of survival to treatment was increased from 28 to 63%. However, the observed treatment effect size is smaller in this trial than the previous trial.

3.2.5.3.2 Exploratory Efficacy Analysis

3.2.5.3.2.1 Survival Time from Spore Challenge or Treatment Initiation

As an exploratory efficacy analysis, time from the initiation of spore challenge to death was compared between the ETI-204 & Levo combination group and the Levo group. For animals that died on study, the deaths occurred within 8 days of spore challenge. The 2 deaths in the ETI-204 & Levo group happened on day 3.1 and day 7.7 following spore challenge, while the 2 deaths in the Levo group happened on day 3.3 and day 5.8 post exposure. The average time from spore challenge to death is 5.4 days in the combination group and 4.6 days in the Levo group. According to the log-rank test, the probability of survival was numerically greater in the ETI-204 & Levo combination group than in the Levo group (Figure 13, P-value = 0.83). For the sake of completeness, Figure 14 illustrated survival times from challenge in all animals challenged and randomized by group.

Analogously, the survival time from the treatment initiation was compared between the combination group and the Levo group. The probability of survival was numerically greater in the ETI-204 & Levo group than the levofloxacin group (P-value = 0.83). Both analyses were performed in the animals who survived to treatment and similar results were obtained in animals challenged and randomized.

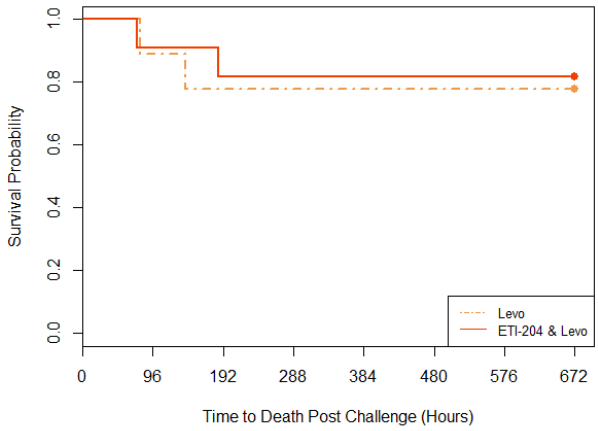

Figure 13 Study 1045: Survival time for animals treated with levofloxacin

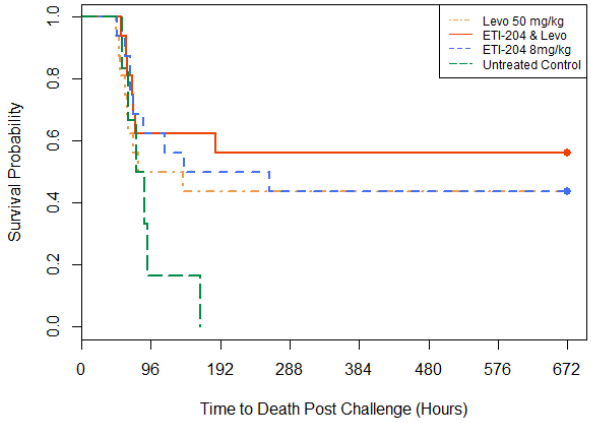

Figure 14 Study 1045: Survival time for all animals randomized and challenged

3.2.5.3.2.2 Analyses by Spore Challenge, PA-ELISA and Bacteremia

Two-sided Spearmen's rank correlation test was conducted to examine the association between the exposure (challenge dose), severity of anthrax infection (PA-ELISA) and time to death. Spearmen's correlation coefficient, r_s, was reported. As PA-ELISA PTT increases, time to death decreases ($r_s = -0.66$, p-value < 0.001). The challenge dose (LD_{50}) was not strongly associated with PA-ELISA ($r_s = 0.42$, p-value = 0.06) and time to death (r_s = -0.39, p-value = 0.09).

Table 30 summarizes survival rates in the Levo group and the ETI-204 & Levo group by exposure and PA-ELISA. These factors were categorized by targeted challenge dose 200 x LD_{50} and median (PA-ELISA). There was no significant association between exposure, PA-ELISA and survival rate. Given the limited sample size for each PA-ELISA group by treatment, regression analysis was not conducted to further explore the association between PA-ELISA and survival rate.

Reference ID: 3857343

Table 30 Study 1045: Survival rates by challenge dose and PA-ELISA

	Levo n = 9	ETI-204 & Levo n = 11	Total n = 20
Challenge dose (LD_{50})			
< 200	5/6 (83%)	3/3 (100%)	8/9 (89%)
200 or higher	2/3 (67%)	6/8 (75%)	8/11 (73%)
PA-ELISA			
< 23	6/6 (100%)	4/4 (100%)	10/10 (100%)
23 or above	1/3 (33%)	5/7 (71%)	6/10 (60%)

3.2.6 Study AR034 (Phase I)

Study AR034: Re-challenge of Rabbits Treated Previously for Inhalational Anthrax with Intravenous ETI-204 to Assess Protective Immunity

3.2.6.1 Study Design

Study AR034 was a parallel-group, open-label, randomized, placebo-controlled two-phase re-challenge study in healthy male and female rabbits to evaluate the added benefit of combination therapy of ETI-204 and levofloxacin (Levo) compared with Levo alone. Animals were challenged with anthrax spores twice, first in Phase I and 9 months later in Phase II. Table 31 provides details of the study design.

The proposed primary objective was to demonstrate that ETI-204 (16 mg/kg, IV) administered alone or in combination with Levo (50 mg/kg, HED) following primary challenge with spores of B. *anthracis* results in the development of protective immunity as measured by increased survival in the absence of treatment following secondary challenge (re-challenge) in NZW rabbits. The secondary objectives of this study were to determine whether rabbits treated with ETI-204 alone or in combination with Levo following primary challenge, as compared to rabbits treated with Levo alone, (a) were more likely to survive a secondary spore challenge, (b) demonstrate longer time to death following secondary challenge, and (c) have significantly higher levels of circulating anti-PA IgGs at the time of secondary challenge.

Table 31 Study AR034: Study design

Group	Treatment	ETI-204 Dose	Levo Dose	Treatment Initiation Post Exposure	Therapy Duration	No. of Animals
Phase I						
1	ETI-204	16 mg/kg	0 (vehicle)	30 hours**	Single dose (IV)	20
2	Levo	0 (saline)	50 mg/kg	30 hours	3 days (oral)	20
3	ETI-204+ Levo	16 mg/kg	50 mg/kg	30 hours	Single dose (ETI-204; IV) + 3 days (Levo; oral)	20
4	Control	0 (saline)	0 (vehicle)	30 hours	Single dose (IV)	8

Reference ID: 3857343

Group	Treatment	ETI-204 Dose	Levo Dose	Treatment Initiation Post Exposure	Therapy Duration	No. of Animals
Phase II (re-challenged 9 month after phase I)						
1	None			N/A	N/A	Survivors Group 1
2	None			N/A	N/A	Survivors Group 2
3	None			N/A	N/A	Survivors Group 3
4	None			N/A	N/A	12 Naive

* 30 hours post-mean challenge
** N/A – Not applicable

Comment: This review focused on the comparison between ETI-204 & Levo and Levo groups in survival rates by Month 9 of the study based on Phase I data with no comments on the proposed objectives for the re-challenge model (Phase I and II). For information on the results of the ETI-204 therapy arm and the re-challenge portion of the study see statistical review by Xianbin Li.

In phase I, a total of 68 eligible rabbits were randomized by weight and sex, before challenge, into three groups of size 20 (Group 1, 2 and 3) and one groups of size 8 (Group 4) with each group consisting of 50% male and 50% female animals. Animals in each group were then randomized to two challenge days (challenge day A and B) with a randomized challenge order per day.

Animals randomized were aerosol exposed to 200 x LD_{50} of *B. anthracis* spores on Day 0 (challenge day) at the (b) (4) ((b) (4) Rabbits in the Levo and combination groups that were alive at 30 hours post median challenge time received the first dose of Levo (50 mg/kg; oral gavage) followed by two subsequent oral Levo doses (50 mg/kg) once daily following the previous dose. Animals in the combination group also received antibody (16 mg/kg; IV bolus) once at 30 hours post median challenge time. Animals were monitored until Month 9 post first challenge and then euthanized.

The primary efficacy endpoint of phase I was 9-month survival, defined as the proportion of animals that survived to Month 9 post initial spore challenge. The primary efficacy analysis was the comparison of survival rates in four study groups with Bonferroni-Holm adjustment (described in Section 3.2.1.1) using one sided Fisher's exact test at an overall alpha level of 0.05. The analysis was performed in animals who survived to receive treatment regardless of bacteremia status. The study also conducted analyses in all animals randomized and challenged.

Comment: This review compared the proportion surviving in the ETI-204 & Levo group to that in the Levo group using a one-sided 0.025 level Fisher's exact test, to evaluate the added survival benefit of ETI-204 when administered in combination with Levo in phase I. The primary population for this review included animals who survived to receive at least one dose of treatment. Sensitivity analyses were performed for all animals randomized and challenged and for animals treated with bacteremia when applicable (Section 3.2.6.3.1).

Reference ID: 3857343

As a secondary efficacy analysis, the log-rank test was used to compare survival time from initiation of spore challenge between Levo only group and the ETI-204 plus Levo group. The analysis was performed in treated animals.

3.2.6.2 Animal Disposition, Demographic and Baseline Characteristics

Table 32 provides a summary of animal disposition in Study AR034. Among the 40 rabbits that were randomized to the Levo or the ETI-204 plus Levo groups and challenged with *B. anthracis* spores, all (40/40) rabbits survived to 30 hours post first exposure and received treatment planned.

Table 32 Study AR034: Animal disposition in Phase I before treatment initiation

	Levo	ETI-204 & Levo	Total
Animals randomized and challenged	20	20	40
Animals who survived to be treated	20	20	40/40 (100%)
Analysis population			
Randomized and challenged	20	20	40
PA-ELISA PTT	1	4	5
Bacteremic PTT	18	17	35
Treated	20	20	40

As shown in Table 33, animals treated in the Levo group and the ETI-204 & Levo group were comparable in sex, weight at baseline, exposure at challenge, quantitative bacteremia, PA-ELISA and time to treatment initiation before treatment. All animals were aged 8 months pre-challenge. Most of the animals were negative for anti-PA IgG ELISA prior to challenge (< BLOQ 50 ng/ml), except for animal L40845 (57.4 ng/ml) in the combination group. For the analyses, PA-ELISA values of <LLOQ were replaced by 4.94 ng/ml for summary statistics; and quantitative bacteremia values of <LOD were replaced by 2 cfu/ml and those of <LLOQ were replaced by 50 cfu/ml. Challenge dose, quantitative bacteremia and PA_ELISA were log transformed with base 10 for comparison between the two treatment groups. The study measured PA-ELISA and bacteremia at 2 days prior to challenge, PTT and Day 7 post exposure, therefore time from challenge to first detectable PA-ELISA and bacteremia was not calculable. Time to treatment initiation ranged from 25.5 to 30.2 hours post challenge. No significant differences were observed between the 2 treatment groups compared for any of the demographic or baseline characteristics. The results were consistent in animals treated and animals treated and bacteremic (results not shown).

Table 33 Study AR034: Demographics and baseline characteristics

	Levo n = 20	ETI-204 & Levo n = 20	P-value*
Sex [n(%)]			
Male	10(50)	10(50)	
Female	10(50)	10(50)	1.00
Weight (kg)			
Mean ± SD	3.3±0.3	3.3±0.3	
Median	3.3	3.4	
(Min, Max)	(2.8, 4.2)	(2.8, 4.1)	0.81
Challenge dose ($\times 10^7$ cfu)			
Mean ± SD	2.2±0.4	2.2±0.4	
Median	2.1	2.2	
(Min, Max)	(1.3, 3.4)	(1.6, 3.1)	0.89
Challenge dose (LD_{50}) [n(%)]			
< 200	8 (40.0)	9 (45.0)	
200 or higher	12 (60.0)	11 (55.0)	1.00
Bacteremia (cfu/mL)			
Geometric Mean	201.6	507.3	
95% CI	(37.1, 1097.1)	(60, 4286.4)	0.58
PA-ELISA (ng/ml)			
Geometric Mean	5.5	6.5	
95% CI	(4.3, 6.9)	(5, 8.5)	0.34
Time to treatment initiation			
Mean ± SD	27.9±1.5	27.5±1.2	
Median	27.9	27.5	
(Min, Max)	(25.7, 30.2)	(25.5, 29.5)	0.11

* P-value was based on chi-square test for categorical data, 1-way ANOVA for continuous data and log-rank test for time to treatment.

3.2.6.3 Results and Conclusions

Study AR034 followed the protocol for a re-challenge study. Overall phase I of this re-challenge study demonstrated that ETI-204 provided a similar survival rate when administered in combination with Levo (95%, 19/20) than that of the Levo alone therapy (100%, 20/20) on the treatment of anthrax in NZW rabbits. This section provides detailed results from primary and exploratory efficacy analyses.

3.2.6.3.1 Primary Efficacy Analysis

Table 34 presents the primary efficacy results in Study AR034. One of the 40 animals that received study agent died, 1 in the combination group. The survival rate in the ETI 204 & Levo

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combination group (95%) was numerically lower than the Levo group (100%), with a difference of 5% (95% CI: -26%, 11%), p-value of 1.00 from one-sided Fisher's exact test. For treated animals with bacteremia PTT, the ETI-204 & Levo group had numerically greater survival rates (100%) than the Cipro group (94%) with survival rates difference (-6%, 95%CI: -29%, 11%). All animals randomized and challenged survived to receive treatment therefore the analysis for the animals treated was the same as that for animals randomized and challenged.

Table 34 Study AR034: Survival rates at Month 9

Animals	Levo	ETI-204 & Levo	Difference (ETI-204 & Cipro – Cipro) 95% CI**	P-value*
Treated	20/20 (100%)	19/20 (95%)	-0.05 (-0.26, 0.11)	1.00
Bacteremic & treated	18/18 (100%)	16/17 (94%)	-0.06 (-0.29, 0.11)	0.49

*P-value based on a 1-sided Fisher's exact test compared to 0.025.
**Difference in % survivors with 95% exact confidence interval (CI).

Comments: The -5% difference in survival rates between the combination group and the Levo group did not reach statistical significance (p = 1.00).

3.2.6.3.2 Secondary Efficacy Analysis

As a secondary efficacy analysis, time from the initiation of spore challenge to death was compared between the ETI-204 & Levo combination group and the Levo group. There was only one death in phase I of the study in the ETI-204 & Levo group which happened on Day 4 following spore challenge. According to the log-rank test, the probability of survival was not significantly greater in the ETI-204 & Levo combination group than in the Levo group (Figure 15, P-value = 0.30). For the sake of completeness, Figure 16 illustrated survival times from challenge in all animals challenged and randomized by group.

This review did not consider subgroup analysis due to only one event on the Levo or ETI-204 & Levo groups in phase I of the study.

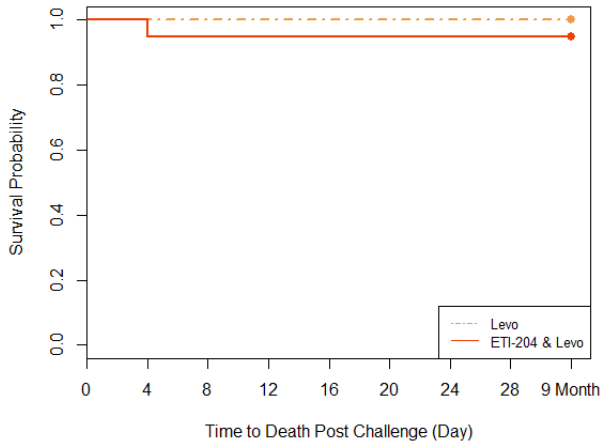

Figure 15 Study AR034: Survival time for animals treated with levofloxacin

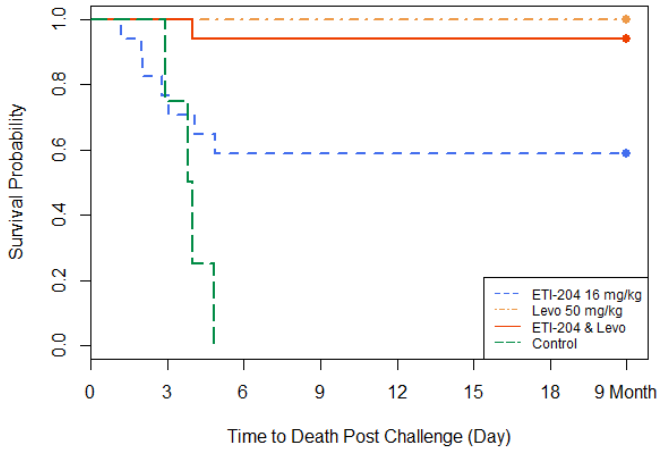

Figure 16 Study AR034: Survival time for treated bacteremic animals

3.2.7 Study AR028

Study AR028: An Exploratory Study to Evaluate the Effects of ETI-204 When Given in Combination with Levofloxacin on Survival in Anthrax-Challenged NZW Rabbits

3.2.7.1 Study Design

Study AR028 was a parallel-group, open-label, randomized, placebo-controlled two-phase study in healthy male and female rabbits to evaluate the added benefit of combination therapy of ETI-204 and Levofloxacin (Levo) compared with Levo alone. Table 35 provides details of the study design. The proposed primary objective was to determine whether ETI-204 (16 mg/kg, IV) improved survival rate when co-administered with levofloxacin (Levo) at a less than HED (6.5 mg/kg), compared to Levo at the same dose with a delayed treatment resulted in 50% survival (72±4 hours post median challenge time).

Table 35 Study AR028: Study design

	Group	Therapy	ETI-204 Dose	Levo Dose	Treatment Initiation	Therapy Duration	Animals Challenged	Animals Randomized
Phase I	1	Control	0 (saline)	0 (water)	72±4 hours post-median challenge time	Levo once daily 3 days (oral) ETI-204 Single dose (IV)	60	8
	2	Levo	0 (saline)	6.5 mg/kg				19
	3	ETI-204+ Levo	16 mg/kg	6.5 mg/kg				17
Phase II	4	Control	0 (saline)	0 (water)			60	4
	5	Levo	0 (saline)	6.5 mg/kg				19
	6	ETI-204+ Levo	16 mg/kg	6.5 mg/kg				17

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Animals, 60 in phase I and 60 in phase II of the study, were aerosol exposed to 200 x LD_{50} of B. *anthracis* spores at the [redacted] (b) (4) ([redacted] (b) (4) All animals survived to receive treatment were randomized by sex into three groups in each phase. For phase I, Group 1 consisted of 8 rabbits. Groups 2 and 3 each consisted of approximately half of the remaining rabbits treated. For phase II, Group 4 had 4 rabbits and Groups 5 and 6 each included approximately half of the remaining rabbits treated.

Rabbits in the Levo group and ETI-204+Levo group received a first dose of Levo (6.5 mg/kg; oral gavage) at 72 hours (±4 hours), followed by two subsequent oral Levo doses (6.5 mg/kg) once daily. Animals in the combination group also received antibody (8 mg/kg; IV bolus) once at 72 hours (±4 hours) post median challenge time. Animals were monitored until Day 28 post challenge and then euthanized.

Comment: This study was well designed. It performed randomization in animals who survived to receive treatment, which avoided deaths occurring between post randomization and prior to treatment.

The primary efficacy endpoint of this study was 28-day survival, defined as the proportion of animals that survived to Day 28 post spore challenge. The primary efficacy analysis was the comparison of survival rates in the ETI-204 & Levo group and the Levo group using a one sided Fisher's exact test at an overall alpha level of 0.05. The analysis was performed in animals who survived to receive treatment. The study also conducted analyses in animals treated with positive bacteremia result prior to treatment. Data for animals challenged but not randomized was not available.

An interim analysis of survival results from phase I was conducted following completion of phase I to select dose for phase II. If a statistically significant difference was found, the plan would have been to lower the ETI-204 in phase II. A statistically significant difference in the survival rates between the ETI-204 & Levo group and the Levo group was not achieved, therefore the dose of ETI-204 in phase II remained as 16 mg/kg. In subsequent analyses, animals treated by the same regimen in phase I and II were combined for the analysis, Groups 1 and 4 (control), Groups 2 and 5 (Levo), and Groups 3 and 6 (ETI-204 & Levo).

Comment: The primary analyses for this review compared the survival proportion of animals in the ETI-204 & Levo and Levo groups using a one-sided 0.025 level Fisher's exact test. The primary analyses population of this review was all animals who survived to receive treatment in phase I & II which includes all randomized animals in this study.

As exploratory efficacy analyses, the log-rank test was used to compare survival time from initiation of spore challenge between the Levo only group and the ETI-204 plus Levo group, and a logistic regression was conducted on the survival outcome by LD_{50}, PA-ELISA and quantitative bacteremia groups in Section 3.2.7.3.3.

The proposed sample size of >30 animals to be challenged in each group was based on the assumptions that the probability of survival was 90% and 60% in the ETI-204 & Levo group and

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Levo group, respectively, using one-sided Fisher's exact test with a 0.05 alpha level. The assumption for the mortality rate prior to treatment at 72 hours post challenge was 35%, which was equivalent to 78 animals surviving to treatment when 120 animals were challenged.

Comment: If a one-sided Fisher's exact test was used for sample size calculation, an alpha level of 0.025 should be used instead and would have still resulted in a power of 80% with 30 animals challenged per group under the same assumptions on survival rates.

3.2.7.2 Animal Disposition, Demographic and Baseline Characteristics

Table 36 provides a summary of animal disposition in Study AR028. One animal (L43701) in the Levo group was never bacteremic and was removed from the treated and bacteremia population. Seventy-three percent (44/60) of animals in phase I survived to treatment at 72 hours. Seventy percent (84/120) of animals in phase I and II combined survived to treatment at 72 hours.

Table 36 Study AR028: Animal disposition before treatment initiation

	Phase I			Total (Phase I & II)		
Treatment group	**Levo**	**ETI-204 & Levo**	**Control**	**Levo**	**ETI-204 & Levo**	**Control**
Animals challenged	60			120		
Animals survived to treatment	19	17	8	38	34	12
Analysis population						
Randomized and treated	19	17	8	38	34	12
Bacteremia at or PTT	18	17	8	37	34	12
PA-ELISA at or PTT	18	17	8	35	34	12

As shown in Table 37, animals treated in the Levo group and the ETI-204 & Levo group were comparable in sex, age (phase I), weight at baseline, exposure at challenge, quantitative bacteremia and PA-ELISA before treatment. Age for animals in Phase II was not provided. PA-ECL was not measured in this study. Most animals survived to Day 28 of the study were reported as negative (< 50 ng/ml) for anti-PA IgG ELISA prior to challenge, except for 3 animals (L43147, L43157, L43721) in the Levo group and 1 animal (L43160) in the ETI-204 & Levo group. For data analyses, challenge dose in LD_{50} was missing for 22 animals and was calculated by the reviewer as challenge dose (cfu) divided by (b) (4) after consulting Dr. Shukal Bala; quantitative bacteremia <LOD (3 cfu/ml) was replaced by 2 cfu/ml and <LOQ (100 cfu/ml) replaced by 50 cfu/ml; PA-ELISA for 24 animals was <LLOQ (9.68 ng/ml) and replaced with 4.84 ng/ml. Challenge dose, PA-ELISA and quantitative bacteremia were log transformed with base 10 for comparison between the 2 treatment groups. Three animals were negative for PA-ELISA PTT and one animal was negative for bacteremia PTT. These four animals were excluded from the corresponding analyses. No significant differences were observed between the 2 treatment groups for any of the demographic or baseline characteristics.

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Table 37 Study AR028: Demographics and baseline characteristics

	Levo n = 38	ETI-204 & Levo n = 34	P-value*
Sex [n(%)]			
Male	20 (52.6)	18 (52.9)	
Female	18 (47.4)	16 (47.1)	1.00
Weight (kg)			
Mean ± SD	3.2±0.1	3.2±0.2	
Median	3.2	3.2	
(Min, Max)	(3, 3.6)	(2.9, 3.6)	0.84
Age in Phase I (month)	n = 19	n = 17	
Mean ± SD	7.8±1.1	8.2±0.8	
Median	8	8	
(Min, Max)	(6, 9)	(6, 9)	0.25
Challenge dose ($\times 10^7$ cfu)			
Mean ± SD	2.3±0.4	2.4±0.4	
Median	2.2	2.4	
(Min, Max)	(1.2, 3.3)	(1.7, 3.4)	0.08
Challenge dose (LD_{50}) [n(%)]			
< 200	14 (36.8)	5 (14.7)	
200 or higher	24 (63.2)	29 (85.3)	0.06
Bacteremia ($\times 10^4$ cfu/mL)			
Geometric Mean	0.7	0.7	
95% CI	(0.3, 1.8)	(0.2, 2.1)	0.52
PA-ELISA (ng/ml)			
Geometric Mean	27.1	37.3	
95% CI	(14.8, 49.6)	(19.2, 72.5)	0.83

* P-value was based on chi-square test for categorical data, 1-way ANOVA for continuous data.

During the course of this study, three rabbits (L43139, L43720 and L43744) treated with Levo only did not become toxemic (detectable PA-ELISA) before treatment initiation by the applicant's definition. Table 38 shows that the average time to toxemia is 31.6 hours in the Levo group and 32.5 hours in the ETI-204 & Levo combination group. Animal L43701 was the sole rabbit treated but negative for bacteremia PTT. The mean time to bacteremia in the Levo group, 42.3 hours, was significantly greater than that in the combination group, 27.5 hours, with p-value < 0.001 using log-rank test. The reviewer's result on time to PA-ELISA was different from that in Table 2 of the study report Appendix-K. A post hoc analysis was conducted to explore possible relationship among the inhaled dose exposure, PA-ELISA PTT and survival status (Section 3.2.7.3.3.2).

There was no difference in the time to treatment initiation between the 2 treatment groups based on log-rank test. The average time to treatment initiation was 72.5 hours and 72.3 hours in the Levo group and the combination group, respectively.

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Table 38 Study AR028: Time to toxemia, bacteremia and treatment initiation in hours

	Levo n = 38	ETI-204 & Levo n = 34	P-value*
Time to first positive PA-ELISA			
Mean ± SD	31.6±16.8	32.5±16.6	
Median	24	24	
(Min, Max)	(24, 72)	(24, 72)	0.95
Time to first positive bacteremia	n = 37		
Mean ± SD	42.3±20.5	27.5±12	
Median	36	24	
(Min, Max)	(24, 72)	(24, 72)	< 0.001
Time to treatment initiation			
Mean ± SD	72.5±1.7	72.3±1.5	
Median	72.6	72.3	
(Min, Max)	(69.2, 75.4)	(69.8, 75.2)	0.30

*P-value was based on log-rank test

Figure 17 demonstrates that PA-ELISA varied in a similar pattern over time in animals who received ETI-204 & Levo combination therapy and the Levo monotherapy from 24 hours post challenge (PC) to 25 days post treatment (PT). The PA level reached a peak around 72 hours post challenge. Some animals died at the peak of PA-ELISA and all animals that survived reduced the PA to the limit of detection except for two animals in the combination arm.

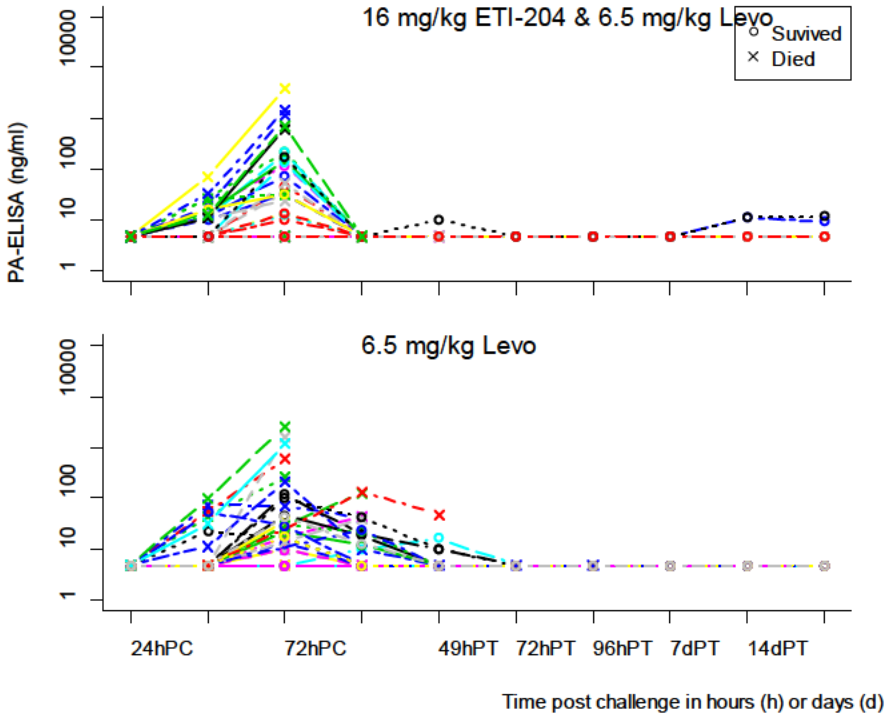

Figure 17 Study AR028: PA-ELISA over time in animals treated with levofloxacin

Quantitative bacteremia in these two treatment regimens changed similarly over time as shown in Figure 18. Most of the animals died at a relative high level of bacteremia and animals that survived cleared serum bacterial load by the end of the study except one animal in the combination group.

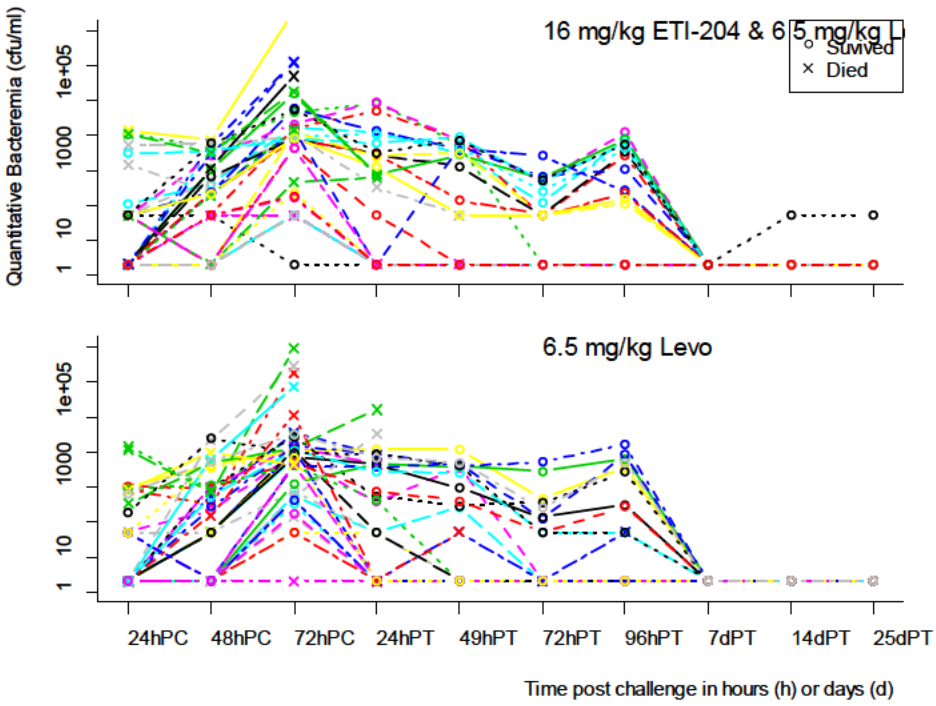

Figure 18 Study AR028: Quantitative bacteremia over time in animals treated with levoflocaxicin

3.2.7.3 Results and Conclusions

Study AR028 followed the protocol and demonstrated that ETI-204 provided a numerical added benefit in survival when administered in combination with Levo (68%, 23/34) over Levo alone therapy (58%, 22/38) in the treatment of anthrax in NZW rabbits. This section provides detailed results from primary and exploratory efficacy analyses.

3.2.7.3.1 Primary Efficacy Analysis

Table 39 presents the primary efficacy results in Study AR028. 27 out of the 72 animals that received study agent died, 16 in the Levo group and 11 in the combination group. The survival rate in the ETI 204 & Levo combination group (68%) was not significantly higher than the Levo group (58%), with a difference of 10% (95% CI: -12%, 32%), p-value of 0.39 from one-sided Fisher's exact test. Similarly, for treated animals with bacteremia, the ETI-204 & Levo group had numerically greater survival rates (68%) than the Cipro group (59%) with survival rates difference (9%, 95%CI: -14%, 30%). All animals randomized were treated by design in this study.

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Table 39 Study AR028: Survival rates at Day 28

Animals	Levo	ETI-204 & Levo	Difference (ETI-204 & Levo – Levo) 95% CI**	P-value*
Randomized and treated	22/38 (58%)	23/34 (68%)	0.10 (-0.12, 0.32)	0.39
Treated and bateremic	22/37 (59%)	23/34 (68%)	0.09 (-0.14, 0.30)	0.47

*P-value based on a one-sided Fisher's exact test compared to 0.025.
**Difference in % survivors with 95% exact confidence interval (CI).

Comments: The 10% difference in survival rates between the 2 treatment groups did not reach statistical significance (p = 0.39). Similarly to study 1045, this study used a HED of Levo and delayed treatment by 72 hours post-challenge. The treatment effect size in treated animals was larger in this trial compared to 1045 (10% vs. 4%) and the sample size was more than doubled in this trial.

3.2.7.3.2 Interim Efficacy Analysis

Table 40 presents the interim efficacy results in Study AR028 after the completion of phase I. Fourteen out of the 36 animals that received study agent died, 8 in the Levo group and 6 in the combination group. The survival rate in the ETI 204 & Levo combination group (65%) was numerically higher than the Levo group (58%), with a difference of 7% (95% CI: -39%, 25%), p-value of 0.68 from one-sided Fisher's exact test. Sensitivity analysis conducted in animals with bacteremia PTT showed similar difference in survival rates, 4% (95% CI: -36%, 28%), as in the animals randomized. The interim analyses did not demonstrate significant difference in survival rates between the Levo group and the ETI-204 & Levo group, therefore dose of Levo was the same as that in phase I, 16 mg/kg.

Table 40 Study AR028: Survival rates at Day 28 in Phase I

Animals	Levo	ETI-204 & Levo	Difference (ETI-204 & Levo – Levo) 95% CI**	P-value*
Randomized and treated	11/19 (58%)	11/17 (65%)	0.07 (-0.39, 0.25)	0.68
Treated and bateremic	11/18 (61%)	11/17 (65%)	0.04 (-0.36, 0.28)	0.82

*P-value based on a 1-sided Fisher's exact test compared to 0.05.
**Difference in % survivors with 95% exact confidence interval (CI).

3.2.7.3.3 Exploratory Efficacy Analysis

3.2.7.3.3.1 Survival Time from Treatment Initiation

As an exploratory efficacy analysis, time from the initiation of treatment to death was compared between the ETI-204 & Levo combination group and the Levo group. For animals that died on study, most deaths occurred within 6 days of treatment. The 16 deaths in the ETI-204 & Levo group happened between 3.1 days and 5.9 days post treatment while the 11 deaths in the Levo group happened between 3.3 days and 7.2 days post treatment. The average time from treatment

to death is 4.1 days in the combination group and 4.4 days in the Levo group. According to the log-rank test, the probability of survival was significantly greater in the ETI-204 & Levo combination group than in the Levo group (Figure 19, P-value = 0.46).

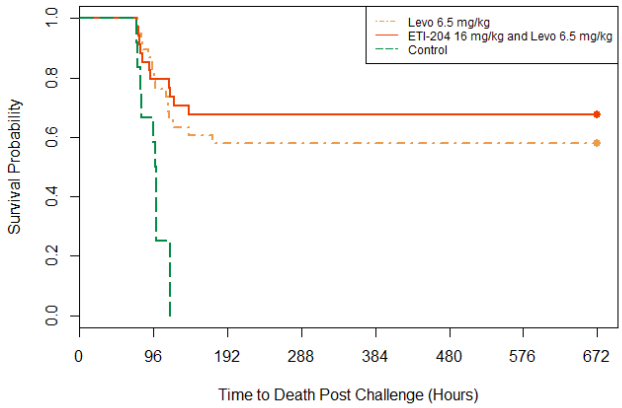

Figure 19 Study AR028: Survival time for animals randomized and treated

3.2.7.3.3.2 Analyses by Spore Challenge, PA-ELISA and Bacteremia

Spearman's correlation test was conducted to examine the association between the exposure (challenge dose in LD50), severity of Anthrax infection (PA-ELISA and quantitative bacteremia) and time to death. As PA-ELISA PTT increases, time to death decreases ($r_s = -0.41$, p-value = 0.04), and level of bacteremia PTT increases ($r_s = 0.83$, p-value < 0.001). Quantitative bacteremia was also significantly negatively associated with time to death ($r_s = -0.44$, p-value = 0.02). The challenge dose (LD50) was not significantly associated with PA-ELISA ($r_s = 0.09$, p-value = 0.45), bacteremia ($r_s = 0.01$, p-value = 0.93) or time to death ($r_s = -0.26$, p-value = 0.19).

An exploratory logistic regression analysis was conducted to examine the association between Day 28 survival and type of treatment received with adjustment of challenge dose PA-ELISA or quantitative bacteremia. When adjusting for challenge dose, neither the dose of exposure nor the type of therapy significantly affected the odds of survival with p-values of 0.07 and 0.64, respectively. Treatment type was not significantly associated with the odds of survival after adjustment for PA-ELISA or quantitative bacteremia in the corresponding logistic regression model. However, a one unit increase in log10 transformed PA-ELISA or quantitative bacteremia resulted in decreased odds of survival by 0.26 (p-value < 0.001) and 0.43 (p-value = 0.002).

In addition, an analysis explored the association among challenge dose, PA-ELISA or quantitative bacteremia, treatment type and time to death in animals randomized and treated. The model including challenge dose violated the proportional hazard assumption and was not performed. A one unit increase in log10 transformed PA-ELISA increased the hazard rate of death by 4.20 (95%CI: 0.24-7.52, p-value < 0.001). As quantitative bacteremia in log10 scale increased by one unit, the hazard rate of death would increase by 2.66 (95%CI: 1.78-3.97, p-value < 0.001). Analogous to the results from logistic regression, treatment type did not reach

statistical significance with p-values of 0.15 and 0.36 in the PA-LISA model and the quantitative bacteremia model, respectively.

3.2.8 Study AP 10-055

Study AP 10-055: Efficacy of Anthim® ETI-204 Monoclonal Antibody as an Adjunct Therapy in a New Zealand White Rabbit Partial Survival Model for Inhalational Anthrax

Of note, this study did not have electronic data available, therefore the following review is exploratory and data was extracted manually from the study report when applicable.

3.2.8.1 Study Design

Study AP 10-055 was a parallel-group, open-label, non-randomized, controlled study in healthy male and female rabbits to evaluate the added benefit of combination therapy of ETI-204 and Doxycycline (Doxy) compared with Doxy alone. Table 41 provides details of the study design.

Table 41 Study AP 10-055: Study design

Group	Treatment	ETI-204 Dose	Doxy Dose	Treatment Initiation Post Exposure	Therapy Duration	No. of Animals
1	Doxycycline	8 mg/kg	2 mg/kg	PA-ECL+ or 30 hours	3 days (oral)	10
2	ETI-204+ Doxy	8 mg/kg	2 mg/kg	PA-ECL+ or 30 hours	Single dose (ETI-204; IV) + 3 days (Doxy; oral)	10
3	Control (Saline)	N/A	N/A	N/A	N/A	4

PA-ECL+: positive protective antigen (PA) result determined by electrochemiluminescence (ECL)
N/A: Not applicable

The proposed primary objective was to assess the efficacy in survival rate of ETI-204 (8 mg/kg, IV) in combination with Doxy at a less than HED (2 mg/kg) compared to Doxy alone at a same dose, when administered upon the first positive PA-ECL result or at 30 hours following inhalational exposure to *B. anthracis,* whichever occurred first, in NZW rabbits.

Animals eligible were aerosol exposed to 200±50 x LD_{50} of B. *anthracis* spores on Day 0 (challenge day) in two iterations at the Center for Aerobiological Sciences United States Army Medical Research Institute of Infectious Diseases. Animals who survived to receive treatment were divided into 2 groups of 10 animals, the Doxy group and the combination group, and 1 group of 4 animals. Rabbits in the Doxy group or combination group received Doxy (2 mg/kg, oral) twice daily for two days. Animals in the combination group also received antibody (8 mg/kg; IV bolus) once at the first positive result PA-ECL or at 30 hours post exposure. Animals were monitored until Day 28 or Day 29 post challenge and then euthanized.

The primary efficacy endpoint of this study was 28/29-day survival, defined as the proportion of animals that survive to Day 28/29 post spore challenge. The primary efficacy analysis was the comparison of survival rates in the three study groups using exact permutation Cochran-

Armitage trend tests stratified by experimental iteration with p-values corrected by permutation to account for multiple comparisons. The analysis was performed in animals who survived to receive treatment and were bacteremic. The study also conducted analyses in animals treated and was bacteremic. Additional comparisons were performed on the mean time to death using a generalized linear model stratified by experimental iteration with p-values corrected by permutation to account for multiple comparisons.

Comment: This review compared the proportion of survival in the ETI-204 & Doxy group to that in the Doxy group using a one-sided 0.025 level Fisher's exact test based on the combined data from two iterations. The primary population for this review contains animals who survived to receive at least one treatment. A secondary survival analysis was conducted to explore the association between time to death and the treatment type (Section 3.2.8.3.2.1). Sensitivity analysis of survival rate was performed for treated animals with bacteremia (Section 3.2.8.3.2.1).

3.2.8.2 Animal Disposition, Demographic and Baseline Characteristics

The 10 rabbits each in Doxy group and ETI & Doxy group all received treatment as planned. Animal 45 in the Doxy group and animal 47 in the ETI-204 & Doxy group were never positive for PA-ECL or bacteremia and were removed from the analyses of treated animals with bacteremia.

As shown in Table 42, animals treated in the Doxy group and the ETI-204 & Doxy group were comparable in sex, spore exposure and bacteremia status before treatment initiation. The study report stated that LD_{50} was calculated by dividing challenge dose by (b) (4). All animals were adults weighting 3-5 kg with no detail information on age. There was no information on anti-PA IgG ELISA prior to challenge. Of note, quantitative bacteremia was not measured in this study. No significant differences were observed between the 2 treatment groups for any of the demographic or baseline characteristics.

Time to treatment initiation was not calculable because no information was available on treatment initiation time or challenge time. Similarly time to PA-ECL and time to bacteremia were not calculable.

Table 42 Study AP 10-055: Demographics and baseline characteristics

	Doxy n = 10	ETI-204 & Doxy n = 10	P-value
Sex [n(%)]			
Male	5 (50.0)	5 (50.0)	
Female	5 (50.0)	5 (50.0)	1.00
Challenge dose ($\times 10^7$ cfu)			
Mean ± SD	4.0±2.5	4.8±1.9	
Median	4.0	4.7	
(Min, Max)	(0.4, 7.4)	(2.2, 8.1)	0.24
Challenge dose (LD_{50}) [n(%)]			
< 200	4 (40.0)	0 (0.0)	
200 or higher	6 (60.0)	10 (100.0)	0.09

***** P-value was based on chi-square or Fisher's exact test for categorical data and 1-way ANOVA for continuous data

Comment: The non-randomized study design could have been the cause of the imbalanced spore exposure between the 2 treatment groups.

3.2.8.3 Results and Conclusions

Study AP 10-055 showed that ETI-204 had numerical added benefit in survival rates when administered in combination with Doxy (90%, 9/10) over Doxy alone therapy (50%, 5/10) on the treatment of anthrax in NZW rabbits, based on the derived data from study protocol. This section provides detailed results from the primary and exploratory efficacy analyses.

3.2.8.3.1 Primary Efficacy Analysis

Table 43 presents the primary efficacy results in Study AP 10-055. Six out of the 20 animals that received study agent died, 5 in the Doxy group and 1 in the combination group. The survival rate in the ETI 204 & Doxy combination group (90%) was not significantly higher than the Doxy group (50%), with a difference of 40% (95% CI: -2%, 72%), p-value of 0.14 from one-sided Fisher's exact test. This comparison was not powered to detect the observed difference in survival rates.

For treated animals with bacteremia, the ETI-204 & Doxy group had numerically greater survival rates (89%, 8/9) than the Doxy group (56%, 5/9) with survival rates difference (33%, 95%CI: -14%, 30%).

Table 43 Study AP 10-055: Survival rates at Day 28 or 29

Animals	Doxy	ETI-204 & Doxy	Difference (ETI-204 & Doxy – Doxy) 95% CI**	P-value*
Treated	5/10 (50%)	9/10 (90%)	0.40 (-0.02, 0.72)	0.14
Treated and bacteremic	5/9 (56%)	8/9 (89%)	0.33 (-0.14, 0.30)	0.47

* P-value based on a one-sided Fisher's exact test compared to 0.025.
** Difference in % survivors with 95% exact confidence interval (CI).

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Comments: The 40% difference in survival rates between the 2 treatment groups did not reach statistical significance (p = 0.14). This result could be conservative because the ETI-204 & Doxy group received imbalanced higher dose of spore exposure compared to the Doxy alone group.

3.2.8.3.2 Exploratory Efficacy Analysis

3.2.8.3.2.1 Survival Time from Spore Challenge

As an exploratory efficacy analysis, time from the initiation of spore challenge to death was compared between the ETI-204 & Doxy combination group and the Doxy group. For animals that died on study, most deaths occurred within 9 days of spore challenge. The 1 death in the ETI-204 & Doxy group occurred on Day 6 following spore challenge while the 5 deaths in the Doxy group happened between 6 days and 9 days following spore challenge. The average time from spore challenge to death is 6 days in the combination group and 7.4 days in the Doxy group. According to the log-rank test, the probability of survival was marginally significantly greater in the ETI-204 & Doxy combination group than in the Doxy group (Figure 20, P-value = 0.07). The analysis was also performed in the treated animals with bacteremia and similar results were obtained with p-value of 0.15.

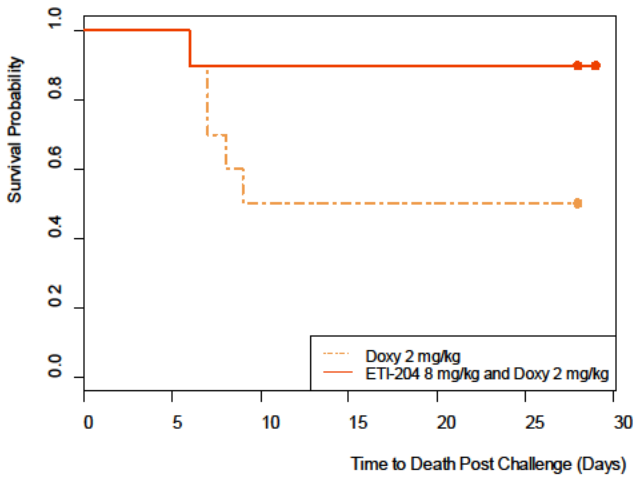

Figure 20 Study AP 10-055: Survival time for all animals treated with doxycycline

3.2.8.3.2.2 Analyses by Spore Challenge

Spearmen's rank correlation test was conducted to examine the association between the exposure (challenge dose) and time to death. The challenge dose (LD_{50}) was not significantly associated with time to death ($r_s = -0.04$). Table 44 summarized survival rates in Doxy group and ETI-204 & Doxy group by exposure in LD_{50}. Most of the death in the Doxy group happened in animals challenged with LD50 <200 (80%, 4/5).

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Table 44 Study AP 10-055: Survival rates by challenge dose

	Doxy n = 10	ETI-204 & Doxy n = 10	Total n = 26
Challenge dose (LD_{50})			
< 200	4/4 (100%)	0/0	4/4 (100%)
200 or higher	1/6 (16.7%)	1/10 (10%)	2/16 (12.5%)

3.3 Evaluation of Safety

The applicant conducted gross necropsy and microscopic pathology on tissues from animals in most of the combination study reviewed. The evaluation for safety data in human trials refers to the medical review of safety data.

Table 45 summarizes safety findings in the brain based on microscopic pathological findings in non-survivors from the 8 combination studies. The brain tissues lesions included but were not limited to bacteremia, hemorrhage inflammation, necrosis or vasculitis. In these trials, similar results were seen between the two treatment arms in the trials. There were three studies where all animals were assessed positive for brain lesions and one trial with no brain lesions found in any animal. Only one trial showed a numerical difference but only 4 animals were assessed and one event found. All survivors had no reported positive pathological findings in the brain when data was provided.

Table 45 Safety results in non-survivors among animals treated in combination studies

Study	ETI-204 Dose IV (mg/kg)	Animals Examined	ETI-204 + Anti	Antibacterial
AR007	8	NA	NA	NA
1030	8	treated	0/0	0/3
1045	8	treated	1/2	0/2
AR034 (Phase I)	16	NA	NA	NA
AR028	16	(selected non-survivors)	4/4	6/6
AP-10-055	8	NA	NA	NA
1056	8	treated	5/5	11/11
2469	8	treated	6/6	9/9

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4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

4.1 Gender, Race, Age, and Geographic Region

This submission included animal studies only, therefore race and geographic region do not apply. Age and weight were in a narrow range within rabbits or monkeys. Gender was balanced through randomization or pre-planning (AP 10-055) between the ETI-204 and antibacterials combination group and the antibacterial alone group in animals randomized. For animals surviving to receive treatment, the difference in treatment effects between the combination group and the antibacterial alone group was similar in general for males and females (details not reported here).

4.2 Other Special/Subgroup Populations

The review team considered categorized spore challenge dose in LD50, PA-ELISA and quantitative bacteremia prior to treatment as possible subgroups. Details on the three subgroup analyses refer to each individual study in Section 3, when data was available.

5 SUMMARY AND CONCLUSIONS

Eight animal studies have been conducted by the applicant to evaluate the efficacy and safety of ETI-204 for the treatment of subjects with (b) (4) *B. anthracis*, including 4 studies conducted in rabbits using antibacterial at a human equivalent dose (HED) (AR007, NIAID 1030, NIAID 1045, AR034 (Phase I)), two rabbit studies using antibacterial at a lower than HED (AR028, AP-10-055), and two monkey studies using antibacterial below HED (NIAID 1056, NIAID 2469).

5.1 Statistical Issues

There are two statistical issues in the review of this BLA submission, the schedule for randomization and the power of primary efficacy analysis in the added benefit studies reviewed.

Given the high mortality of *B. anthracis* when therapy is delayed, as was done for many of the added benefit studies, there are often animals that die prior to receiving treatment. When animals are randomized to treatment prior to challenge, there will be many animal deaths in each treatment arm occurring prior to treatment. A true intent to treat analysis would include these animals as failures. An improved design would be to wait to randomize animals to treatment just prior to treatment initiation, as was done for Study AR028. Because of the difficulty in showing the added benefit of a monoclonal antibody over antibacterial alone, and for ease of interpretation, this review focused on an analysis population that included animals who survived to receive study treatment. A requirement of the use of this analysis population is the assumption that the randomized therapy, including knowledge of the randomized therapy by those conducting the trial, did not affect the initiation of treatment.

As discussed previously, the efficacy of antibacterial therapy in the animal model is high in the treatment if *B. anthracis*, so in order to determine an added effect of ETI-204 over antibacterial

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therapy alone, the efficacy of antibacterial therapy needs to be reduced. This is done by either delaying treatment or administering the antibacterial therapy at a less than human equivalent dose (HED). Both of these methods have their limitations.

When delaying treatment in order to reduce the efficacy of antibacterial treatment, many animals do not survive to treatment. When treatment was delayed to 96 hours, only 28% of the animals survived to treatment. When treatment was delayed to 72 hours, 69% of the animals survived to treatment. This leads to a smaller sample size for analysis and a difficultly in adequately powering the trial. Additionally, it is not clear if the animals that are able to survive are representative of all animals and by extension, do they adequately represent the human disease.

Use of less than a human equivalent dose also has its drawbacks, because the dose can be reduced to a point where it is closer to have an effect of placebo. In the studies contained in this application, most of the antibacterial alone arms did appear to have a survival rate higher than what would be expected from placebo.

5.2 Collective Evidence

Six out of eight combination studies did not reach statistical significance for the added benefit of ETI-204 co-administered with antibacterial compared to the antibacterial alone therapy in survival rates (Table 2). To further evaluate the strength of evidence, exploratory meta-analyses were conducted based on pooled data across combination studies and summarized in Section 5.2.1. Section 5.2.2 summarizes the collective evidence.

5.2.1 Meta-analysis of Combination Studies

The meta-analysis focused on the comparison in survival between the antibacterial monotherapy and the combination therapy of ETI-204 and antibacterial in animals who received treatment from the studies reviewed. The primary endpoint of the meta-analysis was the survival rate difference by the end of each study, Day 28, 29, or 30 days post spore challenge.

The primary analysis method was an exact[2] fixed effect model based on inverse variance weighted estimation approach. An exact method was needed because of small sample sizes and because zero deaths were observed in two of the studies reviewed. The meta-analyses were stratified by animal type (rabbits or monkeys) and dose of antibacterial administered (human equivalent dose or less than HED). Within each stratum, the primary comparison was between the antibacterial group and the combination group on risk difference and reported the associated 95% CI, stratified by study. The heterogeneity across studies included in each stratum was evaluated through the study and treatment group interaction using subject level data.

[2] Tian, L., Cai, T., Pfeffer, M., Piankov, N., Cremieux, P., and Wei, LJ. (2008). Exact and efficient inference procedure for meta-analysis and its application to the analysis of independent two by two tables with all available data but without artificial continuity correction. Biostatistics, 10(2):275-281.

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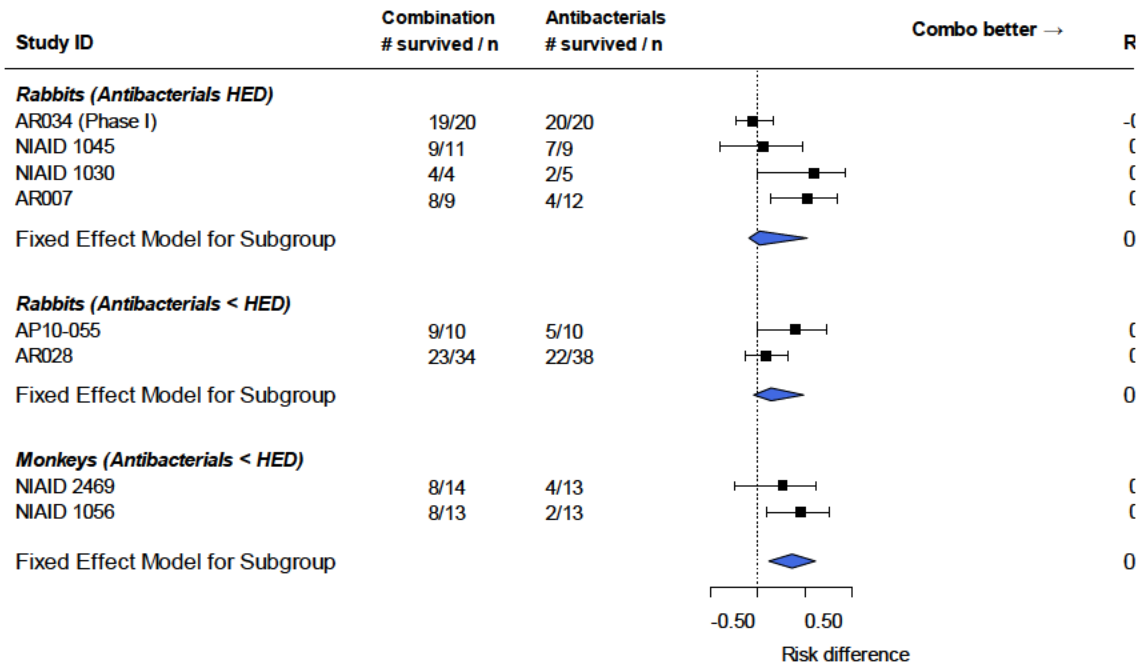
Figure 21 illustrated the survival rate differences for each study and grouped by each stratum from the exact fixed effect model. This meta-analysis was stratified by animal species and doses of antibacterial into three groups, 4 rabbit studies using antibacterial at a HED, 2 rabbit studies using antibacterial below HED and 2 monkey studies using antibacterial below HED.

In rabbits that received antibacterial at HED, animals receiving antibacterial and ETI-204 had similar survival rates to those on antibacterial therapy alone with a risk difference of 2% (95%CI: -0.10, 0.53). As predicted, the studies included were relatively heterogeneous (p-value = 0.052) given various ETI-204 doses administered and different treatment initiation time post exposure.

For studies using less than HED antibacterial in rabbits, the antibacterial and ETI-204 combination group had numerically greater survival rates than the antibacterial alone group with a risk difference of 14% (95%CI: -0.04, 0.48) and the studies included were relatively homogenous (p-value = 0.14).

In monkeys that received antibacterial at a less than HED, those who received antibacterial and ETI-204 had significantly greater survival rates than those on antibacterial monotherapy with a risk difference of 36% (95%CI: 0.11, 0.61). The two studies had similar study designs and were shown homogenous (p-value = 0.38).



Source: created by reviewer

Figure 21 Forest plot of survival differences based on exact fixed effect meta-analyses

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The exact fixed effect model currently does not allow for covariate adjustment, therefore the effect of quantitative PA and bacteremia could not be evaluated in this meta-analysis. The studies in two out of the three strata were relatively homogenous and the rabbit studies using antibacterial at a HED violated the proportional hazard assumption, therefore no further time to event analyses were conducted.

Overall, the meta-analyses demonstrated that when animals received ETI-204 in combination with antibacterial, they had numerically greater probability of survival than those treated with antibacterial monotherapy regardless of the doses for antibacterial based on stratified exact fixed effect model. In addition, the added benefit of ETI-204 in survival for the treatment of inhalational Anthrax in monkeys was significant when co-administered with antibacterial below HED, compared to the antibacterial alone group.

5.2.2 Summary of Collective Evidence

As discussed previously, it is difficult to assess the added benefit of ETI-204 over antibacterial, because antibacterial has a very high rate of survival in the animal model in the treatment setting when given at human equivalent doses (i.e., doses that will give similar exposure in animals that would be seen in humans). Animal models attempting to demonstrate added benefit typically need to reduce the efficacy of antibacterial by either delaying treatment or reducing the dose. This application contains studies that used a human equivalent dose (HED) and that used a lower than HED and treatment was started at various times. Each change in design, either timing of treatment or dose of treatment greatly affected the study outcomes, as is summarized in Table 2. The following discussion reviews the various designs and outcomes.

There were four rabbit studies that dosed animals at a HED of levofloxacin (AR007, NIAID 1030, NIAID 1045, and AR034).

- Study AR007 assessed the added benefit of ETI-204 given as IV 4 mg/kg or IM 8 mg/kg before the development of symptoms (the PEP setting). Levo was given to animal in the combination arm and the antibacterial arm at an HED dose but only given for 5 days, when in the PEP setting, treatment is usually given longer. ETI-204 both IV and IM had significant added benefit in survival when administered concomitantly with Levo over the Levo therapy alone. Though this study assessed Levo at a shorter duration than would be needed in a PEP setting, the efficacy of these lower doses of ETI-204, given either as IV or IM, did show significant increase in survival.
- Study NIAID 1030 assessed the added benefit of ETI-204 given as IV 8 mg/kg. In order to reduce the efficacy of antibacterial alone, treatment was delayed to 96 hours post spore challenge. Only 28% of challenged animals survived to treatment, leaving only 4 animals in the combination therapy group and 5 in the Levo alone group. ETI-204 provided a numerical added benefit in survival rates when administered in combination with Levo (100%, 4/4) over Levo alone therapy (40%, 2/5) for the treatment of anthrax in NZW rabbits. This design is not feasible in assessing the added benefit given the high pre-treatment mortality.

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- Study NIAID 1045 has a similar design as Study NIAID 1030 except that the time for treatment initiation was about 72 hours post exposure. Sixty-three percent of animals (20/32) survived to receive treatment, leaving 11 animals in the combination group and 9 animals in the Levo alone group. Similar survival rates were seen in the ETI-204 and Levo group (82%, 9/11) compared to Levo alone therapy (78%, 7/9) in the treatment of anthrax in NZW rabbits.
- Study AR034 compared 16 mg/kg IV ETI-204 and Levo with Levo alone. Treatment was initiated at 30 hours post challenge. Similar survival rates were seen between these treatment arms (95%, 19/20 for the combination group and 100%, 20/20, for Levo alone).

The results of these studies demonstrate the difficulty in showing the added benefit of ETI-204 over antibacterial alone, when antibacterial is given at a HED in a treatment setting. In the treatment study where treatment was not delayed (given at 30 hours), antibacterial alone had 100% survival. When given as a delayed therapy (given at 72 hours), the efficacy of the antibacterial decreased to 78%. It is still difficult to show added benefit to a treatment with a 78% survival rate, but even more difficult when only 63% of animal survive to treatment. When delayed even further, to 96 hours post challenge, the survival rate on Levo is reduced to a point where it might be possible to show a treatment effect (40%); however, so few animals survive to treatment, it is not feasible to power this type of study. The only study that was able to show a treatment effect when Levo was given at a HED was in the PEP setting. However, the survival rate on antibacterial was low 33% because antibacterial in the PEP setting should be dosed for a longer period of time. Despite these difficulties, taken together along with the studies at lower than HED, this appears to be adequate evidence that ETI-204 does not reduce the efficacy of antibacterial therapy and quite likely adds to the efficacy.

There were four studies that dosed animals at a less than human equivalent dose of antibacterial, 2 monkey studies (NIAID 1056 and NIAID 2469) and 2 rabbit studies (AR028 and AP 10-055).

- Study AR028 delayed treatment to 72 hours post exposure (as was done for NIAID 1045) and 70% of animals survived to treatment (63% survived to treatment in NIAID 1045). This study was more than double the size of NIAID 1045. NIAID 1045 used a Levo dose of 50 mg/kg x 3. AR028 used a much lower Levo dose of 6.5 mg/kg x 3. This lower dose led to survival on the Levo alone therapy arm of 58%, 20% lower than what was seen in NIAID 1045. ETI-204 was dosed at 16 mg/kg IV. But even despite this, only a numerical added benefit of ETI-204 in survival when administered in combination with Levo (68%, 23/34) over Levo alone therapy (58%) was seen. The treatment effect size in treated animals was larger in this trial compared to NIAID 1045 (10% vs. 4%).
- Study AP 10-055 dosed doxycycline, a different antibacterial, at a 2 mg/kg dose twice daily for 3 days. Treatment was initiated at approximately 30 hours post-challenge. This study showed that ETI-204 at 8 mg/kg IV had numerical added benefit in survival rates when administered in combination with Doxy (90%, 9/10) over Doxy alone therapy (50%, 5/10). The 40% difference in survival rates between the 2 treatment groups did not reach statistical significance ($p = 0.14$). This result could be conservative because the ETI-204 & Doxy group received a high dose of spore exposure compared to the Doxy alone group in this non-randomized study.

- Study NIAID 1056 evaluated the added benefit of ETI-204 given as IV 8 mg/kg. This monkey study used an oral ciprofloxacin dose of 10 mg/kg x 4, while the HED of Cipro is about 25.9 mg/kg. To further reduce the efficacy of antibacterial alone, NIAID 1056 initiated the treatments at about 24 hours post the first positive PA-ECL result, equivalent to 48 hours post exposure. The majority of the animals, 81% (26/32), survived to receive treatments. ETI-204 provided significant added benefit in survival rates when co-administered with Cipro (62%, 8/13) over Cipro alone therapy (15%, 2/13) for the treatment of anthrax in cynomolgus monkeys.
- Study NIAID 2469 has the same study design as NIAID 1056. Eighty four percent of the animals (27/32) survived to treatment initiation, leaving 14 animals in the combination group and 13 animals in the Cipro alone group. ETI-204 improved the survival rate numerically when administered concomitantly with antibacterial (57%, 8/14) compared to the antibacterial alone group (31%, 4/13).

Studies using lower than HED achieved the efficacy reduction of antibacterial monotherapy with survival rates ranging from 15% to 58%. These studies using lower than HED demonstrated a clear added benefit of ETI-204 in survival rates, when comparing the combination therapy of ETI-204 plus antibacterial to antibacterial therapy alone.

The meta-analyses of the eight studies reviewed demonstrated that when NZW rabbits receiving ETI-204 in combination with antibacterial, they had numerically greater probability of survival than those treated with antibacterial monotherapy regardless of the doses of antibacterial, based on stratified exact fixed effect model. In addition, the added benefit of ETI-204 in survival for the treatment of inhalational anthrax in monkeys was significant when co-administered with antibacterial below HED, compared to the antibacterial alone group.

5.3 Conclusions and Recommendations

Eight studies were submitted to address the added contribution of ETI-204 when given in combination with antibacterial therapy. These 8 studies varied widely in their design and outcomes allowing for an assessment of the effect of ETI-204 in various study design settings. Four studies at HED showed that ETI-204 does not reduce the efficacy of antibacterial drugs and likely adds to the efficacy in NZW rabbits. Studies at a less than HED, two in NZW rabbits and two in monkeys, clearly demonstrate ETI-204 administered in combination with antibacterial drugs results in higher survival outcomes than antibacterial therapy alone.

In conclusion, the eight combination studies adequately address the added survival benefit of ETI-204 when administered in combination with antibacterial drugs for the treatment of systemic inhalational anthrax disease, compared to the antibacterial therapy alone.

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/s/

LING LAN
12/08/2015

KAREN M HIGGINS
12/08/2015
I concur.

TSAE YIN D LIN
12/08/2015