17 September 2020
EMA/524933/2020
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Obiltoxaximab SFL

Common name: obiltoxaximab

Procedure No. EMEA/H/C/005169/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ADA	Anti-drug antibodies
ADCC	Antibody-dependent cellular cytotoxicity
AE	Adverse event
AIGIV	Anthrax immune globulin intravenous
ATA	Anti-Therapeutic Antibody
AUC	Area under the concentration versus time curve
$AUC_{(0-inf)}$	Area under the concentration versus time curve from time 0 extrapolated to infinity
AVA	Anthrax vaccine absorbed
AVP	Anthrax vaccine precipitated
B. anthracis	*Bacillus anthracis*
BARDA	Biomedical Advanced Research and Development Authority
BDS	Bulk drug substance
CDC	Complement-dependent cytotoxicity
CFU	Colony forming units
Cipro	Ciprofloxacin
CL	Systemic clearance
C_{max}	Maximum concentration
d	Day
Doxy	Doxycycline
DP	Drug product
EC	European Commission
ECG	Electrocardiogram
EF	Edema factor
EMA	European Medicines Agency
ETI-204	Obiltoxaximab
EU	European Union
FDA	Food and Drug Administration
IL-1	Interleukin-1
IM	Intramuscular
IV	Intravenous
kDa	KiloDalton
kg	Kilogram
Levo	Levofloxacin
LF	Lethal factor
LLOQ	Lower limit of quantification
LT	Lethal toxin
MAA	Marketing Authorisation Application
mAb	Monoclonal antibody
mg	Milligram
mITT	Modified intent-to-treat
N/A	Not applicable
NIAID	National Institute of Allergy and Infectious Disease
NZW	New Zealand White
ODD	Orphan drug designation
PA	Protective antigen
PAD4	Domain 4 of protective antigen
PAES	Post-authorisation efficacy study
PD	Pharmacodynamic
PIP	Paediatric investigation plan
PK	Pharmacokinetic
SAE	Serious adverse event
SD	Standard deviation
$t_{1/2}$	Half-life
T_{max}	Time of maximum concentration
TNF	Tumor necrosis factor
UK	United Kingdom

US	United States
USAMRIID	United States Army Medical Research Institute for Infectious Diseases
V_{ss}	Steady-state volume of distribution
V_z	Volume of distribution in the terminal phase

1. Background information on the procedure

1.1. Submission of the dossier

The applicant SFL Regulatory Services GmbH submitted on 3 June 2019 an application for marketing authorisation to the European Medicines Agency (EMA) for Obiltoxaximab SFL, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 20 September 2018.

Obiltoxaximab SFL, was designated as an orphan medicinal product EU/3/18/2065 on 24.08.2018 in the following condition: Treatment of Anthrax.

The applicant applied for the following indication:

Obiltoxaximab SFL is indicated in adults and paediatric patients for treatment of inhalational anthrax due to *Bacillus anthracis* in combination with appropriate antibacterial drugs.

Obiltoxaximab SFL is also indicated in adults and paediatric patients for the post-exposure prophylaxis of inhalation anthrax when alternative therapies are not appropriate or are not available.

The applicant changed to SFL Pharmaceuticals Deutschland GmbH during the procedure at Day 181.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Obiltoxaximab SFL as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/en/medicines/human/EPAR/obiltoxaximab-sfl

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0018/2018 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0018/2018 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with

authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Derogation(s) from market exclusivity

Not applicable

Applicant's request(s) for consideration

Marketing authorisation under exceptional circumstances

The applicant requested consideration of its application for a marketing authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation.

Accelerated assessment

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

New active Substance status

The applicant requested the active substance obiltoxaximab contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Protocol assistance

The applicant did not seek protocol assistance from the CHMP.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Jan Mueller-Berghaus Co-Rapporteur: Filip Josephson

The application was received by the EMA on	3 June 2019
The procedure started on	20 June 2019
The Rapporteur's first Assessment Report was circulated to all CHMP members on	9 September 2019
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	9 September 2019
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	23 September 2019

The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	17 October 2019
The applicant submitted the responses to the CHMP consolidated List of Questions on	24 January 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	02 March 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	12 March 2020
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	26 March 2020
The applicant submitted the responses to the CHMP List of Outstanding Issues on	17 August 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	10 September 2020
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Obiltoxaximab SFL on	17 September 2020

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Obiltoxaximab SFL is proposed in adults and paediatric patients for treatment of inhalational anthrax due to *Bacillus anthracis* in combination with appropriate antibacterial drugs and also in all age groups, for the post-exposure prophylaxis of inhalation anthrax when alternative therapies are not appropriate or are not available.

2.1.2. Epidemiology

Anthrax is a disease caused by the Gram-positive, encapsulated, spore-forming bacterium *B. anthracis*, which is found in soils all over the world. *B. anthracis* exists in two forms, vegetative bacilli and endospores. The spores are remarkably resistant to a range of environmental adverse conditions, ensuring long-term survival and infectivity [Dragon and Rennie, 1995]. Direct contact, ingestion or injection of the vegetative bacilli or spores causes cutaneous, gastrointestinal or injectional anthrax, respectively [Head et al, 2016]. Inhalational anthrax, the most severe form of anthrax, is caused by exposure to aerosolised *B. anthracis* spores.

Sporadic outbreaks of anthrax, most commonly the cutaneous form, occur throughout the world in both animals and humans [WHO, 2017; Vieira et al, 2017; Pillai et al, 2016]; predominantly affect poor, rural communities in anthrax-endemic countries in Africa, Central Asia, the Middle East, and South America are affected; however sporadic cases do occur in industrialised regions including countries in Europe. Typically, humans contract anthrax through direct contact of skin or mucosal membranes with *B. anthracis*–infected animals as they are slaughtered or by handling by-products such as hides. Gastrointestinal anthrax is contracted by consumption of raw or undercooked meat salvaged from infected animals. Although direct contact with an infected cutaneous lesion can transmit anthrax from one person to another, inhalational anthrax requires exposure to aerosolised spores and person-to-person transmission does not occur.

Inhalational anthrax rarely occurs naturally in humans, especially in industrialised regions of the world. In these areas, laboratory sources of inhalational anthrax are a major concern, either due to accidental release or use of *B. anthracis* for bioterrorism. *B. anthracis* spores are readily weaponised and highly virulent and it is not technically challenging to artificially create *B. anthracis* strains carrying antibiotic resistance genes.

2.1.3. Aetiology and pathogenesis

Anthrax is a disease caused by the Gram-positive, encapsulated, spore-forming bacterium *B. anthracis*, which is found in soils all over the world. Inhalational anthrax, the most severe form of anthrax, is caused by exposure to aerosolised *B. anthracis* spores.

Once inside the lung, *B. anthracis* spores are engulfed by alveolar macrophages and transported to mediastinal and peribronchial lymph nodes, where they germinate into vegetative bacilli. Subsequent rapid multiplication of the bacilli causes haemorrhagic mediastinitis and this is followed by haematogenous spread of bacilli throughout the body (systemic disease). In the fulminant phase of the disease, the release of tumour necrosis factor (TNF) and interleukin-1 (IL-1) from macrophages triggered

by bacilli leads to a rapidly deteriorating clinical picture. Anthrax toxins produced by *B. anthracis* have a key role in the pathophysiology of the disease. Early in the course of anthrax, the anthrax toxins suppress innate immune defences, enabling replication of bacilli and preventing development of adaptive immunity through impairment of dendritic cells, macrophages, and T cells [Liu et al, 2010]. In the fulminant stage, significant levels of accumulated anthrax toxin can drive the progression of disease by causing haemorrhagic meningitis and hemodynamic alterations, including severe hypotension. This results in tissue hypo-perfusion, hypoxia and metabolic acidosis, even when bacilli have already been successfully eliminated with antibiotics.

2.1.4. Clinical presentation, diagnosis and prognosis

The median incubation period for inhalational anthrax in humans is 10 days, with a range of 2 to 43 days after exposure to aerosolised *B. anthracis* spores, depending on the size of the inoculum and the immune status of the host [Brookmeyer et al, 2005; Inglesby et al, 2002]. Inhalational anthrax is a bi-phasic disease, with an initial prodromal phase of localised infection characterised by flu-like symptoms. This phase has a median duration of 3.9 days (range: 3.5 to 4.4 days) but then transitions abruptly to the fulminant phase of systemic illness [Holty et al, 2006].

During the initial prodromal phase of localised infection, symptoms include a low-grade fever, malaise and a non-productive cough. These may be accompanied by neurologic symptoms such as dizziness, visual changes or syncope. The transition to systemic infection is accompanied by a high fever, dyspnoea, diaphoresis, and shock.

The fulminant stage of the disease involves bacteraemia with vascular damage, cardiovascular collapse, meningitis, and injury to various vital organs [Abramova et al, 1993; Guarner et al, 2003; Hicks et al, 2012]. Inhalational anthrax is a life-threatening disease, with an overall mortality rate of approximately 50% even with aggressive treatment including multiple antimicrobials [Jernigan et al, 2002]. Patients who progress to the fulminant stage of inhalational anthrax have a mortality rate of 97%, regardless of the treatment they receive [Holty et al, 2006].

2.1.5. Management

Currently, the main treatment for suspected or confirmed inhalational anthrax is antibiotic therapy [EMA, 2014]. Antibiotics are also recommended for post-exposure prophylaxis in individuals with suspected or confirmed exposure to *B. anthracis*. In some cases, immunisation with anthrax vaccines is recommended, and this may reduce the duration of post-exposure prophylactic antibiotic treatment.

Two additional approaches for treatment of inhalational anthrax are currently not authorised in the EU: passive immunisation with anthrax immunoglobulins, and antitoxin therapy with monoclonal antibodies (mAbs) targeting anthrax toxins.

- **Antibiotic treatment**

  Three antibiotics are specifically licensed for anthrax in humans in Europe: ciprofloxacin, levofloxacin and benzylpenicillin.

  - Ciprofloxacin is licensed for post-exposure prophylaxis and curative treatment of inhalational anthrax in children, adolescents and adults. Oral ciprofloxacin 500 mg bid (or 10-15 mg/kg in children) for 60 days is recommended to prevent inhalational anthrax in humans, starting as soon as possible after suspected or confirmed exposure. At least 60 days of prophylaxis after exposure to *B. anthracis* spores is recommended because the spores can potentially remain

dormant for up to 60 days before they are activated. For treatment of inhalational anthrax, it is recommended that the treating physician refer to national and /or international consensus documents.

- ❖ Levofloxacin is licensed for post-exposure prophylaxis and curative treatment of inhalational anthrax, based on in-vitro susceptibility and animal data, together with limited human data. Dosing recommendations are similar, including reference to national and /or international consensus documents for the treatment of inhalational anthrax.
- ❖ Benzylpenicillin is licensed for the treatment of *B. anthracis* infections but no specific recommendations are made in the SmPC.

Ciprofloxacin is recommended as a first line treatment of inhalational anthrax in all age groups [EMA, 2014], and as an alternative for oral follow-up. In addition, the EMA recommends ciprofloxacin as a first line prophylaxis until susceptibility to other agents has been confirmed. Other quinolones (ofloxacin and levofloxacin) can be used as alternative treatment options but dose recommendations are only available for adults. Doxycycline and penicillin provide treatment options when susceptibility has been confirmed although penicillin is not bactericidal against *B. anthracis*.

Natural resistance of *B. anthracis* is known for several antibiotics, including sulfamethoxazole, trimethoprim, cefuroxime, cefotaxime sodium, aztreonam, and ceftazidime. They should not be used for treatment or prophylaxis of anthrax infection [Inglesby et al, 2002].

- **Active immunisation (anthrax vaccines)**

Currently two anthrax vaccines, anthrax vaccine adsorbed (AVA) and anthrax vaccine precipitated (AVP), are approved in the US and/or certain European countries but are not approved for widespread use in the EU.

- ❖ BioThrax

BioThrax and was approved in July 2013 in Germany. In April 2018, it was approved in five additional EU member states (Italy, the Netherlands, Poland, the United Kingdom [UK] and France) via mutual recognition procedure [e.g. MHRA, BioThrax SPC-PIL, 2018]. AVA is indicated for the prevention of disease caused by *B. anthracis* in adults at risk of exposure.

- ❖ AVP

AVP (which has no brand name) has been licensed since September 1991 in the UK [MHRA, Anthrax Vaccine SPC-PIL, 2015]. It is approved for prophylaxis in circumstances where exposure to *B. anthracis* may be expected or anticipated.

In addition to the pre-exposure use of these vaccines, the EMA guidance also recommends anthrax vaccines in certain circumstances for post-exposure immunisation in adults and children, in addition to antimicrobial treatment. With post-exposure vaccination, the need for antibiotic post-exposure prophylaxis can be reduced to 4 weeks.

- **Passive immunisation**

A polyclonal immunoglobulin against protective antigen (PA) (Anthrax Immune Globulin, Intravenous; AIGIV, trade name Anthrasil) is licensed in the US for the treatment of inhalational anthrax in adults and paediatric patients, in combination with appropriate antibacterial drugs. However, AIGIV is not authorised in the EU, and is not listed in the EMA guidance regarding the treatment of inhalational anthrax [EMA, 2014].

- **Antitoxin therapy**

❖ Raxibacumab

Raxibacumab is authorised in the US for the treatment of adult and paediatric 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 [FDA, raxibacumab USPI, 2018].

Currently, there is no antitoxin treatment authorised in the EU for the treatment of inhalational anthrax.

About the product

Type of Application and aspects on development

The CHMP did not agree to the applicant's request for an accelerated assessment as the product was not considered to be of major public health interest. This was based on the following considerations:

- Inhalational anthrax from bioterrorism has to date not yet occurred in the EU and the likelihood of such an event cannot be judged.
- The evidence on efficacy is based on animal models only, which carries inherently higher uncertainties compared to a situation where clinical efficacy data in humans are available.
- Currently there are available therapies that can be used for the treatment of inhalational anthrax and vaccines are available that could be used for prophylaxis in combination with antibiotics.

The applicant requested consideration of its application for a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation based on:

- Inability to provide comprehensive efficacy and safety data due to rarity of the indication.

Inhalational anthrax is encountered very rarely and as an infectious disease, it is sporadic making it impossible to predict when and where it will occur.

- Inability to collect such information because it would be contrary to medical ethics.

Due to the high mortality rate even with standard of care and the anticipated clinical benefit, it would be highly unethical to conduct randomised controlled clinical studies with obiltoxaximab in patients with inhalational anthrax.

Furthermore, the applicant considers that conditional approval is not appropriate for obiltoxaximab, as it is very unlikely that a comprehensive data package can be generated for obiltoxaximab in the future. This is because there is no possibility to conduct prospective randomised controlled clinical studies with obiltoxaximab in patients with inhalational anthrax disease and the opportunity for data collections is limited to open-label single-arm field studies with retrospective elements that can only be conducted upon the occurrence of an anthrax outbreak.

2.2. Quality aspects

The finished product Obiltoxaximab SFL is presented as a 6.0ml concentrate for solution for infusion containing 100mg/ml of obiltoxaximab as active substance.

Other ingredients are: histidine, sorbitol, polysorbate 80, hydrochloric acid (for pH adjustment), sodium hydroxide (for pH adjustment) and water for injections.

The product is available in a Type 1 glass vial with rubber stopper and a polypropylene cap with aluminium seal.

2.2.1. Active substance

General information

Obiltoxaximab (INN) is an affinity enhanced deimmunised anti-anthrax protective antigen (PA) monoclonal antibody (mAb) of the IgG1κ isotype. The VH and VL antibody gene sequences of obiltoxaximab were engineered from the murine mAb 14B7, originally developed by the United States Army Medical Research Institute for Infectious Diseases. The molecule consists of two Heavy Chains and two Light Kappa (κ) Chains. Glycosylation is only at one amino acid site on the heavy chain: Asn (residue 299). The relative molecular mass (glycosylated) is 148.1-148.6 kDa.

Manufacture, process controls and characterisation

Description of manufacturing process and process controls

The manufacturer of the active substance is Lonza Biologics, Inc. (Lonza Portsmouth, USA). A batch of active substance is defined as the material purified from one production bioreactor, cultivated from a single working cell bank (WCB) vial of murine GS-NS0 cells. Cells are cultured in a chemically defined medium which is free of animal derived components. The upstream and downstream manufacturing processes are typical for a therapeutic mAb active substance preparation. After fed-batch cell culture (inoculum expansion, fermentation) and primary recovery through centrifugation, purification is performed by Protein A Affinity Chromatography, Low pH Virus Inactivation, Post-Protein A Ultrafiltration/Diafiltration (UF/DF), Anion Exchange Chromatography, CHT Chromatography, Virus Reduction Filtration, Post-Virus-Reduction UF/DF and Final Concentration, excipient addition and a final 0.2 µm filtration into sterile active substance containers. Bulk purified active substance is tested against the specification. Following testing and review of batch records, the obiltoxaximab active substance is released and shipped to a designated storage facility for storage at the recommended storage condition of -80°C, until further processing into obiltoxaximab finished product.

Control of materials

Details of developmental genetics and the establishment of the cell bank system have been provided, in line with ICH Q5B. Obiltoxaximab is produced from a recombinant DNA-derived murine myeloma cell line (NS0). From the clone, a master seed bank was generated and consequently expanded to a working cell bank. The generation and characterisation of the cell banking system is performed according to the ICH Q5A requirements. Cell bank stability, phenotypic and genotypic characterisation including adventitious agents safety evaluation of the cell bank were investigated during process validation. Pre-harvest and end of production cell bank (EPCB) testing was performed for the extended generation lot and genetic characterisation was performed on the WCB and EPCBs from three active substance lots.

The stability of the genetic construct at the limit of *in vitro* cell age was evaluated. The light and heavy chain copy number remains stable. The evaluation of the final purified protein out of the end of production cell line was performed. A limit of *in vitro* cell age is a generation number has been proposed. The end of production cells are free of bacteria, fungi, mycoplasma and adventitious agents. The analysis also demonstrates that the cell line produces a xenotropic, amphotropic or Mink Cell Focus-Forming (MCF) Murine Leukemia Retrovirus (MLV). This is expected for NS0-cells. Virus testing is performed on each unprocessed bulk and acceptable retrovirus reduction has been provided for the manufacturing process and a satisfactory safety margin was presented for retrovirus. The risk of transmission of retrovirus is therefore considered negligible. Specification for future WCB has been provided and a full panel of virus tests is included as expected since complete panel of virus test was not performed on the MCB. The choice of test is in accordance with ICH Q5A

No animal or human derived materials are used during the obiltoxaximab active substance manufacturing process. All materials are sourced from approved suppliers and purchased of a grade suitable for manufacturing and tested to standards appropriate for their intended use. For non-compendial grade material specifications and tests are provided. These specifications are integrated into the facility's Quality Management System.

Control of critical steps and intermediates

Critical process parameters (CPP), key process performance parameters (KPP) and critical quality attributes (CQA) have assigned acceptance criteria. Potential CPPs and KPPs and their acceptable ranges were identified by gap analysis, risk assessment, literature data, historical data, process limit evaluation studies (PLE) and process characterisation studies. The overall approach is acceptable. It is concluded that key and critical process parameters have been appropriately identified and that the ranges ("acceptable criteria") proposed are adequately supported.

The manufacturing process is in general sufficiently described and the overall control strategy (including in process controls, testing of starting material, monitoring of process parameters etc) and the risk mitigation measures are in general adequate to control the process leading to an active substance of intended and consistent quality.

There are no intermediates isolated during the manufacture of the active substance.

Process validation

The active substance manufacturing process has been appropriately validated following a traditional approach. The process validation (PV) lots meet all CQA´s. The results of CPP´s and KPP´s were within the acceptable ranges. Removal of process and product-related impurities were adequately investigated. Re-processing was validated at small scale in triplicate for the virus reduction filtration step and the bulk fill using final formulation material from three PV lots.

The hold times of intermediates (in-process bulk solutions) were validated. The hold times defined in the manufacturing description are equivalent to or lower than the qualified hold times. Small scale lifetime studies were performed for the chromatography resins. The re-use UF membranes were validated.

The validated state of the process is maintained with an ongoing process verification program. This approach is also endorsed.

Manufacturing process development

Changes to the manufacturing process and site during development have been described. Comparability studies were performed following each change in the manufacturing process. The overall upstream and downstream changes were clearly described. The comparability studies demonstrate that the active substance manufactured at different stages are comparable.

Characterisation

The characterisation included the determination of physicochemical properties such as molecular mass, charged profile, glycosylation, sialic acid content, posttranslational modifications, purity, impurities and biological activity. In general, state-of-the-art methods were applied and most relevant characteristics have been evaluated. The primary structure was determined with N-Terminal and C-Terminal sequencing and peptide mapping. Obiltoxaximab exhibits properties representative of a de-immunised IgG1 murine mAb containing heavy and light chains bound by disulfide linkages with typical levels of heterogeneity in its mass, glycosylation, and charge profiles. The molecular mass of obiltoxaximab was determined both by MALDI-TOF MS and ESI-MS. The masses of the intact protein agreed with the theoretical masses calculated for the mAb with expected glycosylations. Glycoforms at ASN-299 contain several related N-linked glycan structures.

Obiltoxaximab binds to its target antigen which results in neutralisation of the anthrax toxin. Product potency has been confirmed.

The process-related impurities are controlled in the active substance specification. The information regarding process-related impurities is found acceptable.

Specification, analytical procedures, reference standards, batch analysis, and container closure

Specifications were set in accordance with ICH Q6B and includes tests for identity, purity, content, and potency.

Statistical analyses were used to set acceptance limits for several attributes. The proposed active substance specification is adequate.

Analytical methods

Brief descriptions of the analytical methods are provided. The methods are adequate to control the active substance on a routine basis. Analytical validation has been conducted in accordance with guideline ICH Q2(R1) recommendations and for each method, the required characteristics were evaluated. Full method verification and method validation reports are provided and show that the analytical methods are considered validated with respect to accuracy, precision, specificity, and linearity. The defined ranges cover the acceptance criteria in the proposed specifications.

The potency of the active substance is determined using a functional activity assay, the Lethal Toxin Neutralisation Assay (LNA). Cell death is measured using murine monocyte-macrophage cells exposed to the lethal toxin in the presence of obiltoxaximab. The neutralizing capacity of obiltoxaximab is expressed as the half maximal effective concentration (EC50) in ng/mL and as the percent potency relative to a reference standard assigned as 100%.

Batch analysis

Batch release data from several batches has been provided. The data confirm compliance with the proposed commercial specifications and confirm consistency of the product. The presented data indicate that the process is under control.

Reference materials

The establishments and history of in-house reference standards have been outlined. To date, three obiltoxaximab reference standards have been manufactured and qualified. Data from release testing and

characterisation for the reference standards are presented and considered acceptable. Protocols are provided for qualification and annual requalification of primary and secondary reference standard, respectively.

Container closure

The applicant has confirmed that the container closure system conforms to European Pharmacopoeia 3.2.2 Plastic Containers and Closures for Pharmaceutical Use (30202). The applicant further provided the certificate of analysis, the toxicological risk assessment and the extractable study for the container.

Stability

The long-term storage condition of -80°C ± 15°C is supported by real time data. Stability data for three engineering lots and two commercial lots were provided. These were produced at the commercial scale at the proposed commercial manufacturing site.

The forced degradation investigated exhibited no changes when subjected to agitation and freeze/thaw conditions but was affected by photodegradation, deamidation, oxidation, thermal stress and pH hydrolysation.

2.2.2. Finished Medicinal Product

Description of the product and Pharmaceutical Development

Description of the product

The finished product is presented as a 6.0 mL concentrate for solution for infusion containing 100 mg/mL of obiltoxaximab as active substance. Other ingredients are: histidine, sorbitol, polysorbate 80, hydrochloric acid (for pH adjustment), sodium hydroxide (for pH adjustment) and water for injections (WFI). Obiltoxaximab concentrate for solution for infusion is a clear to opalescent, colourless to pale yellow-pale brownish-yellow solution provided as a single-use glass vial stoppered with rubber stoppers and sealed with aluminium flip-off seals.

There are no overages.

All excipients comply with the requirements in the corresponding Ph. Eur. monographs, and pharmacopoeial methods are used for testing. There are no novel excipients and none of the excipients are of human or animal origin.

Pharmaceutical development

The active substance is diluted with formulation buffer to a concentration of 100 ± 10 mg/mL. The buffering component, L-histidine, maintains the pH in the range of 5.0-6.0. Sorbitol functions as the stabiliser and the Polysorbate 80 prevents aggregation. Nitrogen gas is used during the stoppering process.

The changes in the finished product manufacturing process are associated with changes in the active substance manufacturing process. Comparability studies were performed following each change in the active substance manufacturing process. Each comparability study compared finished product from the previous manufacturing process to active substance manufactured from the new process. Although this approach is rather unusual (and does not reflect the differences in the finished product manufacturing process) it can be accepted while the differences between the finished product processes are minor.

Comparability data (release data and extended characterisation) indicate that finished product filled at all manufacturing sites can be considered comparable.

The finished product is supplied in a glass vial with a sterile rubber stopper. The glass vial and the stopper, which are in product contact, meet the requirements of the Ph. Eur. 3.2.1 and Ph. Eur. 3.2.9 respectively. In addition, an extraction study was performed. No significant extractables were observed in the glass vial sample. After treatment of whole stoppers in water under harsh conditions, no significant peaks were observed. Given the aqueous nature of the finished product formulation the possibility of extractables leaching into the finished product is considered low.

Infusion solutions of obiltoxaximab in the intravenous (IV) diluent 0.9% sodium chloride were found to be compatible with common infusion bags of two different materials of construction (polyvinyl chloride and polyolefin) for up to 8 hours at both room temperature and refrigerated temperatures and when administered through different administration-set types simulating the flow conditions of a 90-minute infusion.

With regard to elemental impurities a risk assessment according to Q3D (R1) was conducted. Three batches of finished product were tested confirming that levels of elemental impurities are well below the permitted daily exposure limit provided in ICH Q3D.

A risk evaluation on the potential presence of nitrosamine impurities in Obiltoxaximab SFL was provided. The evaluation of active substance and finished product included raw materials, the process, composition, container closure, stability and facility. No risk of nitrosamine impurities has been identified.

Manufacture of the product and process controls

Manufacture

Batch release is performed by the Qualified Person located at PharmaKorell GmbH, Lörrach, Germany.

The manufacturing process for the finished product consists of thawing concentrated active substance, mixing with formulation buffer, and aseptically filling into vials. The product is manufactured aseptically, and the solution is passed through a bioburden reduction filter (0.22 µm) and subsequently filtered through two 0.22 µm sterile filters in series. Bioburden is tested prior to the bioburden reduction filter and in between the bioburden reduction and the sterilizing filters. Filter integrity testing is performed pre- and post-filtration. No reprocessing procedures are foreseen for the manufacture of the finished product.

In-process controls (IPCs) with adequate acceptance criteria are defined. CPPs are identified and limits are provided.

The process validation activities covered equipment validation, qualification of container closure components and raw materials, IPC analytical method validation or qualification, validation of sterilizing filters, media fill validation, manufacturing process validation and shipping validation. Overall, the activities are sufficiently described. All PV lots met the release acceptance criteria. Shipping validation studies confirm suitable packaging configuration. All acceptance criteria set forth in the pre-approved process validation protocol have been met.

Product specification, analytical procedures, batch analysis

The finished product specifications and analytical methods have been established to adequately control the identity, quality, purity, and potency of the finished product at release and throughout the proposed

shelf life. Specifications were set according to the principles set forth in ICH Q6B. Compendial tests are included as appropriate for the dosage form and route of administration, and limits are set in accordance with Ph. Eur.

Justifications for the compendial and non-compendial methods included in the finished product specification and the corresponding acceptance criteria are provided. The limits for purity and potency are based on the process capability of active substance lots, due to a limited number of finished product lots and the nature of the product that precludes full clinical development. The difficulty to set clinically justified limits for this type of product and indication is acknowledged. The proposed purity and potency limits are acceptable since the levels as such are generally considered safe and potential immunological side effects are less relevant due to the single dose administration of the product.

Analytical methods

Method descriptions and brief method validation summary tables for the assays specific for the finished product are provided. All other methods are either identical to those used for analysis of the active substance or compendial methods.

Batch analysis

Batch release results for the finished product are provided and include batches derived from process validation, batches used for preclinical studies and for clinical trials. All lots met the acceptance criteria in place at the time of release. The batch analysis data demonstrates acceptable batch-to-batch consistency and reproducibility of the manufacturing process proposed for the finished product.

Reference materials

Please see the active substance section. The same reference standards are used for the active substance and finished product.

Stability of the product

Finished product stability studies at the recommended storage condition of 5°C ± 3°C are ongoing for several lots. These lots were filled and finished at the commercial manufacturing site from active substance manufactured at the commercial active substance manufacturing site (Lonza Portsmouth). The PV lots have been shown to be stable up to 72 months at the recommended storage condition of 5°C ± 3°C (studies ongoing) and filled in the intended commercial container.

The claimed shelf-life and storage conditions as per the SmPC of 6 years in a refrigerator (2°C–8°C) is supported by real time data according to ICH Q5C and therefore considered acceptable.

According to GMP requirements one cGMP finished product lot per year (if produced) is placed on stability in support of commercial production.

An in-use microbial study was performed to determine the acceptable storage time for finished product prepared in 0.9% sodium chloride injection in IV bags (ViaFlex). Adventitious microbial proliferation does not occur when the finished product is diluted in 0.9% sodium chloride IV bags for up to 16 hours when stored at either refrigerated or room temperature conditions. The performed studies support the stability claim of 8 hours at either refrigerated or at room temperature conditions as outlined in the SmPC.

Post approval change management protocol(s)

A post approval change management protocol (PACMP) has been proposed for replacement of the current filling line with an optimised filling line.

There will be no changes to the batch size, solution preparation step or sterile filtration; only the filling line will change. A comparison of the current and proposed fill lines is included in the PACMP. A Failure Modes and Effects Analysis (FMEA) risk assessment was conducted to assess the proposed changes to the validated process in order to identify the potential risks to product quality and detail a strategy on how these risks will be mitigated. The control strategy for the new filling process will be based on the IPCs and release tests for the finished product.

As there is no overall change to the batch size, solution preparation step or sterile filtration the proposed data package (appropriate risk assessment, successful validation of the new filling line, batch release data and stability data of the PV batches) might be sufficient to support the implantation of the new filling line. The overall content of the PACMP is found acceptable and consequently a type IB variation may be used for the introduction of the new filling line in accordance with EMA/CHMP/CVMP/QWP/586330/2010 Questions and answers on post approval change management protocols.

Adventitious agents

TSE compliance

Compliance with the TSE Guideline (EMEA/410/01 – rev. 3) has been sufficiently demonstrated. Obiltoxaximab is produced in a serum-free medium and no materials of animal or human origin are used during manufacturing. During cell line development US- and Australian-sourced foetal bovine serum was used in the transfection medium.

Viral safety

Obiltoxaximab is expressed in murine myeloma NS0 cells in the absence of serum. Other than the cells themselves, no material of animal origin is added during fermentation. The cell banking system has been extensively screened for adventitious viruses using a variety of *in vitro* and *in vivo* assays. The tests did not detect presence of any virus contaminants in the cell banks with the exception of intracellular A-type and extracellular C-type retrovirus-like particles as well as reverse transcriptase activity and evidence of infectious retrovirus (in a single assay) which are well known to be present in rodent cells. This is acceptable since there is sufficient capacity within the obiltoxaximab manufacturing process to inactivate/remove such virus particles.

A virus-reduction study was performed in accordance with ICH Q5A *Guidance on Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*. The steps validated for virus reduction are low pH treatment, Planova 20N virus reduction filtration and three chromatography steps in the purification scheme. Relevant model viruses were used in the study. Overall, effective reduction was demonstrated for the obiltoxaximab manufacturing process.

At the end of the obiltoxaximab fermentation procedure, general testing for adventitious viruses is performed, as well a specific PCR test to detect minute virus of mice.

In summary, TSE and viral safety of obiltoxaximab has been sufficiently demonstrated.

2.2.3. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.2.4. Conclusions on chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.3. Non-clinical aspects

2.3.1. Introduction

The pivotal studies (non-clinical efficacy, pharmacokinetics [PK] and toxicity) were conducted in accordance with GLP. Some exceptions to GLP were noted in individual studies. However, these exceptions are not considered to affect the interpretation of study data or the scientific validity of the studies.

2.3.2. Pharmacology

Mechanism of action

Obiltoxaximab *(ETI-204)* is a chimeric mAb with human IgG1/κ constant regions and de-immunised murine variable regions. Obiltoxaximab specifically binds and neutralises the *Bacillus anthracis* PA, the cell-binding component of anthrax toxin.

PA is one of three polypeptides in the tripartite *B. anthracis* toxin, also consisting of the lethal factor (LF), and edema factor (EF). PA and LF combine to produce anthrax lethal toxin (LT), while PA and EF combine to produce edema toxin (ET).

The 83 kDa form of PA binds to anthrax toxin receptors (ATR) on the cell surface (either GMC-2 or TEM-8) and is subsequently cleaved by cell-associated proteases into a receptor-bound 63 kDa fragment and a soluble, inactive 20 kDa fragment. PA63 subsequently assembles with LF or EF and mediates translocation of these factors into the target cells. LF is the predominant cause of severe disease and death following inhalation spore exposure. Because of PA's central role in toxin assembly and intoxication of target cells, PA neutralisation strategies may be effective in preventing the establishment and progression of anthrax disease.

Primary pharmacodynamic studies

In vitro

Using Biacore surface plasmon resonance (SPR) obiltoxaximab was shown to bind to *B. anthracis* PA with a KD of 0.33 nM. This is approximately 10x higher than the affinity of the parental murine mAb

14B7 for PA. The obiltoxaximab affinity for PA lies in the range of the affinity of PA for its cellular receptors (0.17 to 1 nM) as described in the literature.

The reactivity of obiltoxaximab with PA from three *B. anthracis* strains (Ames, Sterne, and Vollum) was shown by immunoprecipitation followed by Western blotting. While this is a limited number of *B. anthracis* strains, obiltoxaximab can be expected to detect PA irrespective of clade based on literature data indicating limited genetic variability of *B. anthracis* in general (Keim et al., 1997) and of the PA gene in particular (Price et al. 1999). Information on the variability in the level of toxin production across *B. anthracis* strains is not available in the literature.

The capacity of obiltoxaximab to prevent binding of PA to the cell surface Anthrax Toxin Receptors (ATRs) was tested by interference/displacement studies in the ELISA format. Obiltoxaximab dose-dependently, at ≥0.5 molar excess, blocked both the binding of PA83 and PA63 binding to the soluble form of CMG-2, the ATR predominantly responsible for the effects of anthrax toxin. Binding to TEM-8 ATR was also investigated but did not work in this assay format, which was assumed to be due to its 1000-fold lower affinity than CMG-2 for PA.

At the cellular level, obiltoxaximab and the murine parent mAb 14B7 was tested for its ability to rescue murine macrophages by neutralisation of anthrax LT. Both mAbs demonstrated improved survival of a murine macrophage cell line incubated with LT, but the EC50 of obiltoxaximab was one fourth versus that of the parental mAb 14B7. This finding reflects the higher affinity of obiltoxaximab for PA compared to the parental mAb.

In vivo

Two in vivo models for inhalational anthrax infection, in rabbit and non-human primate, were used for the non-clinical pharmacodynamic (PD) in vivo evaluation. These rabbit and non-human primate animal models are often cited as generating data with suggested relevance to human inhalational anthrax, at least from a perspective of comparing pathological lesions during the end stage of disease.

The present studies were in general designed based on addressing survival/time to death as primary endpoints and with bacteraemia, PA serum levels and other parameters put as secondary readouts in an exploratory fashion. In the non-clinical pharmacology summary and associated parts of the overview, the applicant has in general focused on the PA serum levels, which in some studies appears to shift the study objectives. In the assessment, survival and bacteraemia data have been extracted from the original reports and are assessed in order to put the PA serum data in its context.

In GLP trigger-to-treat studies, New Zealand White (NZW) rabbits were challenged with spore infection by inhalation of ~200 LD50 equivalents, before treatment with ETI-204 or vehicle. A positive result in a qualitative electro-chemiluminescence PA (PA-ECL) assay or a significant increase in body temperature (SIBT) was used as trigger-to-treat. Positive animals were administered a single IV bolus of ETI-204 at 0 (0.9% NaCl), 1, 4, 8, or 16 mg/kg and evaluated for up to 28 days post challenge (PC). Blood sampling was carried out pre-challenge (i.e., Day -7), at 24, 30, 36, and 42 hours PC prior to treatment (or until decision to treat) with ETI-204, and at 0.25, 4, 8, 24, and 48 hours post-treatment, and on Days 8, 12, 16, 20, and 28 PC. Rabbits that were bacteraemic prior to treatment and treated with ETI-204 at 1, 4, 8, and 16 mg/kg demonstrated survival rates of 17%, 33%, 69%, and 62%, respectively while all saline treated animals succumbed to infection (the 4, 8 or 16 mg/kg groups survival were significant to the control). Ninety percent (63/70) of the challenged animals were bacteraemic before treatment. The rest were treated based on SIBT. Resolution of bacteraemia occurred by 8 days PC for most rabbits that survived. There were significant relationships between ETI-204 dose versus survival as well as quantitative bacteraemia levels at the prior to treatment (PTT) study time point versus survival. PA levels in controls animals increased steadily over time until death, whereas very few samples collected post-treatment with ETI-204 ever rose above the lower limit of

quantification (LLOQ). At 8, 24 and 48 hours post-treatment, there were significantly more animals with PA levels >LLOQ in the saline control treated compared to all ETI-204 treated groups ($p<0.0001$). PK testing showed the terminal half-life ($t_{1/2}$), systemic clearance (CL) and volume of distribution (V_{ss}) were similar across the dose groups, indicating these parameter values were independent of dose. The serum concentrations of ETI-204 declined below LLOQ in surviving animals after approximately 8 days post treatment (PT) which corresponded with the confirmed presence of anti-drug antibodies (ADAs).

In order to test for an add-on effect with antibiotics, ETI-204 (16 mg/kg single IV bolus, concurrently with 1st levofloxacin dose) was administrated in combination with levofloxacin (6.5 mg/kg/day QD x3) in a non-GLP study. Levofloxacin was administered at a suboptimal antibiotic dosing and delayed treatment regimen to allow for identification of possible additive or interference effects of ETI-204. The survival rate in both the levofloxacin and levofloxacin + ETI-204 treated groups were significantly greater than in the control group. However, the survival rate for the levofloxacin + ETI-204 treated group was not significantly different from the levofloxacin only treated group. In the control group PA levels rose sharply through 24 hours post-treatment, and no animals survived past that timepoint. In the Levofloxacin only group, PA levels were quantifiable through 24 hours following the 1st treatment, otherwise below LLOQ after 2nd and 3rd treatment while in the combined treatment PA levels were below LLOQ at all timepoints.

In the non-human primate, cynomolgus, model challenged and monitored in a similar fashion as the rabbit, various doses of ETI-204 was tested with a variable outcome.

In one study, survival rate for 4 or 8 mg/kg were 79 and 73% respectively vs. 14 % in the saline group; complete resolution of bacteraemia was evident at 96 hours post ETI-204 treatment. In contrast, resolution for the two surviving saline treated monkeys occurred by Day 14 post challenge; Total PA levels (including ETI-204 bound PA) declined steadily post-treatment in 4 and 8 mg/kg groups and were < LOD by Day 14 PC. In contrast, PA levels in saline treated group were higher through Day 7 PC. The applicant conclude these results suggest that ETI-204 is efficacious in protecting against death due to anthrax when administrated IV after the onset of disease in cynomolgus monkeys. Moreover, ETI-binds effectively to PA in monkeys and result in a faster return to normal compared to saline treatment. Monkeys with PA levels <100 ng/ml prior to treatment were more likely to survive if treated with ETI-204 than administered saline.

In a second study, survival rate for 4 or 16 mg/kg were 25 and 47% respectively vs. 6 % in the saline group. The difference in survival rates between the ETI-204-treated and the control group were not significant. However, when time to death in addition to survival was considered and the Bonferroni-Holm adjustment was used, the 16 mg/kg ETI-204 group was significantly more protected compared to the control group ($p = 0.0061$). Complete resolution of bacteraemia occurred by 7 days post-treatment for all animals that survived to their scheduled sacrifice. A significantly greater proportion of animals were positive for PA-ELISA in the saline group compared to both the 4 and 16 mg/kg ETI-204 group at 6, 24, 48, and 96 hours post-treatment and at the terminal time point.

In a third study, there was no significant relationship between ETI-204 dose (0, 8 or 32 mg/kg) and survival (13, 6 and 38%). At 48 hours and 96 hours post-treatment, there were statistically significant differences in the proportion of animals positive for bacteraemia between the saline control group and the group treated with 32 mg/kg. Significantly more saline control animals had quantifiable free PA levels when compared to animals administered 8 or 32 mg/kg ETI-204 visible from 15 minutes PT.

Furthermore, the intramuscular (IM) route of administration was used to investigate the protective efficacy of ETI-204 when administered at increasing times following exposure to *B. anthracis* spores. Trigger (PA-ECL)-positive monkeys were administered a single IM dose of ETI-204 at 16 mg/kg at 24, 36, or 48 hours PC and evaluated for up to 28 days PC. Survival rates of 93%, 43%, and 29% were obtained after treatments at 24, 36- or 48-hours PC respectively, while 10% of vehicle treated animals

survived until scheduled termination. As expected, as the time between challenge and IM treatment with ETI-204 increased, the survival probability decreased. For animals treated at 36 or 48 hrs PC, the probability of survival significantly decreased in animals with higher PTT PA-ELISA and bacteraemia levels. Complete resolution of bacteraemia occurred by 7 days PC for all monkeys that survived to scheduled termination. All terminal specimens (blood collected at euthanasia or after animal was found dead) were positive. At start of treatment, free-PA serum levels were as high as in the control in the 48-h group, intermediate in the 36 h group and low in the 24 h group. 24 h post treatment and throughout the study, all ETI-treated groups showed a low PA level as compared to the control. The time of IM administration did not appear to affect ETI-204 PK profiles. These results indicate that ETI-204 is efficacious when administered IM 24 hrs post challenge. When intervention was delayed, the time between exposure and intervention appears critical and inversely proportional to the chance for survival.

To examine whether the anti-PA mAb obiltoxaximab would interfere with the development of a protective anti-PA immune response, animals surviving the challenge with *B. anthracis* were tested for the presence of endogenous anti-PA antibodies. Anti-PA antibodies were detected by ELISA methods that detect both obiltoxaximab and rabbit IgG due to the use of detection reagents that were not species-specific. Thus, these methods can detect endogenous anti-PA antibodies only when levels of obiltoxaximab have sufficiently cleared. Due to the long half-life of obiltoxaximab in cynomolgus monkeys, the development of an anti-PA immune response upon-challenge with *B. anthracis* under treatment with obiltoxaximab was assessed in rabbits only; this is acceptable.

In the rabbit studies discussed by the applicant, treatment was administered via different routes of administration (IV, IM), included obiltoxaximab monotherapy or combination treatment with levofloxacin; treatment time ranged from 35 min prior to challenge up to 72 hrs post-challenge. One study also assessed the development of anti-PA antibodies upon re-challenge; i.e. rabbits surviving an initial *B. anthracis* challenge were re-challenged 9 months later.

In general, serum samples taken prior to *B. anthracis* exposure were negative for anti-PA antibodies while animals surviving the *B. anthracis* challenge had detectable anti-PA antibody levels. In studies where obiltoxaximab was compared to levofloxacin treatment, levels of anti-PA antibodies were not different in the different treatment groups. Results from the re-challenge study further show that the anti-PA antibody response in rabbits surviving an anthrax challenge was long-lasting. Together, these studies demonstrate that the presence of obiltoxaximab (i.e. an anti-PA antibody) does not prevent development of an immune response to PA.

Secondary pharmacodynamic studies

Because ETI-204 is a mAb with no endogenous target(s), no secondary PD studies were conducted.

Safety pharmacology programme

ETI-204 was evaluated in a set of GLP-compliant safety pharmacology studies including assessment of neurobehavioral effects in a study in infected monkeys and two cardiovascular (CV) studies in monkeys. Respiratory safety was assessed in the repeated-dose toxicity studies.

CNS effects were studied in a cynomolgus trigger-to-treat inhalation anthrax study (also assessed in the primary PD section). The animals were administered a single IV bolus of ETI-204 at 0 (0.9% NaCl), 4 or 16 mg/kg. Neurobehavioral examinations were performed on all monkeys pre-test (Day -6) and on all surviving monkeys on Days 28 (interim sacrifice) and 56 PC (terminal sacrifice). A total of 19/48 underwent the PC neurological assessment (at start n=8/sex /group). There were no treatment-related

effects of ETI-204 on any of the neurological assessment parameters. Out of 19 performed neurological assessments PC, 12 were normal for all evaluated parameters. In 6 animals, the only reported finding was a decreased range of motion in thoracic limbs, which was considered an incidental finding with a multi-factorial aetiology in captive non-human primates.

Cardiovascular effects were studied in cynomolgus monkeys (n=12/sex). ETI-204 was administered by a single IV slow bolus injection (3-5 min) at 5 mg/kg or a single IM injection at 5 or 10 mg/kg. A vehicle control group was not included in the study design. Peripheral blood pressure and electrocardiograms (ECG) were recorded for anesthetised monkeys pre-test (Day -1) and 2, 4, and 24 hours post-treatment. There were no biologically significant treatment-related effects on blood pressure or ECG parameters (PR interval, QT interval, QRS duration, and heart rate) after IV or IM dosing of ETI-204. Biologically significant prolongation of corrected QT (QTc) was not observed at any dose or interval. Transient elevations in both systolic and diastolic pressure values were observed at 2 and 4 hours post-treatment on Day 1 in all groups administered ETI-204 when compared to pre-test values. There were no differences noted between dosing routes, and no differences between the administered IM doses of 5 and 10 mg/kg. At 24 hours post-treatment, blood pressure values returned to baseline in all groups. Several animals had slightly shorter RR intervals than 0.27 seconds (0.25, 0.26); however, normal RR intervals have not been established.

In order to verify that the observed CV changes in the anesthetised animal study were due to study procedures and not ETI-204, cardiovascular safety was assessed in conscious, telemetered monkeys that had received 2 IV doses of 10 or 30 mg/kg 2 days apart. There were no test article-related effects on heart rate, body temperature, systolic and diastolic blood pressure, MAP, ECG waveform, or activity. No prolongations of QT or QTc, atrioventricular conduction defects, or premature atrial or ventricular complexes were observed in any animal on any study day. Thus, the observations of shorter RR intervals were considered to be of no biological significance.

Although formal respiratory safety studies were not conducted, detailed daily clinical observations including respiratory monitoring were recorded in the conducted non-clinical toxicology studies with ETI-204. There were no treatment-related clinical observations indicative of respiratory effects after repeat dosing of ETI-204 in tested species (including rat, rabbit and monkey).

In summary, no safety pharmacology concerns were identified.

2.3.3. Pharmacokinetics

Bioanalytical Methods

- Quantification of ETI-204

Serum separator tubes (SST) were used for the collection of blood samples for bioanalysis and immunogenicity analysis but not for the preparation of standards and QCs of the respective methods.

Rat

The assay for quantification of ETI-204 in rat serum is a generic assay. Interferences have been noted with PA63 and PA83.

Rabbit & monkey

The concentrations of free ETI-204 were determined in serum or dose formulations using multiple versions of a sandwich immunoassay that captured ETI-204 with PA83 or PA63 and detected it either using goat antihuman IgG conjugated to Horse Radish Peroxidase (HRP) or ruthenylated protein A/G (detecting also endogenous anti-PA antibodies).

- Anti-drug antibody analysis

ADA analysis follows a tiered approach with a screening assay, confirmation assay and titration. Samples are incubated with both biotinylated-ETI-204 and ruthenylated-ETI-204, allowing any anti-ETI-204 antibodies present in the sample to bind both labelled forms of the drug in a bridging complex. The complex is then captured on streptavidin coated MSD microtitre plates via the biotinylated portion of the complex and readout occurs via ECL. The confirmatory assay includes an additional step, where free ETI-204 is added and disrupts the complex, inhibiting binding to the plate resulting in a reduced signal. No neutralizing antibody assay was used in preclinical studies.

- Quantification of PA

Several assays were developed to quantify free PA63 and/or PA83 in serum of rabbits and monkeys. Early methods had issues with specificity. The later assays using ETI-204 as the solid-phase immobilised capture antibody, followed by either goat anti-PA antisera, and horseradish peroxidase (HRP)-conjugated anti-gamma chain secondary antibody or an HRP-conjugated anti-PA antibody for detection. The assays were able to quantify either PA83 or PA63, but not PA20 and were validated for use in rabbit and monkey serum, respectively. The assays do not tolerate ETI-204 and can only be used for samples before ETI-204 treatment.

Single-dose pharmacokinetics (healthy animals)

The PK of obiltoxaximab were evaluated in single-dose PK studies in rabbits and cynomolgus after IV or IM administration. Toxicokinetics of obiltoxaximab following repeated IV administrations were determined in rats and cynomolgus; as well as in pregnant rabbits as part of the embryofoetal development studies (see below). Thus, the studies reflect the PK of obiltoxaximab when administered according to the proposed clinical route of administration.

After IV administration, ETI-204 concentrations generally declined in a bi- or multi-exponential manner in rabbit and monkey serum, and in rabbit plasma. After IV or IM administration, the terminal $t_{1/2}$ ranged from 2.59 to 5.75 days (IV) and 1.4 to 8.14 days (IM) in rabbits, and 9.20 to 13.9 days (IV) and 6.57 to 12.0 days (IM) in monkeys. There were no gender-related differences in PK noted in any species, and increasing doses produced approximately dose-proportional increases in systemic exposure in all species tested. After a single IM administration, the time to the maximum concentration (T_{max}) ranged from 1.3 to 3.61 days in rabbits and 0.917 to 2 days in monkeys. Absolute bioavailability in rabbits after a single IM administration of ETI-204 was approximately 100% after a 10 mg/kg dose. In a single monkey study, the absolute bioavailability after a single IM dose of ETI-204 at 10 mg/kg was 73%. The volume of distribution of ETI-204 after IV administration extended past the total plasma volume in rats, rabbits, and monkeys, suggesting limited distribution outside of the vascular space. CL of ETI-204 from plasma was limited, representing <1.0% of hepatic and renal plasma flows in rats, rabbits, and monkeys.

Immunogenicity

The potential for development of anti-therapeutic antibodies (ATAs) to ETI-204 was investigated in rabbits and monkeys. In rabbits, 7 total serum samples across 4 studies were confirmed positive for antibodies to ETI-204 with a titre range of 1:5 to 1:125, which had no effect on ETI-204 concentration or PK parameters. In monkeys, 4/6 serum samples were confirmed positive in 1 study (AP116) where only samples from animals in the 10 mg/kg IM dosing group were analysed; however, a second IM study (AP118) in monkeys at 3, 10, and 30 mg/kg where all animals administered ETI-204 were evaluated 5 total confirmed positive samples were identified. The titre range in monkeys across both studies was 1:40 to 1:2560. The development of anti-ETI-204 antibodies may have resulted in decreased exposure in animals at later timepoints in the 10 mg/kg dosing group in study AP116; however, no concurrent decrease in ETI-204 exposure was noted in animals positive for antibodies to

ETI-204 in study AP118, suggesting that the antibodies did not enhance the elimination of ETI-204. However, the number of ADA positive animals and the levels of antibody titre may have been underestimated due to shortcomings in the analysis method.

Distribution, metabolism and excretion

It has been amply demonstrated that the disposition of mAbs generally involves distribution beyond the vascular space with potential uptake into tissues, and catabolism by proteases to small peptides and amino acids which are subsequently incorporated into the endogenous pool or excreted. Therefore, the lack of formal distribution, metabolism, and excretion studies is endorsed.

No specific non-clinical PK drug interaction studies were conducted. This is acceptable, since obiltoxaximab as a mAb is not metabolised via CYP450 enzymes.

2.3.4. Toxicology

The toxicity of obiltoxaximab was assessed in short-term studies in healthy rats and cynomolgus monkeys. These species were selected based on results from tissue cross-reactivity studies, which is considered acceptable, given that obiltoxaximab does not recognise an endogenous target. The reproductive and developmental toxicity was assessed in rabbits. In addition, local tolerance at the IM injection site was assessed as part of the efficacy studies in anthrax-challenged rabbits and cynomolgus monkeys. Neuropathology was evaluated primarily in anthrax-challenged rabbits and cynomolgus monkeys. Taken together, the obiltoxaximab non-clinical safety programme is considered adequate for a mAb, targeting a foreign antigen.

Repeat dose toxicity

The toxicology of ETI-204 has been investigated in repeat-dose toxicity studies in rats and monkeys for up to 14 and 17 days, respectively, to support clinical administration of a single dose of ETI-204 via IV infusion or IM injection.

In the 7-day rat study the only observations were minimal changes in several clinical pathology parameters attributable to individual animal variability and of no toxicologic relevance.

The 14-day pilot repeat-dose toxicity study in Sprague-Dawley rats was conducted to aid in dose selection for the pivotal 14-day repeat-dose study, and also to identify the MTD of ETI-204 administered to healthy (i.e., unexposed to anthrax spores) rats. No adverse effects were observed at all at doses up to 100 mg/kg/dose, and consequently an MTD was not identified. With this result it is questioned why the applicant selected a high dose of only 30 mg/kg/dose in the definitive 14-day repeat-dose study, where again no effects at all were identified.

In the definitive repeat-dose IV toxicology study in rats, there were no gender-related differences in TK parameters, and increases in systemic exposure were approximately dose-proportional. The TK parameters of obiltoxaximab pilot batch were compared to obiltoxaximab commercial batch at the high dose of 30 mg/kg/dose. The only identifiable TK difference in test article from the two batches was a higher maximum concentration (C_{max}) in the material from the pilot batch compared to the material from the commercial batch in both males (1000 versus 667 µg/mL) and females (1140 versus 697 µg/mL), respectively. This was attributed to the high variability in mean C_{max} for the commercial batch at that timepoint (%CV = 62; 0.25 hours postdose). Mean serum concentrations of pilot and commercial obiltoxaximab batches at 4 hours following the first dose were comparable (672 versus 793 µg/mL, respectively). The mean $t_{1/2}$ was similar between dose groups and ranged from 2.46 to 3.57 days. The mean T_{max} was 15 minutes, except in females at 30 mg/kg/dose (commercial batch),

where the T_{max} was 4 hours. Mean V_z (volume of distribution in the terminal phase) values did not vary with dose and ranged from 35.1 to 76.5 mL/kg, which is larger than the total plasma volume in rats, indicating that ETI-204 may have distributed out of the vascular space. Additionally, mean CL values did not vary with dose and ranged from 12.4 to 15.4 mL/day/kg, which represents <0.1% of hepatic and renal plasma flows in rats.

The toxicity of ETI-204 was also investigated in cynomolgus monkeys at doses up to and including 30 mg/kg/dose administered via two 60-minute IV infusions with 8 days between doses. Also, in this study toxicity/adverse effects were not observed at the high dose of 30 mg/kg/dose. It was concluded that IV infusion of obiltoxaximab at doses up to and including 30 mg/kg did not cause any toxicological effects and was not associated with any adverse changes in cardiovascular function in young adult male or female cynomolgus monkeys.

Serum concentrations of obiltoxaximab in the cynomolgus following IV administration generally declined with time in a bi-exponential or multi-exponential manner and measurable concentrations persisted through the last sampling timepoint. Observed increases in systemic exposure were approximately dose-proportional after the first and second dose, and values generally increased after the second dose. Gender-related differences in exposure were limited to 30 mg/kg/dose after the first dose, where male values were approximately 40% higher than females. The $t_{1/2}$ ranged from approximately 6 to 9 days, and mean values were somewhat longer after the first dose. Observed V_z ranged from 58.2 to 76.9 mL/kg after the first dose, which is greater than the total plasma volume in monkeys, indicating that obiltoxaximab may have distributed slightly beyond the vascular space. CL was very low, ranging from 5.088 to 8.808 mL/day/kg, which represents approximately 0.01 to 0.02% of monkey hepatic plasma flow and 0.02 to 0.04% of monkey renal plasma flow.

The applicant concludes that animal exposures to obiltoxaximab at the NOAEL or NOEL in the repeat-dose toxicity studies and embryofoetal development toxicity studies provide adequate safety margins relative to the exposure to a 16 mg/kg dose in humans. It is not agreed that exposure margins were achieved since the exposure at the high dose of 30 mg/kg in the pivotal repeat-dose toxicity studies in the rat and in the monkey is approximately the same as the exposure in humans at the clinical dose based on comparison of area under the concentration versus time curve (AUC). In the embryofoetal developmental study in the rabbit the exposure in the high dose was 2x clinical exposure.

It is however recognised that obiltoxaximab is a mAb directed at a component of the anthrax toxin and without an endogenous target in either humans or laboratory animals. Therefore, toxicity related to exaggerated pharmacology is not expected. As pointed out in the ICH S6 addendum, one short-term study in one species can be considered; no additional toxicity studies, including reproductive toxicity, studies are appropriate. As expected, there were no toxicity findings in any of the conducted studies and the lack of an exposure margin to the highest recommended clinical exposure is accepted.

Genotoxicity and carcinogenicity

No genotoxicity or carcinogenicity studies have been conducted with ETI-204. This is in accordance with ICH S6 guidance based on the nature of the substance being a mAb and no direct or indirect DNA interactions are anticipated. In addition, carcinogenicity studies are not required in support of a single-dose clinical regimen.

Reproductive and developmental toxicity

ETI-204 was evaluated for embryofoetal development in time-mated NZW rabbits administered vehicle or ETI-204 at 16 or 32 mg/kg/dose (30/group) via slow IV bolus on GD 6, 10, 13, and 17. The

frequency of dosing was based on an approximate $t_{1/2}$ of 4 days in rabbits following IV administration. No maternal toxicity or embryofoetal toxicity was observed at any dose level tested. The exposure margin to the human clinical exposure (AUC) in the high dose is 2-fold. Placental transfer was not evaluated.

Toxicity of obiltoxaximab has not been assessed in juvenile animals. This is acceptable and in line with the approved Paediatric Investigation Plan (P/0018/2018), as there are no concerns with regard to paediatric patients which would warrant a juvenile toxicity study.

Local Tolerance

Several nonclinical studies evaluated local tolerance at the injection site in rabbits and monkeys after IM injections. There were no test article-related histopathological findings observed at the injection site neither in healthy rabbits nor in inhalation anthrax challenged rabbits following IM administration. In anthrax infected cynomolgus monkeys, minimal chronic active inflammatory lesions were noted at the injection site and were occasionally associated with myofiber necrosis. Microscopic lesions following IM administration were limited to minimal to mild chronic inflammation confined to the dermis, subcutaneous tissue and/or muscle, with occasional chronic active inflammation and/or bacteria seen at the injection site of moribund or found dead animals. No specific studies investigating local tolerance following IV infusion was presented. The local tolerance following IV infusion was studied in the clinic.

Other toxicity studies

Neuropathology

Neuropathological examination of brain tissues was included in several efficacy studies in anthrax-challenged rabbits and cynomolgus because raxibacumab, another anti-anthrax toxin mAb, was associated with a greater incidence and/or severity of CNS lesions in anthrax animal models. As control, neuropathology was also evaluated in one toxicity study in non-challenged rabbits.

Evaluation of neuropathology after repeated obiltoxaximab IV bolus administrations (32 mg/kg/dose x 4) in uninfected pregnant rabbits revealed no treatment-related microscopic brain lesions indicating that obiltoxaximab per se does not mediate neuropathology. Also, evaluation of neuropathology in anthrax-exposed rabbits and monkeys revealed no treatment-related microscopic brain lesions in animals surviving a single administration of obiltoxaximab at doses up to 16 mg/kg in rabbits (IV and IM bolus) and up to 32 mg/kg in monkeys (IV bolus).

In those animals that succumbed to the infection, an inflammatory response was seen in meninges and brain, including haemorrhage, vasculitis and tissue necrosis. The inflammatory response was more frequent and/or more severe in obiltoxaximab-treated animals than in non-survivors administered placebo. This effect was more pronounced in monkeys but was also present in rabbits. The applicant was asked to comment on the relation of the incidence and/or severity of the neuropathological findings in obiltoxaximab-treatment takin into consideration the underlying disease and the moment of death or euthanasia of the animal. In response, the applicant indicated that link of incidence/severity of CNS lesions with time to death/euthanasia was not established for individual animals. However, all animals succumbed to the infection within 10 days of challenge, while survivors lived to the scheduled necropsies (day 28 or 56 post-challenge). The key to the development of biologically significant CNS lesions was found to be the presence of extravascular bacteria at the time of death/moribund sacrifice indicating bacterial dissemination to the CNS. Thus, the histopathological changes in the CNS of anthrax-challenged non-survivors were in general consistent with haemorrhagic meningoencephalitis. Exacerbated inflammation observed in the meninges of obiltoxaximab-treated non-survivors

(compared to untreated non-survivors) may have resulted from more effective immune activation due to systemic neutralisation of toxin. This argumentation is in line with that in found literature for other anti-PA antibodies and accepted.

Tissue cross-reactivity

Several tissue cross-reactivity studies with obiltoxaximab were performed with obiltoxaximab in human tissue as well as rat and cynomolgus tissues. In all species tested, obiltoxaximab stained vascular smooth muscle cells and epithelial cells in several tissues. In general, the staining observed was cytoplasmic granules and/or filaments. Cytoplasmic targets are not accessible to a mAb *in vivo*. This is confirmed by results from an ex vivo analysis of tissues from obiltoxaximab-treated rats. There was no membrane or intracellular staining in any organ or tissue; but intravascular protein staining was observed, in all evaluated organs, attributed to obiltoxaximab present in serum. Given the intracellular nature of the immunohistochemistry staining and the lack of such staining in tissues from rats treated with obiltoxaximab, the TCR results are not considered toxicologically relevant.

In conclusion, obiltoxaximab has been evaluated in an ICH-compliant (S6, M3) nonclinical development toxicology program, including repeat-dose toxicity studies in the rat and the cynomolgus monkey, embryofoetal toxicity in the rabbit, and local tolerance at the injection site. As expected for a mAb without endogenous targets no specific toxicity of concern was observed in healthy animals and no further toxicity studies are required, even though exposure margins were not achieved.

2.3.5. Ecotoxicity/environmental risk assessment

Obiltoxaximab is a mAb consisting of natural amino acids and is therefore not expected to pose a risk to the environment.

To further justify the absence of ERA studies, the predicted environmental concentration in surface water was calculated based on the amount of obiltoxaximab likely to be used for the treatment of inhalation anthrax. This calculation resulted in a PECsw of 0.00064 µg/L, i.e. a value that is significantly <0.01 µg/L, the trigger value for conducting a Phase II assessment.

2.3.6. Discussion on non-clinical aspects

Pharmacology

The applicant has presented non-clinical *in vitro* data to characterise the binding and *in vitro* function of obiltoxaximab. These data consist of a basic characterisation of binding affinity, competitive inhibition of PA binding to its receptor, and neutralisation of the toxin cytotoxicity in a cell-based assay.

In vivo activity of obiltoxaximab was assessed in animal models of disease, anthrax-challenged rabbits and cynomolgus monkeys. An extensive set of studies was performed, which demonstrate the intended mode of action, i.e. rapid and long-lasting neutralisation of the toxin component PA in anthrax challenged animals. In addition, it was demonstrated, that treatment with an anti-PA mAb does not prevent the development of an endogenous immune response against PA in the anthrax-challenged animals. Due to limitations in the assay that detects endogenous PA, this analysis could not be performed in cynomolgus. This can be accepted.

The combined antibiotic and ETI-204 study in the rabbit did not demonstrate an ETI-204 add-on effect. However, since the levofloxacin treatment was effective when administrated alone it was difficult to show an additive effect. The parallel effects on the bacteraemia, as shown in this study, do not support an additive effect in this model. The importance of the slightly more reduced PA levels after the

combined treatment, remains unclear since it was not reflected in parallel effects on bacteraemia or survival in this study.

In conclusion of the PD studies, cynomolgus as well as the rabbit model appears suitable for testing of ETI-204 therapeutic potential on survival if treatment is accomplished before bacteraemia becomes too high, which in turn depends on degree of initial infection and time from challenge. The rabbit model and the current experimental design did not allow an investigation on whether an add-on effect to levofloxacin exists.

Because ETI-204 is a mAb with no endogenous target(s), it is acceptable that no secondary PD studies were conducted.

To summarise the safety pharmacology profile, ETI-204 appears as expected from a mAb with no endogenous target. However, the tested doses did not provide any clear margins to the clinical exposure.

Pharmacokinetics

- Quantification of ETI-204

A general issue had been raised regarding the use of serum separator tubes (SST) for the collection of blood samples for bioanalysis and immunogenicity analysis but not for the preparation of standards and QCs of the respective methods, as this may lead to a systematic error. To address this issue, the applicant performed an *in vitro* experiment investigating the recovery of ETI-204 spiked into human blood and processed as per the conventional method, or in SST tubes filled at volumes corresponding to what was used in clinical or non-clinical studies. Results for ETI-204 recovery were consistent between clotting procedures, blood volume and the two tested ETI-204 concentrations, indicating that there is no recovery issue.

Rat

The interferences with PA63 and PA83 have no consequences since no studies in infected rats were conducted. The method is otherwise adequately validated, including ISR and parallelism.

Rabbit & Monkey

Some of the methods for quantification of free ETI-204 were only qualified, while others were validated for the matrix of the species, they were intended for but had some outstanding issues. These were resolved with response to the D120 LoQ.

Protein A/G binds IgGs in a non-species-specific manner, thus not distinguishing between ETI-204 and endogenous anti-PA antibodies, thus caution must be exerted when using data from studies AR010, AR014, AR0315, EFT001 pilot and EFT001 (method TLIAM-0204 & VR TNJR11-010). For studies in healthy animals, results may be considered reliable.

Only addendum 1 of the validation report TNRJ-12-093 was provided, without the initial report. The missing report was provided and confirmed the previous conclusion on the method.

The applicant was asked to justify the lack of parallelism data in rabbit and monkey and to discuss the consequences and implications of the lack of such data. The applicant was also asked to complete the validation package by providing parallelism data in all species the method was used in and in both healthy and infected animals, provided samples are still available. The applicant's opinion, that parallelism is not an issue for large molecules is not agreed with. However, since rabbit and monkey samples are no longer available to perform the parallelism investigations, and following 3Rs principles, the lack of parallelism data in rabbit and monkey may be accepted, if all other bioanalysis issues are deemed resolved.

In GCL-250, PA83 was used for capture, whereas PA63 was used in all previous assays. The applicant was asked to discuss the impact of the change of capture reagent on the assay results and their use. The applicant provided data showing that PA63 and PA83 are not directly interchangeable. Nevertheless, there is no direct impact on the PK modelling, since only rabbit data was generated using PA63 and data from the two non-clinical species were asked to be separated.

- Anti-drug antibody analysis

Both the rabbit and the monkey ADA assay were validated but tolerate only low concentrations of ETI-204, leading to a risk of false negative results. However, since obiltoxaximab is intended for single dosing only, these issues will not be pursued.

- Quantification of PA

Early generations of the PA assay were not specific, thus their results should be disregarded (studies NIAID 1030, NIAID 1045, AP 201, NIAID 1056, and NIAID 2456). The later PA assay only has a limited utility given the significant interference by ETI-204. Therefore, only results prior to treatment with ETI-204 can be considered reliable.

Toxicology

The toxicity of obiltoxaximab was assessed in healthy rats and cynomolgus monkeys. Selection of these species for the toxicity studies was adequately justified and accepted. The overall toxicity programme consists of short-term repeated-dose toxicity studies in these 2 species, embryofoetal development studies in rabbits, evaluation of local tolerance and tissue cross-reactivity. As such the programme is in line with recommendations of ICH S6(R1) for a mAb that targets a foreign antigen and is considered adequate.

2.3.7. Conclusion on the non-clinical aspects

The CHMP considers that from a non-clinical point of view, no issues remain which would preclude granting of a Marketing Authorisation.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 1 ***Design of Clinical Studies that Included PK Sampling***

Study Number	Design	Treatment Arms (Number of Subjects)
Single-dose		

Study Number	Design	Treatment Arms (Number of Subjects)
AH101 (Part 1)	Single centre, randomised, double-blind, placebo-controlled, sequential, dose-escalation study in healthy adult subjects. Samples collected over 42 days for determination of ETI-204 PK.	• 19 mg ETI-204 (6) • 57 mg ETI-204 (6) • 114 mg ETI-204 (6) • Placebo (6) 90 min IV infusion
AH102	Single centre, randomised, double-blind, placebo-controlled, sequential, dose-escalation study in healthy adult subjects. Samples collected over 70 days for determination of ETI-204 PK.	• 120 mg ETI-204 (12) • 240 mg ETI-204 (12) • 360 mg ETI-204 (12) • Placebo (9) 60 min IV infusion
AH105	Single centre, randomised, double-blind, placebo-controlled, sequential, dose-escalation study in healthy adult subjects. Samples collected over 71 days for determination of ETI-204 PK.	• 4 mg/kg ETI-204 (30) • 8 mg/kg ETI-204 (30) • 16 mg/kg ETI-204 (30) • Placebo (18) 90 min IV infusion
AH104	Multi-centre, double-blind, randomised, placebo-controlled study in healthy adult subjects. Samples collected over 71 days for determination of ETI-204 PK.	• 16 mg/kg ETI-204 (210) • Placebo (70) 90 min IV infusion
Multiple-dose		
AH109	Multi-centre, double-blind, randomised, placebo-controlled study to evaluate the safety, tolerability, PK, and potential immunogenicity of repeat administration (two doses) of IV ETI-204, either 14 or 120 days following the initial dose in healthy adult subjects. Samples collected over 191 days for determination of ETI-204 PK.	Sequence A: • 16 mg/kg ETI-204 Days 1 and 14; Placebo on Day 120 (35) Sequence B: • 16 mg/kg ETI-204 Days 1 and 120; Placebo on Day 14 (35) 90 min IV infusion
Drug-drug interaction		
AH101 (Part 2)	Single centre, randomised, double-blind, placebo-controlled study in healthy adult subjects to evaluate the interaction between ETI-204 and ciprofloxacin. Samples collected over 42 days for determination of ETI-204 PK. Samples collected for 12 hours after the first ciprofloxacin dose and at sequential pre-dose times through Day 14 for determination of ciprofloxacin PK.	• 114 mg ETI-204 with ciprofloxacin (6) • ETI-204 placebo with ciprofloxacin (6) 90 min IV infusion Ciprofloxacin PO 500 mg every 12 hours for 14 days starting on the day of ETI-204 administration.
AH110	Single centre, randomised, open-label, parallel group study in healthy adult subjects. Samples collected over 71 days for determination of ETI-204 PK. Samples collected for 24 hours after the first IV ciprofloxacin dose and last oral ciprofloxacin dose (Day 9) for determination of ciprofloxacin PK.	• 16 mg/kg ETI-204 with ciprofloxacin (20) • 16 mg/kg ETI-204 without ciprofloxacin (20) 90 min IV infusion Ciprofloxacin 400 mg IV immediately after ETI-204 dose on Day 1, followed by 750 mg po every 12 hours from Days 2-8, with a final dose in the morning of Day 9.
• Source: adapted from 2.7.2 table 15		

2.4.2. Pharmacokinetics

Since controlled clinical trials in humans with anthrax are neither ethical nor feasible, clinical pharmacology of obiltoxaximab was evaluated in clinical studies with healthy human adults, as well as

in two well-characterised animal models of inhalational anthrax (NZW rabbits and cynomolgus monkeys), and in healthy Sprague-Dawley and Fischer 344 rats.

Obiltoxaximab is to be administered as a single IV 16 mg/kg infusion over 90 minutes.

Human PK data was collected from 7 clinical trials (AH101, AH102, AH105, AH104, AH110, AH109, and AH106) including also IM mode of administration. Obiltoxaximab was evaluated at different IV dose strength ranging from 19 mg to 16 mg/kg, single and multiple dose (two doses), and with and without concomitant treatment (ciprofloxacin, diphenhydramine).

PK from the individual studies is summarised in Table 2; Table 3 compares healthy human PK parameters to those of the efficacy models, in their healthy and infected versions.

Table 2: ETI-204 PK parameters following IV administration to healthy humans (Mean, CV%)

Study	Dose (mg/kg)	N	C_{max} (µg/mL)	T_{max} (d)	$AUC_{(0\text{-}inf)}$ (µg·d/mL)	$t_{1/2}$ (d)	CL (L/d)	Vd (L)	V_{ss} (L)
AH101[a]	0.23[b]	6	6.1 (16)	NR	49.9 (28)	10.9 (63)	0.404 (25)	5.77 (47)	NR
	0.75[b]	6	19.7 (19)	NR	273 (41)	16.9 (34)	0.228 (25)	5.18 (9.0)	NR
	1.55[b]	6	34.9 (18)	NR	448 (26)	15.3 (22)	0.270 (27)	5.71 (14)	NR
	1.55[b,c]	6	37.9 (18)	0.104 (73)	504 (26)	15.5 (22)	0.252 (27)	5.08 (14)	NR
AH102	1.49[d]	12	39.3 (19)	NR	507 (28)	21.9 (41)	0.252 (24)	7.47 (24)	NR
	3.04[d]	12	89.5 (36)	NR	1090 (21)	20.9 (18)	0.229 (21)	6.95 (32)	NR
	4.69[d]	12	154 (37)	NR	1680 (23)	16.8 (14)	0.225 (23)	5.43 (26)	NR
AH105	4	30	94.0 (21)	0.283 (NR)	1080 (23)	18.2 (4.3)	0.279 (23)	7.08 (18)	NR
	8	30	210 (41)	0.267 (NR)	2390 (22)	20.8 (4.4)	0.270 (24)	7.89 (20)	NR
	16	29	330 (19)	0.259 (NR)	4410 (23)	20.4 (5.0)	0.287 (28)	8.05 (18)	NR
AH104	16	202	400 (23)	0.178 (NR)	5170 (26)	20.2 (26)	0.270 (33)	7.41 (26)	6.34 (24)
AH109	16[e]	35	384 (27)	0.134 (122)	4690 (29)	21.5 (31)	0.300 (35)	8.75 (24)	7.20 (21)
	16[f]	31	402 (33)	0.122 (136)	4400 (18)	18.6 (19)	0.313 (24)	8.33 (29)	7.01 (33)
	32[g]	32	NA[h]	NA[h]	10,300 (25)	22.8 (26)	0.274 (31)	8.69 (31)	NA[h]
AH110	16	20	402 (23)	0.206 (134)	4891 (18)	19.5 (21)	0.268 (22)	7.57 (34)	6.28 (27)
	16[c]	18	397 (16)	0.161 (133)	4990 (19)	19.0 (16)	0.247 (30)	6.59 (20)	5.68 (17)

[a]: Samples analysed with a non-validated assay
[b]: Nominal doses were 19, 57, and 114 mg; mg/kg dose estimated using mean body weight in each group
[c]: Coadministered with ciprofloxacin (500 mg orally twice daily for 14 days in AH101; 400 mg IV single dose followed by 750 mg orally twice daily for 8 days in AH110)
[d]: Nominal doses were 120, 240, and 360 mg; mg/kg dose estimated using mean body weight in each group
[e]: Day 1 dose in Sequence B
[f]: Day 120 dose in Sequence B (not considered definitive)
[g]: Two 16 mg/kg doses in Sequence A (Day 1 and Day 14)
[h]: Not valid for comparison (split dose)
Source: 2.7.2 table 42

Table 3: ETI-204 PK Parameters in Rabbits, Monkeys and Healthy Humans (Mean ± SD)

	Healthy Rabbits	Spore-Challenged Rabbits*	Healthy Monkeys	Spore-Challenged Monkeys *			Healthy Humans
Study	AR010	AR033	AP116	AP204	AP202		AH105, AH104,& AH110[a]
Dose (mg/kg)	10 mg/kg (n = 10)	16 mg/kg (n = 9)	10 mg/kg (n = 6)	16 mg/kg (n = 8)	Pilot batch 16 mg/kg (n = 6)	Commercial batch 16 mg/kg (n = 5)	16 mg/kg (n = 269)
C_{max} (µg/mL)	342 ± 112	391 ± 65.5	292 ± 21.4	459 ± 80.1	336 ± 73.1	292 ± 40.3	392 ± 89.3
$t_{1/2}$ (d)	4.17 ± 2.66[b]	1.60 ± 0.48[c]	12.4 ± 4.03[g]	5.13 ± 2.11	7.17 ± 2.68	5.75 ± 3.15[b]	20.1 ± 5.03[d]
$AUC_{(0\text{-}inf)}$ (µg·d/mL)	1200 ± 243[b]	958 ± 67.1[c]	2520 ± 641[g]	1860 ± 169	2110 ± 345	1830 ± 240[b]	5050 ± 1290[e]
CL (mL/d/kg)	8.72 ± 2.03[b]	16.8 ± 1.18[c]	4.18 ± 1.05[g]	8.66 ± 0.739	7.82 ± 1.64	8.83 ± 1.13[b]	3.43 ± 0.964[e]
V_z (mL/kg)	46.5 ± 24.4[b]	38.0 ± 9.35[c]	71.3 ± 13.2[g]	63.0 ± 22.6	76.3 ± 21.0	69.7 ± 31.6[b]	95.3 ± 24.3[e]
V_{ss} (mL/kg)	53.9 ± 17.3[b]	43.2 ± 7.52[c]	NR	60.3 ± 12.5	56.2 ± 16.4	53.4 ± 16.6[b]	80.1 ± 19.6[f]

* animals that survived only; [a]AH110 includes subjects who received ETI-204 alone (n = 20) and with IV (400 mg single dose) and oral (750 mg twice daily for 8 days) ciprofloxacin (n = 18); [b] n = 8; [c] n = 6; [d] n = 257; [e] n = 255; [f] n = 226; [g] n = 4. Source 2.5 Table 7 & 8

Methods

- Quantification of ETI-204

Serum separator tubes (SST) were used for the collection of blood samples for bioanalysis and immunogenicity analysis but not for the preparation of standards and QCs of the respective methods.

A qualified method was used to analyse samples from study AH101 (first in man).

All other clinical studies were analysed using method GCL-160. The concentrations of free ETI-204 were determined in human serum using a sandwich immunoassay that captured ETI-204 with PA83 and detected it using a monkey adsorbed sheep anti-human IgG HRP conjugate. The method had several issues; however, based on additional data provided, the method can be considered sufficiently validated.

- Anti-drug antibody analysis

Samples for ADA analysis were also collected in SST tubes.

ADA analysis follows a tiered approach with a screening assay, confirmation assay and titration. After a 1:10 MRD, and acid dissociation, followed by neutralisation, ADAs are captured with biotinylated ETI-204. The biotinylated ETI-204/ADA complex is subsequently immobilised on streptavidin coated plates. Following a wash and re-acidification, the ADA solution is transferred to an MSD plate and subsequently neutralised and incubated with ruthenylated ETI-204 for ECL detection.

The assay was validated in two steps using either rabbit anti-ETI-204 antiserum or later affinity purified rabbit anti-ETI-204 IgG as the positive control. ETI-204 is tolerated up to 200 µg/mL.

The confirmatory assay includes an additional step, where free ETI-204 is added and disrupts the complex, inhibiting binding to the plate, thus resulting in a reduced signal.

An assay for neutralizing antibodies was developed but was only used in study AH105 due to issues of interference with ETI-204.

- **Population pharmacokinetic analysis**

The objectives of the Population Pharmacokinetic Analysis Modelling of ETI-204 in Healthy and Anthrax-Infected Subjects were:

- To characterise ETI-204 population PK in healthy and infected NZW rabbits.
- To characterise ETI-204 population PK in healthy and infected cynomolgus monkeys.
- To characterise ETI-204 population PK in healthy humans.
- Bridge infected cynomolgus monkey PK model to humans using the healthy human model for the prediction of ETI-204 exposures in humans infected with inhalational anthrax.
- Perform population PK simulations using the bridged human model to derive ETI-204 human exposures for the proposed preclinical dose.

Population analyses were conducted using the nonlinear mixed effects modelling (NONMEM) software.

Data

There were ten studies used for population PK modelling consisting of one in healthy rabbits (AR010), one in anthrax infected rabbits (AR033), one in healthy cynomolgus monkeys (AP116), four in infected cynomolgus monkeys (AP201, AP202, AP203, AP204) and three in healthy humans (AH102, AH104, AH105). As the model in healthy and infected rabbits (AR010 and AR033) is not used for prediction of exposure in infected humans or dose selection, the focus in this report is on the Cynomolgus Monkey and Healthy Human models.

After reducing the population PK datasets, 96 NZW rabbits with 791 observation data records (ETI-204 serum concentrations) remained for the combined healthy and infected rabbit data set. For the cynomolgus monkey PK dataset, 150 monkeys with 929 observations remained. For the human population PK data set, 303 subjects contributed 2830 observations. Body weight was the only covariate considered for preclinical studies. For human studies, bodyweight, age and race (white vs. non-white) were used for covariate modelling. The number of excluded samples from each study was not presented.

Covariate modelling

The primary covariates of interest had been predefined for this analysis in the Modelling and Simulation Plan. They included age, body weight, and race (white vs. non-white) as predictors of variability in ETI-204 PK. A covariate modelling approach emphasizing parameter estimation rather than stepwise hypothesis testing was implemented for this population PK analysis.

Model for Healthy and Infected Cynomolgus Monkeys

The final monkey structural model consisted of a two-compartment model parameterised in terms of CL, Vc, Vp and Q. Absorption kinetics following IM administration were described by ka and F1. TMDD in infected cynomolgus monkeys was approximated via parallel nonlinear elimination for infected animals only, parameterised in terms of Vmax and Km. Volume and clearance terms were allometrically scaled (fixed allometric exponents of 0.75 for CL and Q, and 1 for Vc and Vp), normalised to a reference weight of 2.88 kg.

The population model to describe ETI-204 PK in healthy and infected cynomolgus monkeys is shown below:

$$CL = \theta_{CL} \cdot \left(\frac{WT_i(\text{kg})}{2.88(\text{kg})}\right)^{0.75} \cdot \exp^{\eta_{CL}} \qquad healthy$$

$$CL = \theta_{CL} \cdot \left(\frac{WT_i(\text{kg})}{2.88(\text{kg})}\right)^{0.75} \cdot \exp^{\eta_{CL}} + \left(\frac{V_{\max} \cdot \exp^{\eta_{Vmax}} \cdot C_p}{K_m + C_p}\right) \qquad infected$$

$$V_c = \theta_{Vc} \cdot \left(\frac{WT_i(\text{kg})}{2.88(\text{kg})}\right)^{1.0} \cdot \exp^{\eta_{Vc}}$$

$$V_p = \theta_{Vp} \cdot \left(\frac{WT_i(\text{kg})}{2.88(\text{kg})}\right)^{1.0}$$

$$Q = \theta_Q \cdot \left(\frac{WT_i(\text{kg})}{2.88(\text{kg})}\right)^{0.75}$$

$$k_a = \theta_{ka}$$

$$F1 = \theta_{F1}$$

Parameter estimates from the final model are presented in Table 4. Shrinkage estimates were 26%, 14%, 26%, and 49% for CL, Vc, Vp, and Vmax respectively.

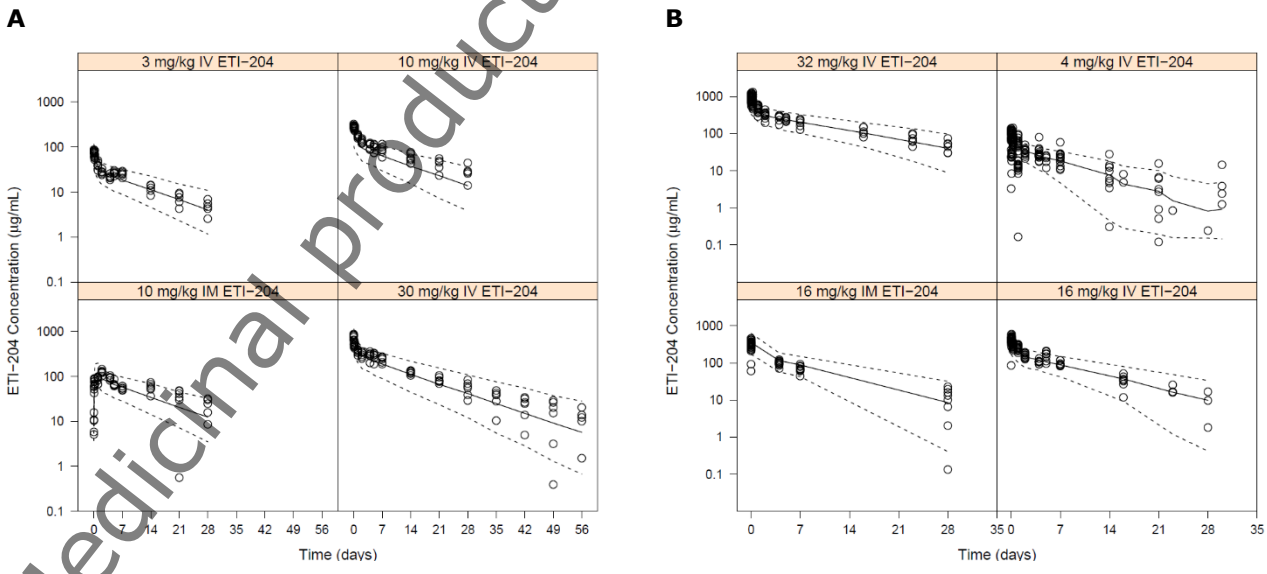
Table 4. Cynomolgus Monkey Population PK Modelling Parameter Estimates and %RSE from *Population PK Model, 95% CI from Bootstrap*

Description	Model	Estimate	%RSE	95% CI	Variability
clearance	$CL \sim \theta_1 \cdot (WT/2.88)^{0.75} \cdot e^{\eta_1}$	0.0191 L/d	5.1	(0.0162, 0.0223)	
central volume of distribution	$V_c \sim \theta_2 \cdot (WT/2.88)^{1.0} \cdot e^{\eta_2}$	0.134 L	3.39	(0.127, 0.141)	
peripheral volume of distribution	$V_p \sim \theta_3 \cdot (WT/2.88)^{1.0} \cdot e^{\eta_3}$	0.123 L	3.89	(0.109, 0.138)	
intercompartmental clearance	$Q \sim \theta_4 \cdot (WT/2.88)^{0.75}$	0.089 L/d	11.2	(0.0785, 0.103)	
maximum velocity for nonlinear clearance	$V_{max} \sim \theta_5 \cdot e^{\eta_4}$	0.275 $ug/mL/d$	17.6	(0.0671, 0.903)	
ETI-204 concentration to reach half Vmax	$K_m \sim \theta_6$	3.21 ug/mL	20.5	(1.12, 35.6)	
first-order absorption rate	$k_a \sim \theta_7$	3.89 d^{-1}	12.8	(2.96, 5.08)	
bioavailability	$F \sim \theta_8$	0.895	18.6	(0.777, 1.02)	
interindividual variability of CL	$IIV_{CL} \sim \Omega_{1.1}$	0.111	30.1	(0.0323, 0.198)	%CV = 34.3
interindividual CL-Vc covariance	$cov_{CL,Vc} \sim \Omega_{2.1}$	0.0497	35.7	(0.0121, 0.085)	CORR = 0.626
interindividual variability of Vc	$IIV_{Vc} \sim \Omega_{2.2}$	0.0569	20.5	(0.0311, 0.0885)	%CV = 24.2
interindividual CL-Vp covariance	$cov_{CL,Vp} \sim \Omega_{3.1}$	-0.147	72.4	(-0.326, 0.00372)	CORR = -0.471
interindividual Vc-Vp covariance	$cov_{Vc,Vp} \sim \Omega_{3.2}$	-0.0436	125	(-0.163, 0.0666)	CORR = -0.195
interindividual variability of Vmax	$IIV_{Vmax} \sim \Omega_{3.3}$	0.883	55.9	(0.227, 2.16)	%CV = 119
proportional error	$err_{prop} \sim \Sigma_{1.1}$	0.0779	3.44	(0.0635, 0.0963)	%CV = 27.9

RSE: relative standard error
CI: Confidence Interval
CV: Coefficient of Variation
CORR: correlation

Results from the VPC for the healthy and infected cynomolgus monkeys demonstrate good agreement between simulated and observed ETI-204 concentrations (Figure 1), although the median prediction for 10 mg/kg dose regimens somewhat under-predicts the central tendency of the observed data.

Figure 1. Model Evaluation Results: Population PK Model Visual Predictive Check for Healthy (A) and Infected (B) Cynomolgus Monkeys. Solid line is the median ETI-204 concentration from 200 simulated trials, dashed lines are the simulated 90% prediction interval, and open circles are observed values.



Model for Healthy Subjects

The initial human population PK structural model consisted of a two-compartment model with first-order elimination, parameterised in terms of CL, Vc, Vp and Q. No absorption model was necessary, since all subjects received IV ETI-204 administration. A nonlinear clearance component was not

included, since there was no need to account for target-mediated drug disposition (TMDD) in uninfected humans. Inter-subject random effects were included for all structural model parameters. Allometric exponents on clearances and volumes of distributions were estimated.

Once the population PK model was developed for healthy humans, it was necessary to bridge the cynomolgus monkey model to humans to predict likely exposures in healthy and infected humans.

Population PK simulations for healthy and projected infected paediatric subjects

A bridged human population PK model that approximates TMDD for infected subjects was used to simulate healthy infected human exposures in adult and paediatric subjects. ETI-204 is eliminated by non-specific proteolysis, and a minimal effect of maturation on ETI-204 CL is expected. Therefore, no maturation effect was included in the simulation model. Although paediatric simulations would typically be performed using an adult population PK model that is allometrically scaled, simulations for this study were performed using the estimated adult weight effect exponents. Simulations were performed for 500 typical human subjects (weight = 75 kg) administered 16 mg/kg ETI-204 for both healthy and infected populations. Although 70 kg was the reference value for human PK modelling, a weight of 75 kg was chosen for the simulations since this was closer to the median human weight for all studies. These simulation results were compared to those performed for infected monkeys (3 kg) administered 16 mg/kg.

Absorption

Since ETI-204 is given IV, bioavailability is 100% by definition. Since ETI-204 is given IV, no food effect is expected.

During the ETI-204 nonclinical and clinical development program, changes to the manufacturing process for ETI-204 bulk drug substance (BDS) and drug product (DP) were introduced to prepare for large-scale production.

With respect to human studies, AH104, AH109, AH110, and AH106 were conducted with the commercial formulation of ETI-204, while AH101, AH102, and AH105 were conducted with investigational material.

No comparative PK study has been designed to demonstrate equivalence between the different formulations with regard to key PK parameters, however comparison within and across studies revealed overall comparability:

TOX001:

Data from Study TOX001 indicate that overall, ETI-204 exposures (commercial formulation) over the first three days post-dose were lower (19%) compared to the pilot formulation, which might be attributed to imprecise measurement of the first sampling points after IV bolus injection.

AP202:

Similarly, data from cynomolgus monkey following 16 mg/kg IV SD in different groups within study AP202 indicate comparative exposure following the pilot or commercial formulation in this species.

Comparison of 16 mg/kg IV SD (AH 105, AH104) in human (pilot vs. commercial formulations) and comparison of two fix doses, 114 mg (~ 1.55 mg/kg) and 120 mg (~1.49 mg/kg) pilot formulation, did not reveal any major differences in PK that could be attributed to the different formulations.

PK data following the two most recent formulations (pilot and commercial) have been included in population PK analyses of human and animal data but has not been tested as a covariate

Distribution

No protein binding studies were conducted.

In humans, mean Vd (V_z) values ranged across studies from 5.08 to 8.75 L, and mean V_{ss} values ranged from 5.68 to 7.20 L. Overall mean (± SD) V_z and V_{ss} values were 7.0 (± 1.2) and 6.5 (± 0.61) L, respectively, across studies (Table 2).

Elimination

No metabolism and excretion studies have been conducted with ETI-204, since degradation by proteolysis is expected.

Following single IV administration of ETI-204 16 mg/kg in healthy subjects, the mean C_{max} and area under the concentration-time curve from time zero to infinity [$AUC_{(0\text{-inf})}$] were 400 ± 91.2 mcg/mL and 5170 ± 1360 mcg·day/mL, respectively.

Mean ETI-204 $t_{1/2}$ values ranged from approximately 15 to 23 days at doses of approximately 0.75 mg/kg to 32 mg/kg (Table 2).

Mean CL values ranged across studies from 0.225 to 0.313 L/d at doses of approximately 0.75 mg/kg to 32 mg/kg, with an overall mean (± SD) in this dose range of 0.268 (± 0.024) L/d across studies (Table 2).

Affinity of ETI-204 to FcRn has not been evaluated.

Dose proportionality and time dependencies

The PK of ETI-204 is linear over the dose range of 1.49 mg/kg to 16 mg/kg following single IV administration in healthy subjects (excluding study AH101 that was measured using a non-validated method). Mean C_{max} and $AUC_{(0\text{-inf})}$ at 16 mg/kg values ranged from 330 to 402 µg/mL and 4400 to 5170 µg·d/mL, respectively.

Exposure in healthy human subjects following single dose IV infusion over 90 minutes increased with dose; however dose-proportionality could not be proven by statistical analyses in most of the human dose-ranging PK studies (AH102, AH105, AH106).

The characterisation of PK following 16 mg/kg IV (SD infusion over 90 minutes) was consistent across the studies (AH105, AH104, AH109, AH110).

The ETI-204 $AUC_{(0\text{-inf})}$ following two 16 mg/kg doses 2 weeks apart was approximately twice that after a single 16 mg/kg dose on day 1 or day 120 (study AH109). No significant differences in mean estimates of C_{max}, $AUC_{(0\text{-inf})}$, CL, or half-life of ETI-204 between the 2 doses administered ≥4 months apart was observed (see Table 2).

The incidence of ADA occurrence in humans was low. Approximately 3% (14/470) of subjects were positive for treatment-emergent ADA after a single IV dose. A total of 4/65 human subjects were positive for treatment-emergent ADA in those who received two IV ETI-204 doses. Titres ranged from 1:20 to 1:320 and from 1:20 to 1:80 after single and repeat doses, respectively. After IV administration, the presence of ADA had no discernible effect on ETI-204 PK. There were no adverse events (AEs) coincident with the development of ADA in human subjects.

No accumulation of ETI-204 was seen after multiple dose administration. ETI-204 is intended for single dose administration only, thus time-dependency has a low relevance.

Intra – and intervariability

Inter-individual variability (CV%) in CL/F, Vc and Vp was estimated to be 25.4%, 19.8% and 27.8% respectively. As ETI-204 is going to be administered as a single dose, intra-individual variability was not reported.

Pharmacokinetics in target population

- **Pharmacokinetics in healthy subjects**

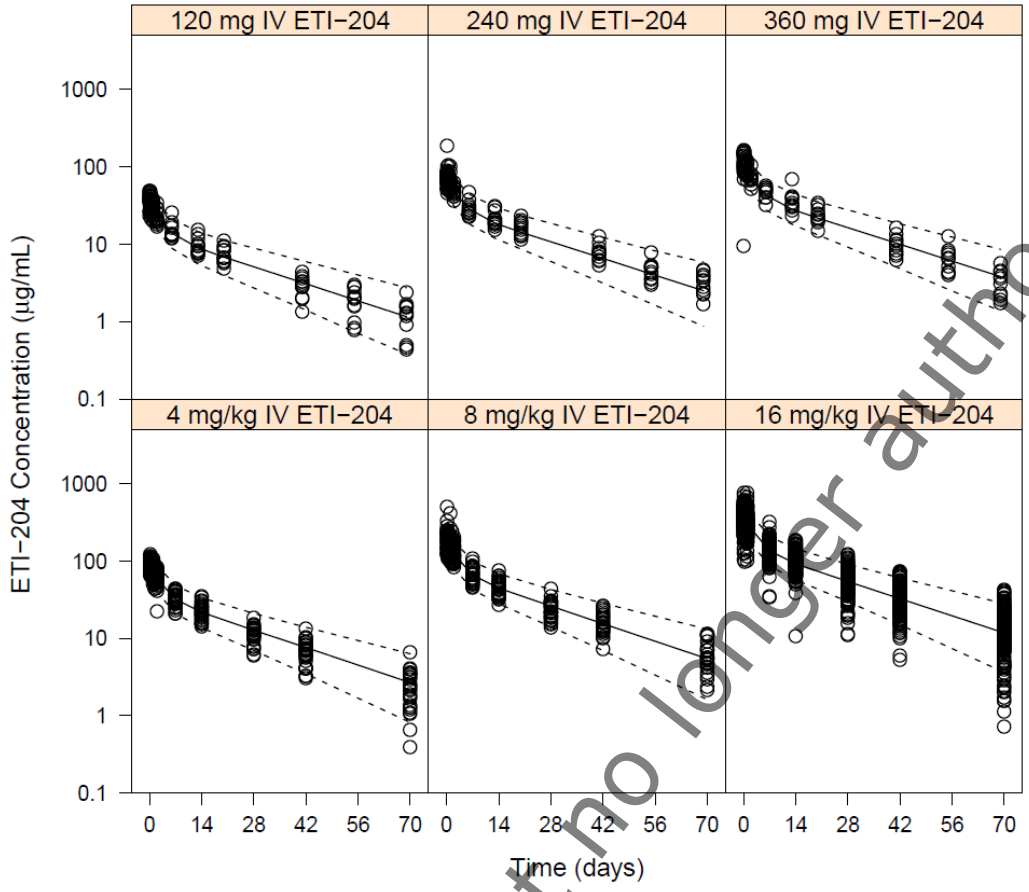
The human population PK data set was comprised of 303 subjects with 2830 observation records. Mean (range) body weights were 78.8 (50.0–96.7) kg for study AH102, 79.2 (48.4–149.5) kg for study AH104 and 75.2 (50.4–102.4) kg for study AH105. Mean (range) ages were 33.8 (19.0–51.0), 42.6 (18.0–79.0), and 30.1 (18.0–58.0) years for AH102, AH104, and AH105, respectively. There were 83 non-white subjects included in the data set.

The final human structural model consisted of a two-compartment model parameterised in terms of CL, Vc, Vp and Q. Allometric exponents for volume and clearance terms were estimated instead of fixing to theoretical values. Inter-subject random effects were included for CL, Vc, and Vp, with a full block covariance matrix for all random effects. Shrinkage estimates were 2%, 5%, and 15% for CL, Vc, and Vp respectively. Residual error was described by a proportional error model.

Table 5. Human Population PK Modelling Parameter Estimates and %RSE from Final Population PK Model, 95% CI From Bootstrap

Description	Model	Estimate	%RSE	95% CI	Variability
clearance	$CL \sim \theta_1 \cdot \theta_{10^{RACE.}} (WT/51)^{\theta_6} (WT/70)^{\theta_6} \cdot e^{\eta_1}$	0.233 L/d	2.02	(0.224, 0.242)	
central volume of distribution	$V_c \sim \theta_2 \cdot (WT/70)^{\theta_7} \cdot e^{\eta_2}$	3.21 L	1.44	(3.12, 3.29)	
peripheral volume of distribution	$V_p \sim \theta_3 \cdot (WT/70)^{\theta_8} \cdot e^{\eta_3}$	2.73 L	2.33	(2.62, 2.84)	
intercompartmental clearance	$Q \sim \theta_4 \cdot (WT/70)^{\theta_9} \cdot e^{\eta_4}$	0.473 L/d	5.67	(0.427, 0.516)	
body weight effect on on CL	θ_6	0.677	10.3	(0.531, 0.815)	
body weight effect on on Vc	θ_7	0.55	11.1	(0.426, 0.67)	
body weight effect on on Vp	θ_8	0.486	23	(0.33, 0.703)	
body weight effect on on Q	θ_9	0.174	167	(0.01, 0.666)	
non-white race effect on CL	θ_{10}	1.21	2.95	(1.15, 1.29)	
age effect (greater than 50 years) on CL	θ_{11}	-0.298	44.8	(-0.58, -0.041)	
interindividual variability of CL	$IIV_{CL} \sim \Omega_{1.1}$	0.0623	12.5	(0.049, 0.0777)	%CV = 25.4
interindividual CL-Vc covariance	$cov_{CL,Vc} \sim \Omega_{2.1}$	0.0277	21.2	(0.0183, 0.0408)	CORR = 0.568
interindividual variability of Vc	$IIV_{V_c} \sim \Omega_{2.2}$	0.0384	13.6	(0.0288, 0.0491)	%CV = 19.8
interindividual CL-Vp covariance	$cov_{CL,Vp} \sim \Omega_{3.1}$	0.0243	29.9	(0.0109, 0.0387)	CORR = 0.358
interindividual Vc-Vp covariance	$cov_{Vc,Vp} \sim \Omega_{3.2}$	0.0316	19.9	(0.0204, 0.0432)	CORR = 0.592
interindividual variability of Vp	$IIV_{Vp} \sim \Omega_{3.3}$	0.0743	17.6	(0.0499, 0.102)	%CV = 27.8
proportional error	$err_{prop} \sim \Sigma_{1.1}$	0.0172	7.88	(0.0146, 0.0199)	%CV = 13.1

Figure 2. Model Evaluation Results: Population PK Model Visual Predictive Check for Humans. Solid line is the median ETI-204 concentration from 200 simulated trials, dashed lines are the simulated 90% prediction interval, and open circles are observed values.



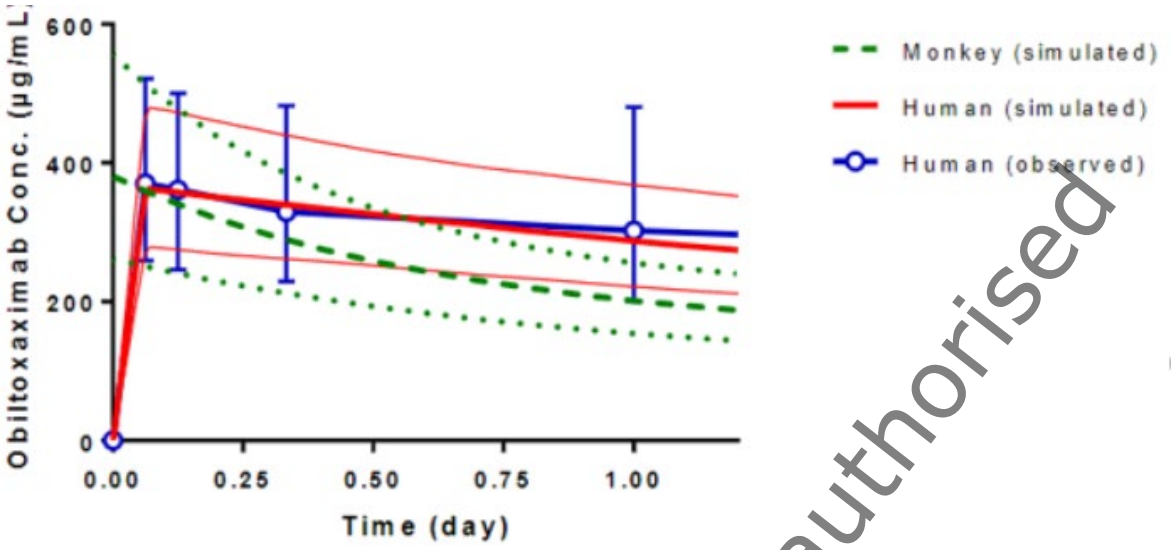
Data from simulated populations of 1000 healthy monkeys and humans and observed exposure data in healthy humans from Study AH104 are compared after a 16mg/kg dose of ETI-204 (obiltoxaximab).

Comparison of obiltoxaximab serum concentration

	C_{max} (µg/mL)	$AUC_{0\text{-}inf}$ (µg·day/mL)
Healthy Monkeys (simulated) [a]	380 (261, 557)	2600 (1540, 4440)
Healthy Humans (simulated) [a]	362 (278, 479)	4470 (3180, 6550)
Healthy Humans (observed)	390 (270, 555) [b]	5050 (3270, 7890) [c]

Values are Median (5th and 95th Percentile)
[a] N = 1000 simulated profiles
[b] N = 202
[c] N = 191

vs. time profiles following 16 mg/kg showed that the C_{max} is similar between healthy NHP and humans. The average $AUC_{(0\text{-}inf)}$ is higher in humans compared to healthy NHP given 16 mg/kg, however the distributions overlap.



- **Population PK Simulations of infected human exposures and Extrapolated Human Dose Predictions**

The bridged human population PK model that approximates TMDD was used to simulate infected human exposures:

$$CL = \theta_{CL} \cdot \left(\frac{WT_i(\text{kg})}{70(\text{kg})}\right)^{\theta_{CL,WT}} \cdot \exp^{\eta_{CL}} + \left(\frac{V_{\max} \cdot \left(\frac{\text{WT(kg)}}{2.88(\text{kg})}\right)^{0.75} \cdot \exp^{\eta_{Vmax}} \cdot C_p}{K_m + C_p}\right)$$

$$V_c = \theta_{Vc} \cdot \left(\frac{WT_i(\text{kg})}{70(\text{kg})}\right)^{\theta_{Vc,WT}} \cdot \exp^{\eta_{Vc}}$$

$$V_p = \theta_{Vp} \cdot \left(\frac{WT_i(\text{kg})}{70(\text{kg})}\right)^{\theta_{Vp,WT}} \cdot \exp^{\eta_{Vp}}$$

$$Q = \theta_Q \cdot \left(\frac{WT_i(\text{kg})}{70(\text{kg})}\right)^{\theta_{Q,WT} \cdot \exp^{\eta_Q}}$$

where all parameters were defined as the estimates from the uninfected human model except for Vmax, Km, and ηVmax, which were obtained from the cynomolgus monkey population PK model.

Population PK simulations results to compare a 16 mg/kg IV dose in infected monkeys and humans are shown in Figure 3 and Figure 4 and Table 6 and Table 7. Median C_{max} for healthy and projected infected humans were slightly higher than those for monkeys, although overall distributions were generally comparable. $AUC_{(0\text{-inf})}$ was almost twice that for projected infected humans when compared to infected monkeys, while $AUC_{(0\text{-inf})}$ for healthy humans was greater than double.

Figure 3: Population PK Simulation Results: Comparison of ETI-204 Concentrations Following a 16 mg/kg Dose in Infected Cynomolgus Monkeys and Healthy Humans. Results are shown as the median concentration (solid lines) and 90% prediction interval (dashed

lines) from 1000 simulated typical humans (weight = 75 kg) or monkeys (3 kg). Left Panel: linear scale; Right Panel: semi-log scale

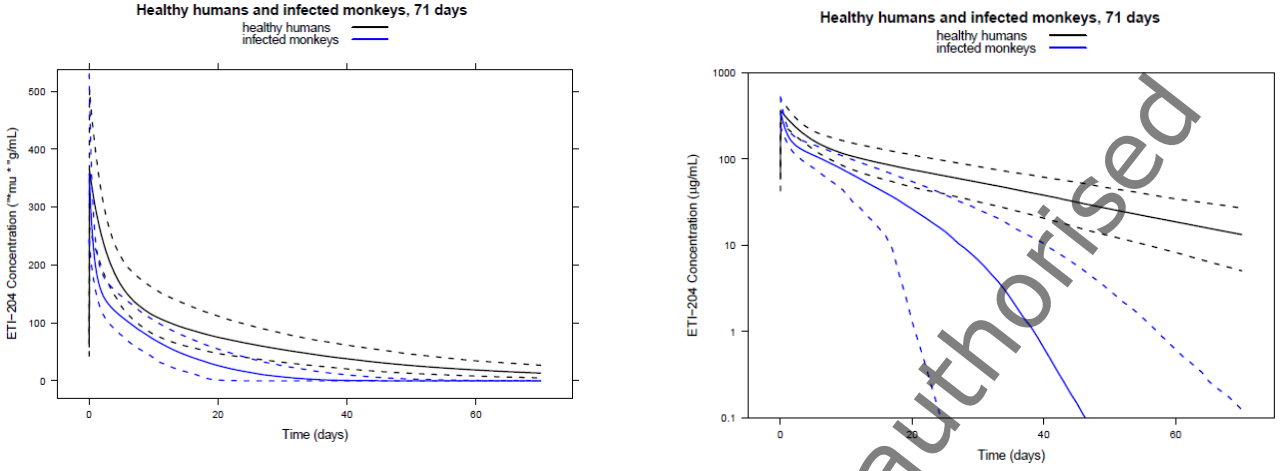


Figure 4. Population PK Simulation Results: Comparison of ETI-204 Concentrations Following a 16 mg/kg Dose in Infected Cynomolgus Monkeys and Projected Infected Humans. Results are shown as the median concentration (solid lines) and 90% prediction interval (dashed lines) from 1000 simulated typical humans (weight = 75 kg) or monkeys (3 kg). Left Panel: linear scale; Right Panel: semi-log scale

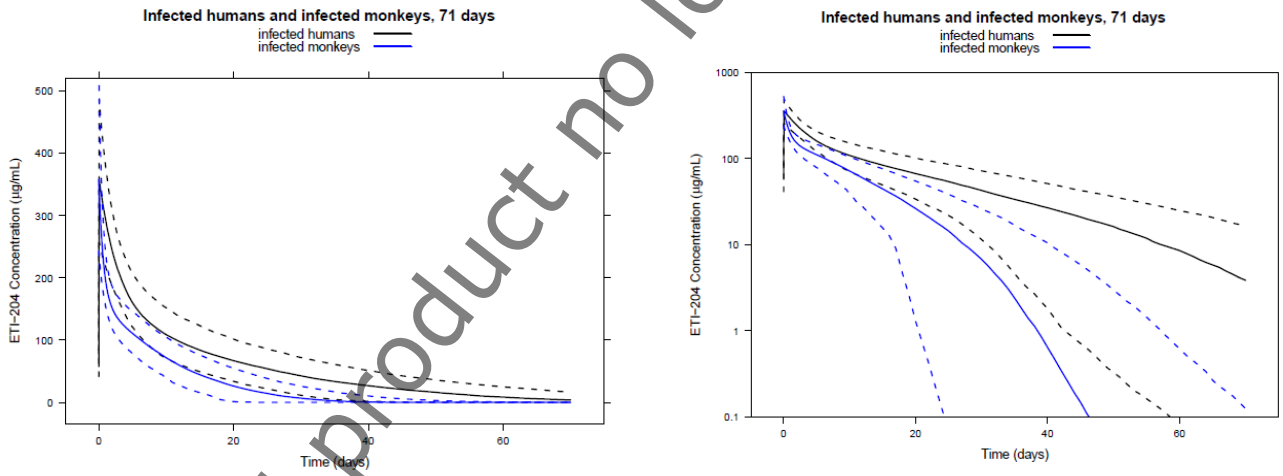


Table 6. Summary of simulated C_{max} (ug/mL) for humans (75 kg, infected and healthy) and monkeys (3 kg, infected) administered 16 mg/kg ETI-204

Population	N	Q5	Q25	median	mean	Q75	Q95
Healthy Humans	500	297	348	386	388	423	490
Infected Humans	500	298	342	377	382	419	481
Infected Monkeys	500	262	310	366	371	420	512

Table 7. Summary of simulated $AUC_{(0\text{-}inf)}$ (ug/mL·d) for humans (75 kg, infected and healthy) and monkeys (3 kg, infected) administered 16 mg/kg ETI-204

Population	N	Q5	Q25	median	mean	Q75	Q95
Healthy Humans	500	3240	4210	4910	4980	5630	6960
Infected Humans	500	2370	3270	4010	4070	4740	6040
Infected Monkeys	500	1160	1600	1920	1970	2270	3010

Special populations

No dedicated formal intrinsic factor PK studies were conducted.

The effects of intrinsic factors on ETI-204 PK were evaluated using both non-compartmental and modelling approaches. Population PK modelling was utilised to assess the impact of age, gender, body weight, and race.

- **Impaired renal and hepatic function**

No conclusion of impact of hepatic or renal disease on ETI-204 PK can be drawn from the human PK data (healthy adult subjects) provided.

- **Gender**

After adjusting for body size, a post hoc analysis was performed where gender effects were estimated on the exponent for body size effects on ETI-204 CL. For females, the body weight effect was estimated to be 13% (95% CI: 72 %, 154%) higher when compared to males. The small difference and inclusion of the null value of 100% in the 95% CI indicates that ETI-204 CL is similar for males and females.

- **Race**

ETI-204 CL was increased in non-white subjects (n=83) when compared to whites, with an effect estimate of 1.21 (1.16, 1.30). This suggests that ETI-204 exposure in non-white subjects will be 79% of that in white subjects. The majority of the CL distribution for non-white subjects lies within the no effect range. Using the updated human pop PK model (including study data from AH110), obiltoxaximab CL appeared to be 17% (11%, 23%) higher in non-white subjects when compared to white subjects. Estimates of these covariate effects were comparable to those from the previous final model.

The applicant conducted backwards deletion of the covariates age and race in the model. Age and race were found to be statistically significant, however, the age distribution was skewed in the race groups. According to the model non-white subjects would have 79% of the exposure compared to that in white subjects. The applicant claims that this is not an issue because the majority of the CL distribution for non-white subjects lies within the no effect range. While this range has not been justified, the results should be interpreted with caution as they are based on an explorative analysis. While the covariates

may improve the predictability of the model, the clinical relevance is not known. This is reflected in the SmPC. A conclusion on impact of race cannot be made based on this analysis.

- **Weight**

The effects of body weight on ETI-204 CL were derived from simulations using a population PK approach. Allometric effects on structural population PK parameters (CL, Vc, Vp, Q) were estimated separately with estimates (95% CI) of 0.677 (0.531, 0.815), 0.550 (0.426, 0.670), 0.486 (0.330, 0.703), and 0.174 (0.010, 0.666) for CL, Vc, Vp, and Q respectively. Distributions of ETI-204 CL estimates from the bootstrap analysis are plotted for the reference subject (CL, 70 kg, white race, age less than 50 years). Eighthly percent and 100% of the typical 100 and 125 kg subjects are outside the range. For the typical 100 kg subject, the expected CL would be approximately 1.5 times that of the reference (70-kg subject). This indicates that ETI-204 AUC would be approximately 33% lower in the heavier subjects. The human pop PK model has been updated with PK data collected from AH110. Recalculated estimates for body weight effects on structural parameters in the same range, with estimates of 0.720 (0.595, 0.845), 0.608 (0.498, 0.718), 0.447 (0.254, 0.640), and 0.268 (-0,216, 0.753) for CL, Vc, Vp, and Q respectively. Clearance at a high body weight (109 kg) was approximately 38% higher than in a reference population. Following weight-based dosing (16 mg/kg) this results in an increase in $AUC_{(0\text{-inf})}$ of 12%, which is deemed not clinically meaningful.

- **Elderly**

All clinical studies were conducted in adult (≥18 years of age) healthy volunteers. No alteration of dosing is needed for patients ≥65 years of age, however few subjects aged 65 years and over were included in the studies to determine whether their PK differs from younger subjects. ETI-204 CL decreased with increasing age above 50 years old. Of note, data with respect to age seem not to be balanced with most PK collected from patients below the age of 50. The effect estimate was -0.298 (-0.580, -0.041). This translates to CL values of 0.217 L/day (93% of the reference CL) and 0.208 L/day (89% of the reference CL) for the typical 65 and 75-year-old subject, respectively.
CL decreased with increasing age above 50 years old, with an re-calculated effect estimate of -0.361 (-0.619, -0.102) using the updated pop PK model.

- **Children**

No PK data in paediatric patients or healthy subjects are available. All PIP measures are deferred until a confirmed outbreak of anthrax.

The bridged human population PK model that approximates target-mediated drug disposition (TMDD) for infected subjects was used to simulate healthy infected human exposures in adult and paediatric subjects. A minimal effect of maturation on ETI-204 CL is expected. Therefore, no maturation effect was included in the simulation model. Simulations for this study were performed using the estimated adult weight effect exponents. Five hundred paediatric subjects were simulated for each weight bin from 5 to 50 kg, in 5 kg increments. A new weight cut-off (40 kg instead of 50 kg) has been introduced in the paediatric posology. Switching the cut-off from 50 to 40 kg will result in subjects in the 40-50 kg range to be under-exposed: the median $AUC_{(0\text{-inf})}$ and C_{max} for a 45 kg subject would be 4070 µg/mL·day and 299 µg/mL compared to a median of 4600 µg/mL·day and 349 µg/mL for the typical 70 kg subject. These values result in changes of -12% and -15% for $AUC_{(0\text{-inf})}$ and C_{max}, respectively. A cut-off of 50kg would result in an $AUC_{(0\text{-inf})}$ and C_{max} for a 50-kg individual to be 29% and 16% greater than the median in adults. The differences are unlikely to have a clinical impact. The new proposed posology is considered acceptable.

$AUC_{(0\text{-inf})}$ and C_{max} (simulated median, 5th and 95th percentiles) were plotted versus body weight. Simulated and observed adult (75 kg) exposure metrics are overlaid for comparison to paediatric

distributions. Simulated values for adult exposure metrics are shown in Table 8. Plotted simulated values may vary slightly, since adult values were simulated with each paediatric scenario.

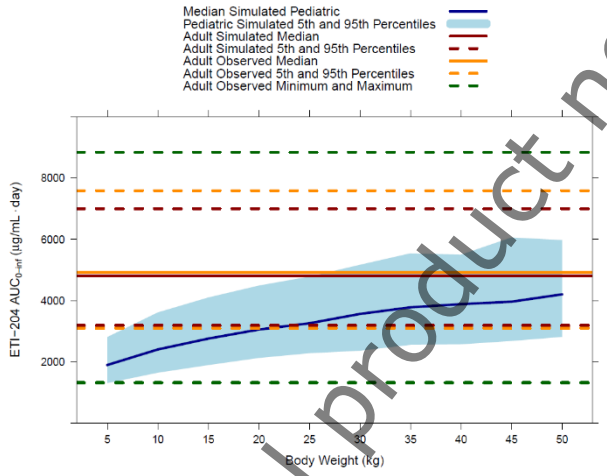
Table 8. Representative observed (Obs) and simulated (Sim) exposure summary for adults administered 16 mg/kg ETI-204

	C_{max} (μg/mL)			AUC_{inf} (μg/mL · h)		
Statistic	Healthy Obs	Healthy Sim	Infected Sim	Healthy Obs	Healthy Sim	Infected Sim
Minimum	106			1320		
5th Percentile	259	258	255	3097	3190	2363
Median	384	359	359	4921	4810	3866
95th Percetile	547	493	487	7588	7000	6349
Maximum	769			8845		

For a 16 mg/kg IV dose in healthy paediatric subjects, $AUC_{(0\text{-inf})}$ and C_{max} increased with increasing body weight. Figure 6 and Figure 7 show results for ETI-204 IV doses of 24 mg/kg (15–50 kg) and 32 mg/kg (< 15 kg).

Figure 5. Simulated ETI-204 $AUC_{(0\text{-inf})}$ (A) and C_{max} (B) vs. Body Weight in Healthy Paediatric Subjects Administered 16 mg/kg IV ETI-204. Results are compared to simulated and observed exposures for healthy adults.

A



B

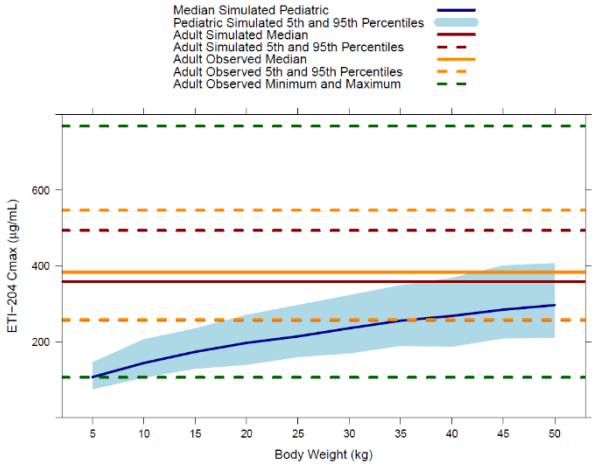


Figure 6. Simulated ETI-204 $AUC_{(0\text{-}inf)}$ (A) and C_{max} (B) vs. Body Weight in Healthy Paediatric Subjects Following Dose Adjustment. Results are compared to simulated and observed exposures for healthy adults.

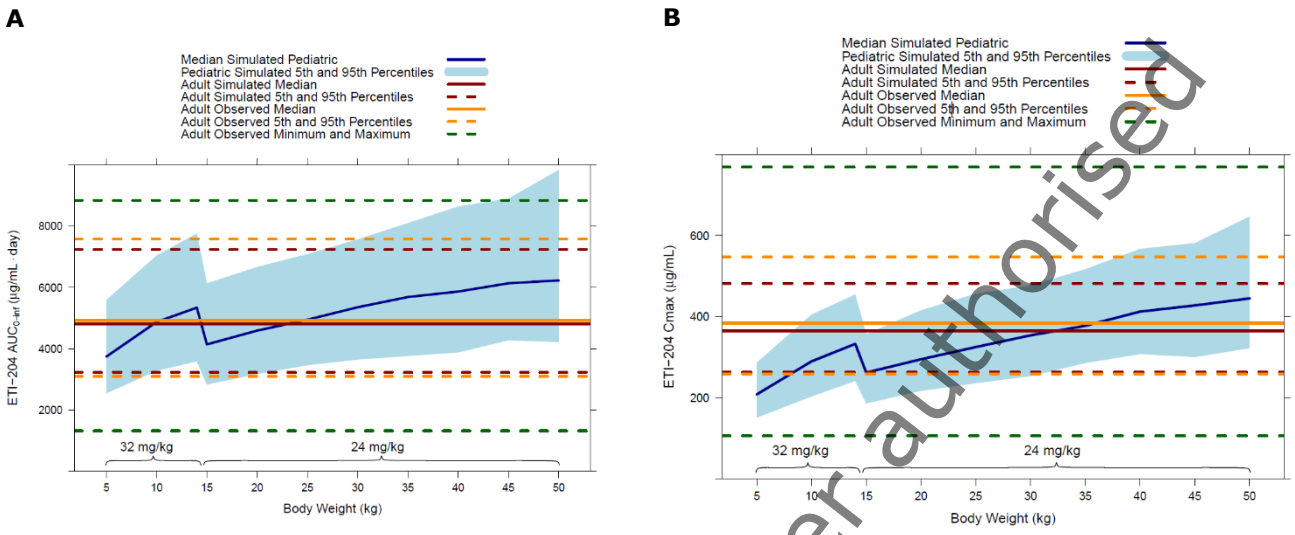
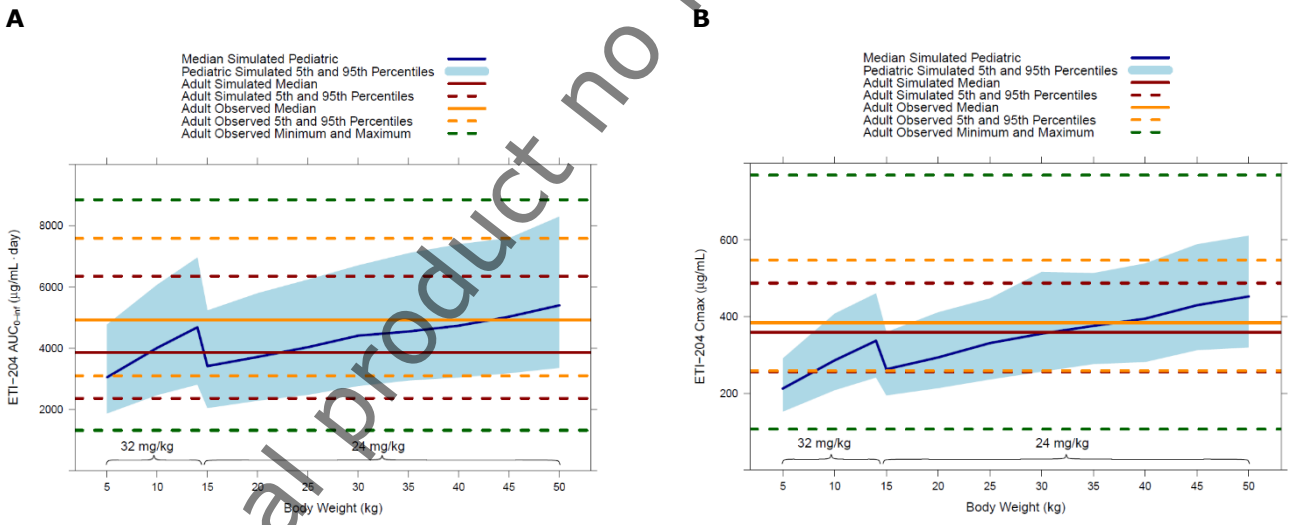


Figure 7. Simulated ETI-204 $AUC_{(0\text{-}inf)}$ (A) and C_{max} (B) vs. Body Weight in Infected Paediatric Subjects Following Dose Adjustment. Results are compared to simulated infected and observed healthy adult exposures.



Interactions

No *in vitro* studies were carried out with ETI-204.

For a mAb, no direct drug-drug interaction via CYP enzymes is anticipated.

ETI-204 PK was evaluated after IV administration when given concomitantly with ciprofloxacin. Ciprofloxacin did not affect the PK of ETI-204. ETI-204 did not affect cytokine concentrations.
No other antibiotic treatment than ciprofloxacin has been investigated with regard to its interaction potential in human.

Pre-treatment of humans with diphenhydramine to minimise the risk of hypersensitivity reactions had also no apparent effect on the PK of ETI-204 based on study results from AH104.

2.4.3. Pharmacodynamics

Mechanism of action and primary pharmacology

B. anthracis secretes binary A2/B-type bacterial exotoxins comprising the enzyme (A) moiety LF or EF, each associated with PA, the common binding (B) component. The virulence of *B. anthracis* is attributed to the tripartite toxin complex, and toxic activity is expressed only when PA is combined with LF (forming LT) or EF (forming oedema toxin).

ETI-204 humanised murine variable region sequences that targets *B. anthracis* PA, the common cell-binding component of both anthrax toxins which are key for *B. anthracis* virulence and pathogenesis. The affinity-enhanced, de-immunised immunoglobulin G subclass 1 (IgG1) mAb has a molecular weight of 148 kilo Dalton (kDa) and is produced in cultures of stably transfected non-secreting GS NS0 myeloma cells.

ETI-204 binds to domain 4 of PA (PAD4), which is the domain responsible for the binding of PA to cell surface receptors. Obiltoxaximab possesses a very high affinity for PA with a dissociation constant (KD) of 0.33 nM, which is similar to the PA receptor KD (i.e., 0.17 to 1 nM). Binding of ETI-204 to PAD4 (Little et al, 1996; Leysath et al, 2009) prevents the cell binding of PA63-EF and PA63-LF complexes and prevents the entry of EF and LF into the cytosol, thereby preventing the downstream deleterious effects of anthrax toxin.

Mode of action has been investigated *in vivo* (NZW rabbit, cynomolgus monkey) and is not expected to differ from between the species in humans. In addition, the ability of ETI-204/obiltoxaximab to neutralise LT-mediated cell death was shown in an *in vitro* LT neutralisation assay with murine macrophages (Study PCRPT0005).

Special Studies

Because controlled clinical trials in humans with anthrax are neither ethical nor feasible, no human study including PD endpoints regarding efficacy has been conducted. The planned field study AH501 will further assess the clinical pharmacology including PK and immunogenicity of ETI-204 in the human target population including paediatric subjects.

Immunogenicity in humans was low with a proportion of 3.0-8.8% subjects positive for a treatment-emergent ATA response. ETI-204 will be administered as single dose IV, which limits the ATA-related effects and immunogenic potential of the antibody to be clinically relevant.

The effect of ETI-204 on the release of pro-inflammatory cytokines (interleukin (IL)-1β, IL-2, IL-6, tumour necrosis factor-α (TNF-α), and interferon γ (IFNγ)) was evaluated in clinical study AH104. No clinically relevant change in the cytokines could be detected.

Pharmacodynamics and Pharmacokinetics-Pharmacodynamics (PK/PD)

As ETI-204 is a mAb for which the target (*B. anthracis* PA) does not exist in healthy subjects, PD studies cannot be conducted in healthy subjects. As controlled clinical trials in humans with inhalational anthrax are not feasible due to the rarity of this disease, no PD and PK/PD studies of ETI-204 SFL were conducted in patients.

Relationship between plasma concentration and effect

A robust PK/PD model that adequately describes the exposure-response relationship for anti-PA mAbs in inhalational anthrax has not been established in animals or humans. In the absence of such a model, the selection of an effective human dose has been driven by the goal of achieving ETI-204 exposures in humans that are comparable to or exceed those in animals.

Relationship between dose and effect

Survival Modelling

The objectives of the Survival Analysis Modelling of ETI-204 in Anthrax-Infected Subjects were:

- Develop survival models to describe ETI-204 dose-response in rabbits and cynomolgus monkeys.
- Identify an ETI-204 dose, via modelling, that is likely to be maximally efficacious for preclinical efficacy trials for treatment of inhalational anthrax.

Data

Infected animal studies (rabbits: AR033, cynomolgus monkeys: AP201, AP202, AP203, and AP204) where animals were given only ETI-204 treatment (i.e. no antibiotic co-medication) were used for survival modelling (time-to-event model that describes the observed survival in these studies). There were 259 animals included in the survival data set. Studies AR033, AP202 and AP203 were GLP-compliant as well as blinded. Studies AP201 and AP204 were GLP-compliant and blinded to group only.

In the ETI-204 monotherapy treatment studies, study drug treatment was triggered by the following clinical signs or symptoms of systemic anthrax disease:

- AR033: A significant increase in body temperature (SIBT, defined as a ≥ two standard deviation increase from baseline temperature either three consecutive times or two consecutive times twice), positive results in a PA-ECL assay (≥ 1 ng/mL positive control) developed by NIAID or 54 hours
- AP201, AP202, AP203 and AP204: Positive results in a PA-ECL assay (≥ 2 ng/mL positive control) developed by NIAID or 54 hours after challenge in monkeys (whichever occurred first).

Results

The original dose-response (survival) model could not be supported because it was developed using pooled data from monotherapy ETI-204 studies; from Study AR033 (rabbits who received placebo (n=14), 1 mg/kg (n=14), 4 mg/kg (n=14), 8 mg/kg (n=14) or 16 mg/kg (n=14) ETI-204) and Studies AP201, AP202, AP203 and AP204 (Cynomolgus monkeys who received placebo (n=63), 4 mg/kg (30), 8 mg/kg (n=31), 16 mg/kg (n=50) or 16 mg/kg (n=16) ETI-204). However, the disease progression in rabbits is rapid and rabbits are more sensitive to infection of inhalational anthrax compared to primates. In addition, the exposure [$AUC_{(0\text{-}inf)}$] is twice as high in monkeys compared to rabbits, when given 16 mg/kg dose, which made the interpretation of results difficult.

The applicant was asked to run the models separately for rabbits (AR033) and monkeys (AP201, AP202, AP203 and AP204) and present the results in the same manner as was done for the pooled analysis along with a discussion of the results and the selected optimal dose. For this purpose, The Weibull cure-rate model was fit to survival data. The survival model also included an Emax dose-response and an exponential effect of log10(PTT bacteraemia) on logit(*p*surv). Preliminary models demonstrated that ETI-204 dose amount did not affect rate of death.

As rabbit data are deemed only supportive with regard to dose selection and to exposure relevant for human dosing, only the NHP model and results are presented in the following.

The NHP model and results:

The NHP model estimates are in the range of those resulting from the original pooled analysis. The estimated ED50 is 1.990 mg/kg (95% confidence interval: 0.351–11.2). The final parameter estimates, and 95% confidence intervals are shown in Table 9.

Table 9 Final parameter estimates from the dose-response survival model estimated from *monkeys. Confidence intervals are Wald confidence intervals based on the asymptotic approximation to the standard error of the parameter estimate.*

Parameter	Units	Estimate	95% CI
θ_0		0.579	(-0.654 , 1.81)
E_{max}		3.460	(1.81 , 5.11)
ED_{50}	mg/kg	1.990	(0.351 , 11.2)
θ_1	$\log_{10}$ cfu^{-1}	0.298	(0.19 , 0.406)
θ_2		1.240	(0.425 , 2.06)
λ_0		-2.340	(-2.63 , -2.05)
λ_1	$\log_{10}$ cfu^{-1}	0.193	(0.134 , 0.252)
α		3.000	(2.58 , 3.49)

θ_0 - Baseline logit for P(cure)
E_{max} - Logit for maximum P(cure)
ED_{50} - ETI-204 dose to reach half maximum effect
θ_1 - Rate for log10(bact) P(cure)
θ_2 - exponent for quantitative bacteremia effect
λ_0 - placebo P(cure)
λ_1 - Slope effect of log10(bact) on λ
α - shape parameter

Internal validation using Kaplan-Meier plots has been conducted, predicting the percent surviving in all studies (*Figure 8*– studies following 16 mg/kg).

Figure 8 . Internal validation of dose-response relationship comparing data from Study AP202 and from Study AP204 to model-based predictions from the monkey survival mode. The results for the 16 mg/kg ETI-204 dose are from combined groups treated with the pilot and commercial formulations. Model prediction (red lines) and 90% prediction interval (blue region) with observed Kaplan-Meier estimates of survival (black lines) overlaid.

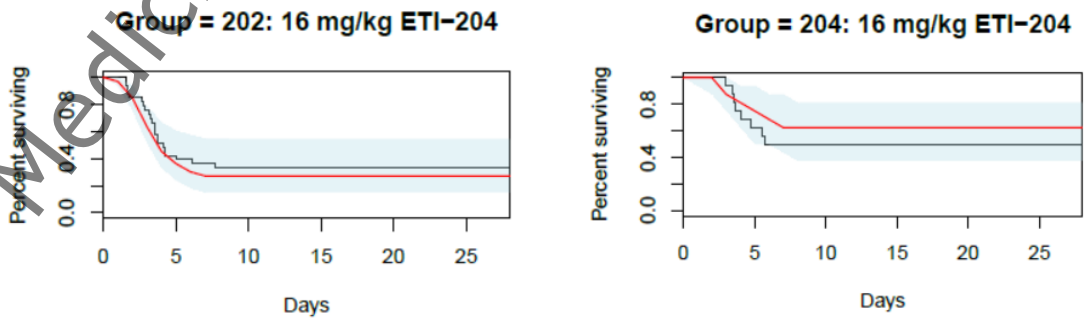


Table 10 ***Predicted proportion of monkeys surviving to the end of the study as a function of dose and prior-to-treatment bacteraemia quantile, based on dose-response survival model. Model predictions shown for doses*** **of 4, 8, 16, and 32 mg/kg. PTT bacteraemia groups are defined as quartiles of the PTT bacteraemia distribution across the studies AP201, AP202, AP203 and AP204.**

PTT group	Dose (mg/kg)	Estimate	95% CI
[BLQ,3.31] (log10 cfus)	4	0.73	(0.50 , 0.85)
[BLQ,3.31] (log10 cfus)	8	0.80	(0.62 , 0.89)
[BLQ,3.31] (log10 cfus)	16	0.84	(0.67 , 0.92)
[BLQ,3.31] (log10 cfus)	32	0.87	(0.68 , 0.94)
(3.32,4.16] (log10 cfus)	4	0.45	(0.22 , 0.65)
(3.32,4.16] (log10 cfus)	8	0.54	(0.34 , 0.73)
(3.32,4.16] (log10 cfus)	16	0.62	(0.43 , 0.80)
(3.32,4.16] (log10 cfus)	32	0.67	(0.44 , 0.85)
(4.17,5.17] (log10 cfus)	4	0.19	(0.07 , 0.53)
(4.17,5.17] (log10 cfus)	8	0.26	(0.11 , 0.65)
(4.17,5.17] (log10 cfus)	16	0.32	(0.16 , 0.74)
(4.17,5.17] (log10 cfus)	32	0.38	(0.18 , 0.80)
(5.18,8.56] (log10 cfus)	4	0.03	(0.00 , 0.39)
(5.18,8.56] (log10 cfus)	8	0.05	(0.01 , 0.49)
(5.18,8.56] (log10 cfus)	16	0.06	(0.01 , 0.61)
(5.18,8.56] (log10 cfus)	32	0.07	(0.01 , 0.67)

PTT: prior-to-treatment
BLQ: below the limit of quantitation
cfu: colony-forming unit

Figure 9 and *Figure 10* show the average predicted dose-response and combined survival data from all preclinical NHP studies. While, both figures show a close alignment of the model predictions and observed data, the observed surviving proportion for the 16 and 32 mg/kg groups in are below the average model prediction. This difference is likely because the 16 mg/kg group is largely influenced by Study AP202 and the 32 mg/kg group comes solely from Study AP203 which both had higher PTT bacteraemia values when compared to other studies.

Figure 9 Predicted proportion of monkeys surviving to the end of the study as a function of dose averaged across the prior-to-treatment bacteraemia levels in the studies (AP201, AP202, AP203 and AP204). Grey band represent 90% confidence interval.

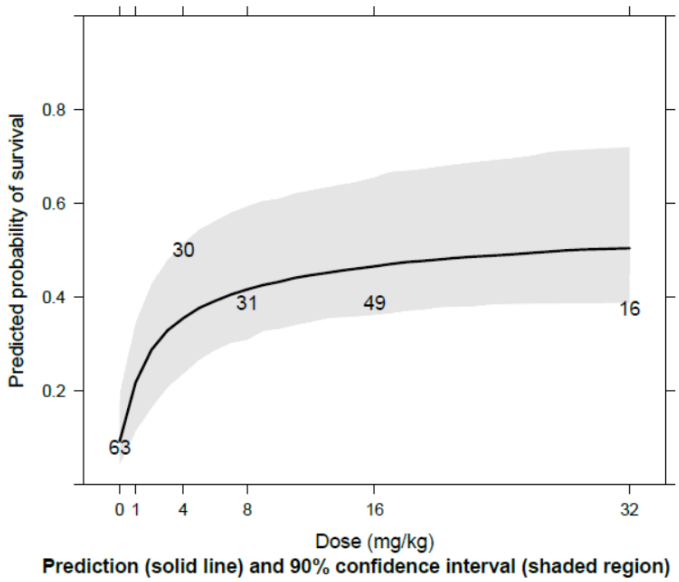
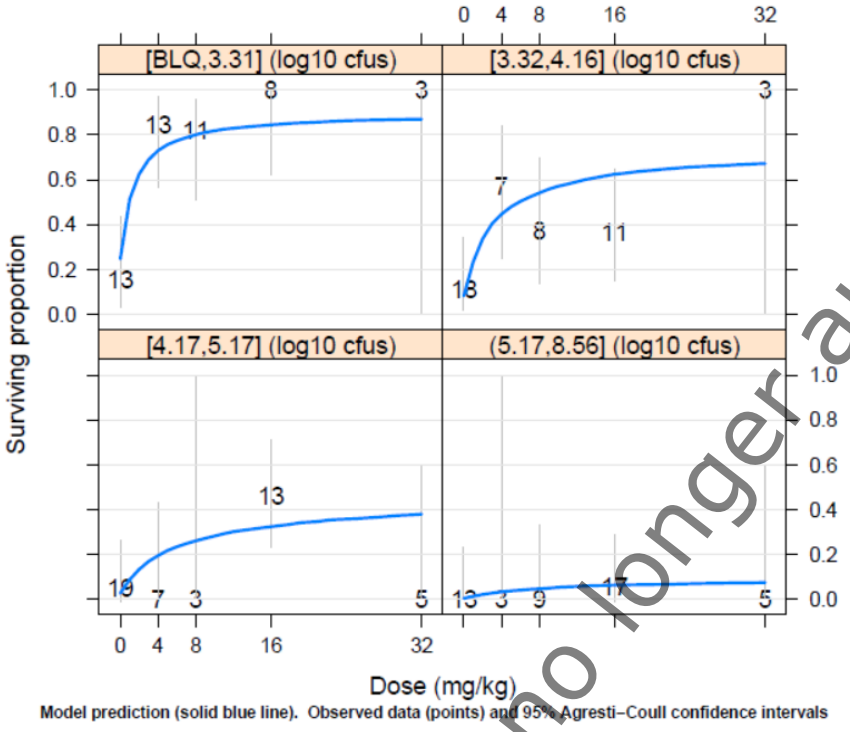


Figure 10 emphasises the strong relationship between both dose and PTT bacteraemia on the probability of survival. After separating the effects of both dose and PTT bacteraemia, we see that the model accurately predicts the observed survival rates in all dose groups.

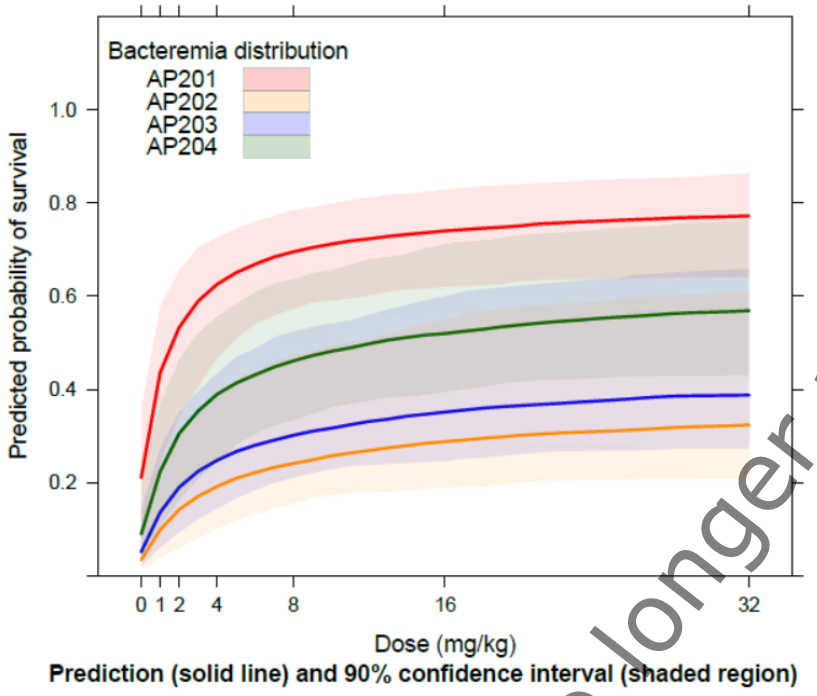
Figure 10 Comparison of the observed and predicted relationship between dose and the probability of surviving to the end of the study. Data are pooled across studies AP201, AP202, AP203, and AP204. Symbols in the plot represent the number of animals at the observed value at that combination of dose level and PTT bacteraemia grouping, with gray vertical lines representing 95% CI's. Blue lines represent the model predictions. Panels are defined as quartiles of the PTT bacteraemia distribution across the 4 studies.



The model implies that higher PTT bacteraemia levels are associated with lower survival rates and faster rates of death among animals that die. Higher doses are associated with higher survival rates, with an estimated dose to give half the maximal survival benefit (ED50) being 1.99 mg/kg. Rate of death was similar over the range of ETI-204 doses studied.

Figure 11 shows the expected survival rate as a function of dose for PTT bacteraemia distributions similar to those in studies AP201, AP202, AP203, and AP204. If the PTT bacteraemia values are high (similar to studies AP202 and AP203), the maximum expected survival is approximately 30% and if the PTT bacteraemia levels are lower (similar to AP201) the maximum expected survival is approximately 80%. At a PTT bacteraemia level of approximately 10^5 log10 CFU/mL, there is almost zero probability of survival, indicating that animals at this level are beyond a "point of no return" for survival. Regardless of the PTT bacteraemia levels, doses above 8 mg/kg are predicted to have relatively small increases in survival, with a dose of 16 mg/kg having a marginal difference in survival when compared to 8 and 32 mg/kg. A dose of 16 mg/kg appears to be adequate for optimal predicted probability of survival.

Figure 11 **Predicted proportion of animals surviving to the end of the study as a function of dose for prior-to-treatment bacteraemia levels in the AP201, AP202, AP203 and AP204 studies. Coloured bands represent 90% confidence intervals. Mean [range] PTT bacteraemia levels (log10 CFU/mL) for each study were: AP201(2.42 [1-6.38]), AP202 (5.06 [2.76-8.56]), AP203 (4.76 [1-7.72), AP204 (3.92 [1-5.68]).**



2.4.4. Discussion on clinical pharmacology

Methods

- ***Quantification of ETI-204***

Given the pivotal role of both preclinical and clinical PK in terms of bridging the efficacy seen in animal models to the human situation, a group of bioanalytical issues have been raised at the level of major objections. The consequence of the bioanalytical issues raised is uncertainty regarding the reliability of the measured concentrations, exposure and PK parameters of ETI-204 in human and animals. As PK data are critical in terms of showing that the proposed human dose is adequate, i.e. that the exposure in humans following a 16 mg/kg dose is not lower than the exposure in primates dosed 16 mg/kg.

All blood to be used for PK analyses (clinical and non-clinical) has been collected in serum separator tubes (SST) that use gel to facilitate serum separation from the blood clot. Several publications and tube manufacturers warn for incomplete recovery in drug quantitation settings (Bowen 2014, Karppi 2000). It is unknown to which extent ETI-204 concentration may be affected by the tube used for blood collection. The applicant was requested to justify, preferably with data, that the recovery of ETI-204 from those tubes is complete, especially considering the fact that both calibration standards and QCs have been prepared differently (by spiking ETI-204 directly into serum), thus giving the possibility of a systematic error in serum concentration. The influence of the collected blood volume had also to be considered.

It was acknowledged that without further testing of controls prepared in whole blood and subject to the same pre-analytical step as the study samples, absolute recovery cannot be assured. However, since samples from past studies were no longer available, and the assay is currently inactive, the applicant included the missing investigations on recovery in the protocol of the post authorisation efficacy field study AH501, using scavenged blood samples, if available. Also, the applicant performed an *in vitro* experiment investigating the recovery of ETI-204 spiked into human blood and processed as per the conventional clotting method, or in SST tubes filled at volumes corresponding to what was used in clinical or preclinical studies. Calibration curves and QCs were prepared as previously with normal serum, without SST tube. Results for ETI-204 recovery were consistent between clotting procedures, blood volume and the two tested ETI-204 concentrations, indicating that there is no recovery issue.

Based on supplementary data provided on raised concerns, the method can be considered sufficiently validated, although a few issues remain outstanding:

- The applicant acknowledges that interference by PA63 and other *B. anthracis* toxin components has not been addressed as part of the validation of method GCL-160. This omission can currently be accepted considering that the human samples analysed were from healthy subjects not exposed to *B. anthracis*. It is acceptable that the validation package for the assay in human serum is completed as part of field study AH501 (post-authorisation efficacy study [PAES]) with regards to the interference with PA (63 and 83), EF, and LF (specific obligation).
- Interference by ADA to be evaluated, preferably using human ADA rather than rabbit anti-obiltoxaximab antiserum. Since the samples are no longer available and only the PK data from healthy animals and humans will be used for the dose selection, it is acceptable that the missing data is generated in the planned PAES study AH501 (specific obligation).
- Parallelism data had not been provided for the phase I studies in human. Parallelism should be performed with incurred samples from the planned open-label field study (specific obligation).
- In the absence of data in haemolytic and lipemic serum, data for such samples had to be excluded. The applicant clarified that of all clinical samples analysed, only two were noted as haemolytic and none was lipemic. For the two haemolytic samples, it was considered that they fitted within the PK profile of the concerned subject and they were not excluded. This can be accepted, provided the validation in haemolytic and lipemic serum is performed as outlined in the protocol of the PAES study AH501 (specific obligation).

Anti-drug antibody (ADA) analysis

SST tubes were used for immunogenicity samples, thus the same concerns as for the bioanalysis apply. The applicant discussed the implications of the results from recovery experiments from SST tubes with regards to the assessment of immunogenicity, since similar tubes were used for those samples. It was argued that due to the low biological variability between healthy individuals, and signals close to the instruments background, it is unlikely that differences will be shown in serum collected by different methods (SST vs. clot and centrifugation). This is agreed with.

ADA analysis follows a tiered approach with a screening assay, confirmation assay and titration.

The assay was validated in two steps using either rabbit anti-ETI-204 antiserum or later affinity purified rabbit anti-ETI-204 IgG as the positive control. ETI-204 is tolerated up to 200 µg/mL.

The confirmatory assay includes an additional step, where free ETI-204 is added and disrupts the complex, inhibiting binding to the plate, thus resulting in a reduced signal.

An assay for neutralizing antibodies was developed but was only used in study AH105 due to issues of interference with ETI-204.

- ***Population Pharmacokinetic analysis***

The objectives of the Population PK Analysis were to characterise ETI-204 population PK in healthy and infected NZW rabbits and cynomolgus monkeys, and healthy humans. The infected cynomolgus monkey PK model was bridged to humans using the healthy human model for the prediction of ETI-204 exposures in humans infected with inhalational anthrax and PK simulations were performed using the bridged human model to derive ETI-204 human exposures for the proposed preclinical dose. The PK datasets consisted of 150 cynomolgus monkey with 929 observations, and 303 human subjects with 2830 observations. Body weight was the only covariate considered for preclinical studies. For human studies, bodyweight, age and race (white vs. non-white) were used for covariate modelling. During assessment, outstanding issues on the modelling method were justified.

ADME

Absorption

The PK of obiltoxaximab are linear over the dose range of 4 mg/kg (0.25 times the lowest recommended dose) to 16 mg/kg following single IV administration in healthy subjects. Following single IV administration of obiltoxaximab 16 mg/kg in healthy, male and female human subjects, the mean C_{max} and $AUC_{(0\text{-}inf)}$ were 400 ± 91.2 mcg/mL and 5170 ± 1360 mcg·day/mL, respectively. The half-life of obiltoxaximab was approximately 20 days (mean).

Distribution

Mean obiltoxaximab steady-state volume of distribution was 79.7 ± 19.2 mL/kg and greater than plasma volume, suggesting some tissue distribution.

Biotransformation

No formal metabolism studies have been conducted with obiltoxaximab.

However, the disposition of mAbs generally involves distribution beyond the vascular space with potential uptake into tissues, and catabolism by proteases to small peptides and amino acids which are subsequently incorporated into the endogenous pool or excreted.

Elimination

Mean obiltoxaximab CL values were 3.35 ± 0.932 mL/d/kg and much smaller than the glomerular filtration rate, indicating that there is virtually no renal clearance of obiltoxaximab.

Pharmacokinetics in target population

The final human structural model consisted of a two-compartment model parameterised in terms of CL, Vc, Vp and Q. Shrinkage on CL, Vc and Vp was low. The model minimised successfully with 3 significant digits. Relative standard error was <30% for all parameters except bodyweight on Q (167%) and age effect on CL (45%). The goodness-of-fit plots do not indicate model misspecification. The bootstrap derived 95% CI are acceptable, except for Q and age effect on CL. The covariates included in the final model were bodyweight, with estimated exponents, age and race. The nonlinear

clearance model component from the cynomolgus monkey model was added to the human population PK, allometrically scaled to human body size. To predict ETI-204 exposures in infected humans, the nonlinear clearance model component from the cynomolgus monkey model was added to the human population PK, allometrically scaled to human body size.

The tables of population PK simulations results (Table 6 and Table 7) of a 16 mg/kg IV dose in infected monkeys and humans show that the simulated exposure is higher in healthy and infected humans compared to infected monkeys. However, conflicting results have been presented in the different reports submitted where in some the simulated C_{max} is lower for healthy human compared to the observed C_{max} in infected monkeys given 16 mg/kg. This indicates that either the model cannot capture C_{max} or that the simulations were not carried out with enough simulated subjects. As the concentration measured is free ETI-204 it was deemed important to compare exposures in uninfected (healthy) monkeys to healthy humans. In response, the applicant provided table and plots which showed that C_{max} is similar between healthy NHP and humans. The average $AUC_{(0\text{-}inf)}$ is higher in humans compared to healthy NHP given 16 mg/kg, however the distributions overlap.

The applicant used the dissociation constant (Kd) to calculate a target human serum concentration needed to bind 99.9% of PA and compared it to the simulated concentration in human with a dose of 16 mg/kg. KD is an equilibrium constant that equals the concentration of free ETI-204 at which half of the total PA is associated with ETI-204. It is not independent of the concentration of PA changing. The applicant presented the concentration PA at which the KD was determined in the comparative binding affinity study and discussed it in relation to the concentrations PA observed in infected animals with or without antibiotics and in all cases without ETI-204 due to the analytical interference.

As single doses are administered as bolus in animal studies and as an IV infusion over 90 minutes in human, C_{max} values might be different. It is however agreed that this is not considered to be of clinical consequence. The applicant stated that based on population modelling, as well as modelling of mean concentration vs. time data from Study AH104, simulated administration of ETI-204 in humans by bolus injection instead of by 90-minute infusion would be expected to have a negligible effect on C_{max} (<1%) and would have no impact on $AUC_{(0\text{-}inf)}$. The applicant showed these analyses in detail as C_{max} is a pivotal PK measure for PK comparison across species.

Special populations

- ***Renal and Hepatic impairment***

No dedicated formal intrinsic factor PK studies were conducted. As obiltoxaximab is a mAb, significant differences in PK or safety are not anticipated in subjects with renal or hepatic impairment.

- ***Gender, Elderly, Race and Weight***

Obiltoxaximab PK were evaluated via a population PK analysis using serum samples from 370 healthy subjects who received a single IV dose across 4 clinical trials. Based on this analysis, gender (female versus male), race (non-Caucasian versus Caucasian), or age (elderly versus young) had no meaningful effects on the PK parameters for obiltoxaximab. However, clinical studies of obiltoxaximab did not include sufficient numbers of subjects aged 65 years and over to determine whether their PK differs from younger subjects. Of the 320 subjects in clinical studies of obiltoxaximab, 9.4% (30/320) were 65 years and over, while 2% (6/320) were 75 years and over.

Clearance at a high body weight (109 kg) was approximately 38% higher than in a reference population. Following weight-based dosing (16 mg/kg) this results in an increase in $AUC_{(0\text{-}inf)}$ of 12%, which is not clinically meaningful.

- ***Children***

Obiltoxaximab PK have not been evaluated in children. The dosing recommendations in Table 2, section 4.2 of SmPC (Recommended paediatric dose of obiltoxaximab [weight-based dosing]), are derived from simulations using a population PK approach designed to match the observed adult exposure to obiltoxaximab at a 16 mg/kg dose.

Pharmacodynamics and PK/PD

Obiltoxaximab binds free PA with an affinity equilibrium dissociation constant (Kd) of 0.33 nM.

In vitro, obiltoxaximab binds to PA from the Ames, Vollum, and Sterne strains of *B. anthracis*. The epitope on PA to which obiltoxaximab binds is conserved across reported strains of *B. anthracis*.

In vitro studies in a cell-based assay, using murine macrophages, suggest that obiltoxaximab neutralises the toxic effects of LT, a combination of PA + LF.

In vivo efficacy studies in NZW rabbits and cynomolgus macaques challenged with the spores of the Ames strain of *B. anthracis* by the inhalational route, showed a dose-dependent increase in survival following treatment with obiltoxaximab. Exposure to *B. anthracis* spores resulted in increasing concentrations of PA in the serum of NZW rabbits and cynomolgus macaques. After treatment with obiltoxaximab there was a decrease in PA concentrations in a majority of surviving animals. PA concentrations in placebo animals increased until they died.

NHP model implies that higher prior to treatment bacteraemia levels are associated with lower survival rates and faster rates of death among animals that die. Higher obiltoxaximab doses are associated with higher survival rates, with an estimated dose to give half the maximal survival benefit (ED50) being 1.99 mg/kg. Rate of death was similar over the range of ETI-204 doses studied.

Regardless of the PTT bacteraemia levels, doses above 8 mg/kg are predicted to have relatively small increases in survival, with a dose of 16 mg/kg having a marginal difference in survival when compared to 8 and 32 mg/kg. A dose of 16 mg/kg appears to be adequate for optimal predicted probability of survival.

2.4.5. Conclusions on clinical pharmacology

The major objection and all other remaining concerns were successfully resolved.

A PAES, i.e. the phase 4 open-label field study AH501 is planned to be conducted upon an anthrax outbreak in a country where obiltoxaximab is authorised and available. As a stated secondary objective, clinical pharmacology assessments will include the PK evaluation of obiltoxaximab and the incidence of ATAs.

The validation of the method in human serum is however not yet considered as fully adequate. The following aspects should be validated prior to use of the assay for sample analysis for clinical study AH501: interference by PA (63 and 83), EF, LF and ADAs, and assay performance in haemolytic and lipemic serum. Parallelism should be performed with incurred samples from the planned PAES study.

It is agreed that outstanding aspects on the method validation will be performed as part of the planned field study AH501 (PAES).

2.5. Clinical efficacy

Since controlled clinical efficacy trials exposing humans to anthrax spores would neither be ethical nor feasible, the efficacy of ETI-204 was evaluated in two established animal models of inhalational anthrax, NZW rabbits and cynomolgus monkeys. The pathophysiology of anthrax toxin in both rabbits and monkeys is considered similar to humans, even though a more rapid development of the disease can be expected in rabbits that are herbivores and highly sensitive to anthrax infection.

Several studies have been executed in rabbits and cynomolgus monkeys challenged with *B. anthracis* spores, where ETI-204 has been administered both as monotherapy treatment of anthrax and as concomitant treatment with antibiotic.

Table 11. Overview of efficacy studies conducted with ETI-204 (obiltoxaximab)

Study ID GLP status Product (batch)	Combination with antibiotics (Y/N)	Doses mg/kg	Route of administration	Species	Study design
Treatment studies					
AP202 GLP Pilot vs. commercial	N	0, 16 (pilot), 16 (commercial) n=16-17/group	IV	Cynomolgus monkey	Randomised, blinded, placebo-controlled, parallel-group, trigger-to-treat (dosing upon positive PA-ECL or time) study of drug products made from commercial and pilot batches.
AP204 GLP Pilot	N	0, 4, 16 n=16/group	IV	Cynomolgus monkey	Randomised, blinded-to-group, placebo-controlled, parallel-group, trigger-to-treat (dosing upon positive PA-ECL or time), dose-ranging study in anthrax-challenged animals.
AP201 GLP Pilot	N	0, 4, 8 n=14-15/group	IV	Cynomolgus monkey	Randomised, blinded-to-group, placebo-controlled, parallel-group, trigger-to-treat (dosing upon positive PA-ECL or time), dose-ranging study in anthrax-challenged animals.
AP203 GLP Commercial	N	0, 8, 32 n=16/group	IV	Cynomolgus monkey	Randomised, blinded, placebo-controlled, parallel-group, trigger-to-treat (dosing upon positive PA-ECL or time), dose-ranging study in anthrax-challenged animals.
AR021 GLP Pilot	N	0, 1, 4, 16 n=9 (0, 1) n=17 (4, 16)	IV	NZW rabbit	Randomised, open-label, placebo- and positive-controlled (levofloxacin), parallel group, trigger-to-treat (dosing upon positive PA-ECL, elevated body temperature, or time), dose-ranging study in anthrax-challenged animals.
AR033 GLP Pilot	N	0, 1, 4, 8, 1 n=14/group	IV	NZW rabbit	Randomised, blinded, placebo-controlled, parallel group, trigger-to-treat (dosing upon positive PA-ECL, elevated body temperature, or time), dose-ranging study in anthrax-challenged animals.
1030 Non-GLP Pilot	Y (human equivalent dose)	Untreated (N=6), 8 8 + Levofloxacin 50 x 3d Levofloxacin 50 x 3d n=16/group	IV PO (Levofloxacin)	NZW rabbit	Randomised, blinded, placebo-controlled, parallel group, trigger-to-treat (dosing upon positive PA-ECL, elevated body temperature, or time), dose-ranging study in anthrax-challenged animals. Exploratory study.
1045 Non-GLP Pilot	Y (human equivalent dose)	Untreated (N=8) 8 mg/kg 8 + Levofloxacin 50 x 3d Levofloxacin 50 x 3d n=16/group	IV PO Levofloxacin	NZW rabbit	Randomised, controlled, open-label, parallel-group, factorial design study; dose received 72 hours after anthrax exposure. Exploratory study.
1056 Non-GLP Pilot	Y (lower than HED)	Untreated, 8 mg/kg, 8 + Cipro 10 x 4 days Cipro 10 x 4 days n=8-16/group	IV PO Ciprofloxacin	Cynomolgus monkey	Randomised, controlled, open-label, parallel-group, factorial design study; dose received upon positive PA-ECL (ETI-204 only treatment) or 24±12 hours after positive PA-ECL (ETI-204 + cipro; cipro alone). Exploratory study.
2469 Non-GLP Pilot	Y (lower than HED)	Untreated 8 mg/kg + cipro10 mg/kg/day x 4 days Cipro 10, 26 mg/kg/day x 4 days	IV PO	Cynomolgus monkey	Randomised, controlled, open-label, parallel-group, factorial design study; dose received 24±12 hours after positive PA-ECL. Exploratory study.

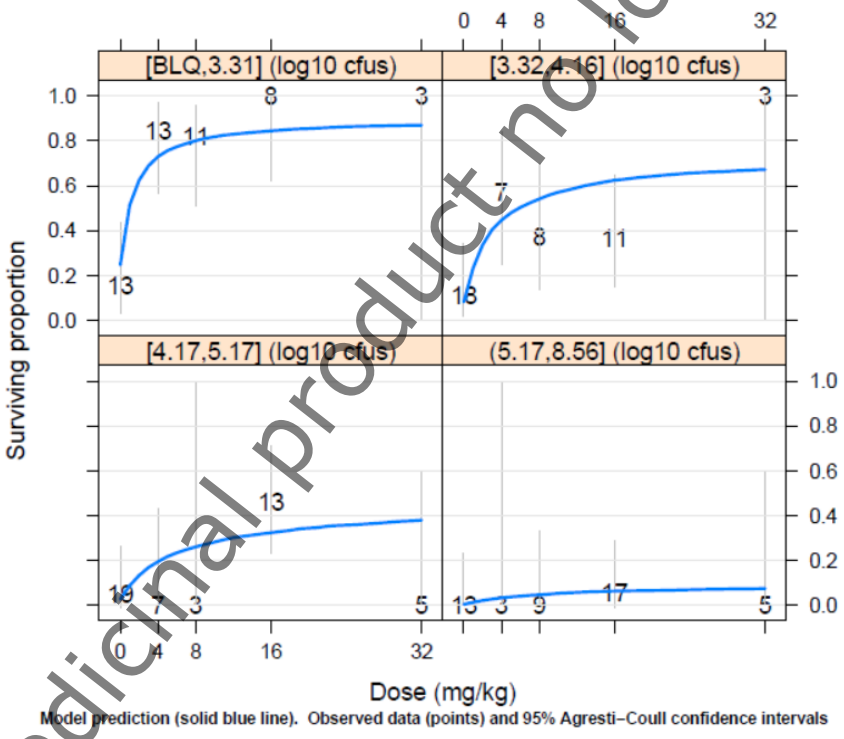
Study ID GLP status Product (batch)	Combination with antibiotics (Y/N)	Doses mg/kg	Route of administration	Species	Study design
AP-10-055 Non-GLP Pilot	Y (lower than HED)	Saline control (n=4) Doxy 2 mg/kg/day x 3 days bid IV ETI-204 8 + doxy 2 mg/kg/day x 3 days bid IV n=10/group	IV IV Doxycycline	NZW rabbit	Randomised, controlled, open-label, parallel-group, factorial design study; dose received at detection of elevated PA. Exploratory study.
AR028 Non-GLP Pilot	Y (lower than HED)	0 (n=12) 16 + Levo 6.5 x 3d (n=34) Levo 6.5 x 3d (n=38)	IV PO Levofloxacin	NZW rabbit	Randomised, controlled, open-label, parallel-group, factorial design study; dose received 72 hours after anthrax exposure. Exploratory study.
Post-exposure prophylaxis studies					
AP107 GLP Pilot	N	0, 2, 8 mg/kg, IV 4, 8 mg/kg, IM n=8-9/group	IV/IM	Cynomolgus monkey	Randomised, open-label, placebo-controlled, parallel group, IV and IM ETI-204 dose-ranging study (dosing at 24 hrs following *B. anthracis* spore exposure)
AP301 GLP Commercial	N	0, 8, 16 mg/kg n=6/group	IM	Cynomolgus monkey	Randomised, blinded, placebo-controlled, parallel group, IM ETI-204 dose-ranging study (dosing at 18, 24, and 36 hrs following *B. anthracis* spore exposure). Primary objective was assessment of PK not to evaluate efficacy.
AP307 Non-GLP Commercial	N	0, 16 mg/kg n=14-16/group	IM	Cynomolgus monkey	Randomised, open-label, placebo-controlled, parallel group, IM ETI-204 study (dosing at 24, 36, and 48 hrs following *B. anthracis* spore exposure)
AR004	N	4 mg/kg	IV	NZW rabbit	24, 36, 48h post-exposure – exploratory only
AR012	N	1, 2, 4, 8, 15 mg/kg	IV/IM	NZW rabbit	24h post-exposure – exploratory only
AR0315	N	4, 16 mg/kg	IM	NZW rabbit	18, 24h post-exposure – exploratory only
AR035	N	16 mg/kg	IM	NZW rabbit	18, 24, 36h post-exposure – exploratory only
AR037	N	8, 16, 32 mg/kg	IM	NZW rabbit	24h post-exposure – exploratory only
AR007 GLP	Y (human equivalent dose)	0, 4, 8 Levofloxacin 50 x 5d	IV/IM PO Levofloxacin	NZW rabbit	Randomised, controlled, open-label, parallel-group, factorial design study; dose received 9 hours after anthrax exposure
Rechallenge study					
AR034 Non-GLP Commercial	Y (human equivalent dose)	0 plus no Levo 0 + Levo 50 mg/kg/day 16 16 + Levo 50 mg/kg/day x 3d	IV PO Levofloxacin	NZW rabbit	Randomised, controlled, open-label, parallel-group, factorial design study; dose received 30 hours after anthrax exposure
Pre-exposure prophylaxis studies					
AP305	N	16 mg/kg	IM	Cynomolgus monkey	24, 48, 72h pre-exposure – out of scope for this application
AR001	N	4 mg/kg	IV	NZW rabbit	45 min pre-exposure – out of scope for this application
AR003	N	0.5, 1, 2, 4, 8 mg/kg	IV/IM	NZW rabbit	35 min pre-exposure – out of scope for this application

2.5.1. Dose selection

The maximally efficacious dose was identified empirically across the range of IV doses that were evaluated in monotherapy studies in both species.

The selection of 16 mg/kg as the maximally efficacious dose in animals was, as stated by the applicant, supported by population PD modelling which included an assessment of the effect of covariates, including dose, species, and prior-to-treatment bacteraemia on survival. The modelling suggests an inverse correlation between survival and prior-to-treatment quantitative bacteraemia (figure below). Two of the included studies (AP201 and AP204) were not completely blinded and the applicant was asked to clarify whether this could have had any impact on the outcome of the studies. Based on the applicant's response to this issue, it appears that there was no obvious impact of the two blinding methods on survival outcomes for the control group albeit some effects were observed for euthanised animals (difference of 21%). Overall, the survival in the control groups across the performed studies in monkey was stated to be consistent with expected survival in untreated animals based on published literature and natural history studies and the blinding schema did not appear to impact the overall survival outcomes for the control group. Thus, the inclusion of all four monkey studies (AP201, AP204, AP203 and AP202) and separate analysis from rabbit data in the PK modelling appears acceptable.

Figure 12 **ETI-204 dose-response in monkeys (studies AP201, AP204, AP203 and AP202) stratified by level of prior-to-treatment bacteraemia**



The four quadrants represent quartiles of the prior-to-treatment bacteraemia distribution across all 4 studies. Markers indicate the total number of animals (monkeys) at each observed mean data point; the prediction based on modelling of survival data and dose (solid blue line) is overlaid. Grey vertical lines represent 95% confidence intervals. CFU=colony forming units/mL.

Based on the models, it has been suggested that 8 mg/kg is close to the plateau of the modelled dose-response relationship, providing approximately 90% of the maximal response. Therefore, an 8 mg/kg dose should also be expected to confer a near-maximal efficacious response; however, the 16

mg/kg dose exceeds the minimum effective dose as well as the ED90. The selected human dose of 16 mg/kg administered IV was based on a comparison of systemic exposures in humans to those in animals (NHP) at the maximally efficacious animal dose of 16 mg/kg IV.

After IV administration of a 16 mg/kg dose of ETI-204 in humans, serum ETI-204 C_{max} values were comparable to the exposure in animals (rabbit and monkey, pooled results), suggesting that this dose provides maximum initial concentrations was sufficient to neutralise systemic PA. After a 16 mg/kg IV dose, $AUC_{(0\text{-}inf)}$ values were at least twice as high in humans as in infected animals and ETI-204 serum concentrations are sustained at higher levels over a longer period of time in humans than in animals.

Table 12. Comparison of ETI-204 PK Parameters after a 16 mg/kg IV Dose in Humans (from a Simulated Population of 500 Healthy and Infected Subjects) to Observed Values in Infected Monkeys (Studies AP204 and AP202) and Infected Rabbits (Study AR033)

	C_{max} (µg/mL)	$AUC_{(0\text{-}inf)}$ (µg·d/mL)
Infected monkeys[a]	408 (237, 589)[b]	1870 (613, 2458)[c]
Infected rabbitsa	402 (279, 517)[d]	958 (867, 1042)[e]
Healthy humans	363 (265, 503)	4980 (3240, 6960)
Infected humans	357 (257, 486)	4070 (2370, 6040)

Values are mean (5th and 95th percentile).
[a] All animals at 16 mg/kg (survivors and nonsurvivors)
[b] N = 27.
[c] N = 19.
[d] N = 14.
[e] N = 6.

The exposure in healthy NHP has not been presented for the recommended dose 16 mg/kg, therefore a direct comparison between exposure in infected and healthy animals is not possible without further calculations. In addition, due to bioanalysis issues (e.g. interference by target) such comparisons must be done with caution. The dose selection is further discussed in the clinical section for PK/PD.

2.5.2. Main studies

Monotherapy studies (AR033, AP204, AP202 and AP301)

To evaluate activity of the mAb ETI-204, **four monotherapy studies** including rabbit and monkey were provided which were GLP-status and blinded. Those are considered to constitute the pivotal efficacy studies and are listed in the table below. Of these, Study AP204 was only blinded to group but the applicant showed that this did not have any impact on the outcome of the study and this study is therefore considered as pivotal. Other monotherapy studies were also provided, however, since they investigated dose levels other than 16 mg/kg, they were unblinded or without GLP-status, they are considered supportive (see section of supportive studies).

Table 13. Overview of the Main Efficacy Studies Evaluating ETI-204 Administered as Monotherapy

Study Number GLP status Product (batch)	Species/Strain	Study Design	Treatment Regimen and Dose	Total No. Animals[1]
AP204 GLP Pilot	Monkey/cynomolgus	Randomised, blinded-to-group, placebo-controlled, parallel-group, trigger-to-treat (dosing upon positive PA-ECL or time), dose-ranging study in anthrax-challenged animals. ETI-204 was administered 39.2 hrs to 44.4 hrs post-challenge among the groups. Follow-up period was 28 or 56 days.	ETI-204 IV: 0 mg/kg 4 mg/kg 16 mg/kg	 16 16 16
AP202 GLP Commercial, Pilot	Monkey/cynomolgus	Randomised, blinded, placebo-controlled, parallel-group, trigger-to-treat (dosing upon positive PA-ECL or time) study of drug products from commercial and pilot batches. ETI-204 was administered 38.9 hrs to 39.3 hrs post-challenge among the groups. Follow-up period was 28 days.	ETI-204 IV: 0 mg/kg 16 mg/kg (pilot) 16 mg/kg (commercial)	 17 17 16
AP301 GLP Commercial	Monkey/cynomolgus	Randomised, blinded, placebo-controlled, parallel group, IM ETI-204 dose-ranging study (dosing at 18, 24, and 36 hrs following *B. anthracis* spore exposure). Follow-up period was 28 days Primary objective of this study was to examine the PK of two different doses (16 and 8 mg/kg) of ETI-204 when administered IM as a single injection.	ETI-204 IM: 0 mg/kg (18 hrs) 8 mg/kg (18 hrs) 16 mg.kg (18 hrs) 8 mg/kg (24 hrs) 16 mg/kg (24 hrs) 8 mg/kg (36 hrs) 16 mg/kg (36 hrs)	 6 6 6 6 6 6 6
AR033 GLP Pilot	Rabbit/NZW	Randomised, blinded, placebo-controlled, parallel group, trigger-to-treat (dosing upon positive PA-ECL, elevated body temperature), dose-ranging study in anthrax-challenged animals. Treatment was initiated 25.8 hrs to 27.7 hrs post-challenge. Follow-up period was 28 days.	ETI-204 IV: 0 mg/kg 1 mg/kg 4 mg/kg 8 mg/kg 16 mg/kg	 14 14 14 14 14

[1]Total number of animals randomised to treatment. BDS: bulk drug substance; GLP: Good Laboratory Practices; IM: intramuscular; IV: intravenous; kg: kilogram; mg: milligram; NZW: New Zealand White; PA-ECL: protective antigen electrochemiluminescence; PK=pharmacokinetics; po=oral.

Methods

The efficacy of ETI-204 as monotherapy treatment for inhalational anthrax was investigated after a single IV dose in one study in NZW rabbits (AR033, dose range: 0, 1, 4, 8 and 16 mg/kg, IV) and in three studies including cynomolgus monkeys (AP202, AP204 and AP301, dose range: 0, 4, 8, 16 mg/kg, IV). The studies were stated to be randomised, placebo-controlled, parallel-group studies in which IV ETI-204 alone was administered to rabbits or monkeys, which were exhibiting clinical signs or symptoms of systemic anthrax. All studies had GLP status and were blinded. Study AP204 was only blinded to group but the applicant showed that this did not have any impact on the outcome of the studies and this study is thus considered as pivotal. Two different formulations of ETI-204 were used (pilot and commercial). In two monkey studies (AP202 and AP301) animals were treated with the

commercial formulation whereas ETI-204 in the pilot formulation was administered in rabbits (AR033) as well as in monkeys (Studies AP204 and AP202).

Animals in the ETI-204 monotherapy studies were aerosol challenged with an approximate *B. anthracis* (Ames strain) spore dose of 200 LD50 (±50%).

The animals were observed every 6 hours beginning 18 hours (rabbits) or 24 hours (monkeys) after the median challenge time for all animals on a given challenge day and ending at either 168 hours (7 days; rabbits) or 192 hours (8 days; monkeys) after the median challenge time, for mortality and outward clinical signs of anthrax disease such as lethargy, respiratory distress, moribundity, activity (i.e., recumbent, weak, or unresponsive), seizures. After study Day 7 in rabbits and study Day 8 in monkeys, animals were observed twice daily until the day of euthanasia when they were observed once immediately prior to euthanasia. All surviving animals were euthanised on Day 28.
Animals who exhibited any of the following criteria during the monitoring period were immediately euthanised:

- Presence of any hyperactivity or seizure denoting primary central nervous system disease
- Respiratory distress, dyspnoea, or forced abdominal respirations
- Unresponsive to touch or external stimuli
- Moribundity

If all following clinical signs listed below were observed in any of the treatment studies in monkeys, illness was considered irreversible and the animal was euthanised:

- Recumbency and weakness
- ≥20% body weight loss
- Total anorexia with a duration > 48 hours

Criteria for euthanasia because of irreversible disease were not established in the rabbit studies because of the rapid progression of disease and death in this species.

Treatments

In study AP301 treatment was initiated at different time points; 18 h, 24 h, 36 h post exposure.

In the studies AP202, AP204 and AR033, treatment of ETI-204 was triggered by the following clinical signs or symptoms of systemic anthrax disease:

- A significant increase in body temperature, defined as ≥ two standard deviation (SD) increase from baseline temperature either three consecutive times or two consecutive times twice (SIBT). SIBT was not used as a treatment trigger in monkeys because of their strong diurnal temperature rhythms
- A SIBT, positive results in a PA-ECL assay (≥ 1 ng/mL positive control) developed by NIAID or 54 hours (AR033) or 72 hours (AR021) after challenge in rabbits (whichever occurred first). SIBT was defined as a temperature reading
- Positive results in a PA-ECL assay (≥ 2 ng/mL positive control) developed by NIAID or 54 hours after challenge in monkeys (whichever occurred first)

These triggers were selected based on results from the natural history studies which demonstrated that SIBT was a reliable indicator of an inhalational anthrax infection in NZW rabbits and detectable

serum PA coincides with detection of bacteraemia, without the time lag associated with bacterial cultures, in both NZW rabbits and cynomolgus monkeys.

Objectives

To evaluate efficacy of ETI-204 in monotherapy studies for the treatment of inhalational anthrax after single IV administration in cynomolgus monkeys and rabbits.

Outcomes/endpoints

Primary endpoint

The primary efficacy endpoint in the monotherapy treatment studies was the survival rate at the end of the study.

Secondary endpoint

The secondary efficacy endpoint was survival time.

Bacteraemia

Quantitative bacteraemia (CFU/mL blood) was assessed in studies AR033, AP202, AP204 and AP301 as a PD parameter since the appearance of bacteria in the blood signifies a systemic anthrax infection and likely infection of peripheral organs. Thus, bacteraemia is also considered a reliable marker of the severity of disease [Weis et al, 2011].

Randomisation and blinding (masking)

The studies AR033, AP202 and AP301 were blinded according to the following procedure. The Study Director, Sponsor, microbiologists, pathologist, neuropathologist (i.e., Tox Path Specialists, LLC), technicians performing the dosing, and all technicians assessing the animals were blinded to the contents of the dosing vials and animal group assignments. Once animals were randomised to a group, challenge day, and challenge order, the results were sent to the Study Coordinator and Quality Assurance (QA) Auditor. The QA Auditor verified the randomisation and then provided the Study Director with the randomisation information regarding animal ID, challenge day, and challenge order but not the group assignments. Group assignments remained blinded to the Study Director and Sponsor until sample analysis was complete and data summary tables were audited ("database lock"). Once the summary tables audit was complete, the group assignments were released to the Study Director, but the Study Director did not release the group assignment information to the study pathologist or neuropathologist.

Study AP204 was blinded to group only. The applicant was asked to clarify whether this could have had any impact on the outcome of the studies. Based on the applicant's response to this issue, it appears that there was no obvious impact of the two blinding methods on survival outcomes for the control group albeit some effects were observed for euthanised animals (difference of 21%). Overall, the survival in the control groups across the performed studies in monkey was stated to be consistent with expected survival in untreated animals based on published literature and natural history studies and the blinding schema did not appear to impact the overall survival outcomes for the control group. Thus, the inclusion of study AP204 as pivotal study appears acceptable.

Statistical methods

Sample Size

The sample size for each study was based upon the ability to detect a statistically significant difference in survival rate between the control and ETI-204 groups. Table below summarises for each study the hypothesised survival rate for each group, the number of animals per group, and the resultant power based upon a specific statistical test at a predetermined significance level.

Table 14. Hypothesised Survival Rates for the IV Administration of ETI-204 as Monotherapy

Study	Statistical Test	Hypothesised Survival Rate	Number per Group	Power
AR021	Fisher's exact test versus control one-sided, α=0.05	Control: 5% 4, 16 mg/kg: 55% Levofloxacin: 65%	10 17 10	- 81.3% 86.1%
AR033	Fisher's exact test versus control two-sided, α=0.05	Control: 5% 1 mg/kg: not specified 4, 8, 16 mg/kg: 70%	14 14 14	- - 83.4%
AP201	Fisher's exact test versus control one-sided, α=0.05	Control: 10% 4, 8 mg/kg: 65%	15 15	- 82.5%
AP204	Fisher's exact test versus control two-sided, α=0.05	Control: 10% 4, 16 mg/kg: 65%	16 16	- 80.9%
AP203	Fisher's exact test versus control two-sided, α=0.05	Control: 10% 8, 32 mg/kg: 65%	16 16	- 80.9%
AP202	Boschloo's exact test versus control one-sided, α=0.025	Control: 10% 16 mg/kg[1]: 55%	17 17	- 83.5%

[1]Drug products made from pilot and commercial batches.

Calculations were conducted using the statistical package StatXact. Sample size calculations did not include adjustments for multiple comparisons.

Analysis populations

Statistical analyses for the monotherapy treatment studies were conducted using a modified Intent-to-Treat (mITT) population, defined as all randomised animals that were positive for bacteraemia prior to study treatment.

Primary analysis

The intended primary statistical method for the primary endpoint was either Fisher's exact test or the Boschloo's exact test with a Berger-Boos correction of gamma = 0.001. Statistical significance was declared at either the 0.025 (one-sided) or 0.05 (two-sided) level.

The secondary efficacy endpoint was survival time. In the monotherapy treatment studies, survival time was summarised both from the time of spore challenge and from the time of treatment.

Survival curves were plotted for each group. Pairwise comparisons to the control group were conducted and p-values for both the Wilcoxon test and the log rank test were presented.

Results

Results, main monotherapy studies (AP204, AP202, AP301 and AR033)

Survival rate (primary endpoint)

Table 15. Survival rates in IV ETI-204 monotherapy studies in Cynomolgus monkeys (AP202 and AP204)

	Monkeys (56 days)[1] Study (AP204)			Monkeys (28 days)[1] Study (AP202)		
	Number (%) Survival	p-value[4]	95% CI[5]	Number (%) Survival	p-value[4]	95% CI[5]
Placebo	1/16 (6.3%)	-	-	0/17 (0%)	-	-
ETI-204 4 mg/kg IV	4/16 (25.0%)	0.1074	(-0.090, 0.473)	-	-	-
ETI-204 16 mg/kg IV	7/15 (46.7%)	0.0068*	(0.089, 0.681)	5/16 (31.3%)[2] 6/17 (35.3%)[3]	0.0085* 0.0055*	(0.079, 0.587) (0.113, 0.617)
Geometric mean bacteraemia at PTT (95% CI) (CFU/mL)**	8.27×10^3 (3.89×10^3, 1.76×10^4)			1.50×10^5 (6.26×10^4, 3.59×10^5)		

CI: confidence interval; IV: intravenous; PTT: prior to treatment.
[1]Survival assessed after spore challenge (28-56 days).
[2]Commercial product.
[3]Clinical product.
[4]p-value is from 1-sided Boschloo Test (with Berger-Boos modification of gamma=0.001) compared to placebo.
[5]Exact 95% confidence interval of difference in survival rates.
*Denotes statistical significance at the 0.025 level.
**Only 1 monkey with blood bacteria concentrations $\geq 1 \times 10^5$ CFU/mL survived to the scheduled sacrifice in any of the studies.

Table 16. Survival rates in IM ETI-204 monotherapy studies in Cynomolgus Monkeys (AP301)

	Monkeys (28 or 56 days)[1] Study AP301			
	Number (%) Survival	p-value[2]	95% CI[3]	Proportion (%) Bacteraemic Animals
Placebo[4]	0/6 (0%)	-	-	0/6 (0%)
8 mg/kg IM at 18 hours	6/6 (100%)	0.0012*	(0.471, 1.000)	0/6 (0%)
16 mg/kg IM at 18 hours	6/6 (100%)	0.0012*	(0.471, 1.000)	0/6 (0%)
8 mg/kg IM at 24 hours	5/6 (83%)	0.0042*	(0.230, 0.996)	1/6 (16.7%)
16 mg/kg IM at 24 hours	5/6 (83%)	0.0042*	(0.230, 0.996)	1/6 (16.7%)
8 mg/kg IM at 36 hours	0/6 (0%)	1.0000	-	6/6 (100%)
16 mg/kg IM at 36 hours	3/6 (50%)	0.0345	(-0.037, 0.882)	5/6 (83.3%)

IM: intramuscular.
[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.
[2]p-value is from 1-sided Boschloo Test (with Berger-Boos modification of gamma=0.001) compared to control.
[3]Exact 95% confidence interval of difference in survival rates.
[4]Treatment with placebo was at 18 hours for AP301 and 24 hours for AP307.
*Denotes statistical significance at the 0.025 level.

Table 17. Survival rates in IV ETI-204 monotherapy studies in NZW Rabbits (AR033)

	AR033	
	% (proportion)	p-value[1] Fisher Boschloo
Placebo	0 (0/13)	
1 mg/kg ETI-204 IV	16.7% (2/12)	0.220 0.1187
4 mg/kg ETI-204 IV	33.3% (4/12)	0.0391 0.0232*
8 mg/kg ETI-204 IV	69.2% (9/13)	0.0002* 0.0011*
16 mg/kg ETI-204 IV	61.5% (8/13)	0.0008* 0.0013*
Levofloxacin 50 mg/kg[2]		

[1]Compared to placebo.
[2]Once daily for 3 days.
*Denotes statistical significance at the 0.025 level. IV: intravenous; kg: kilogram; mg: milligram.

Survival time (secondary endpoint)

The survival time plots by treatment groups (from treatment to death) for the main efficacy studies are shown below.

Figure 13 Survival time in IV ETI-204 monotherapy studies in NZW Rabbits (AR033)

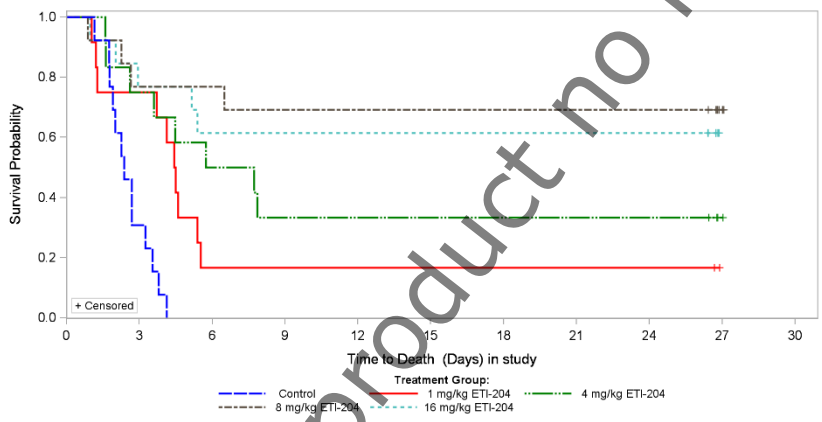


Figure 14 Survival time in IV ETI-204 monotherapy studies in monkeys (AP204)

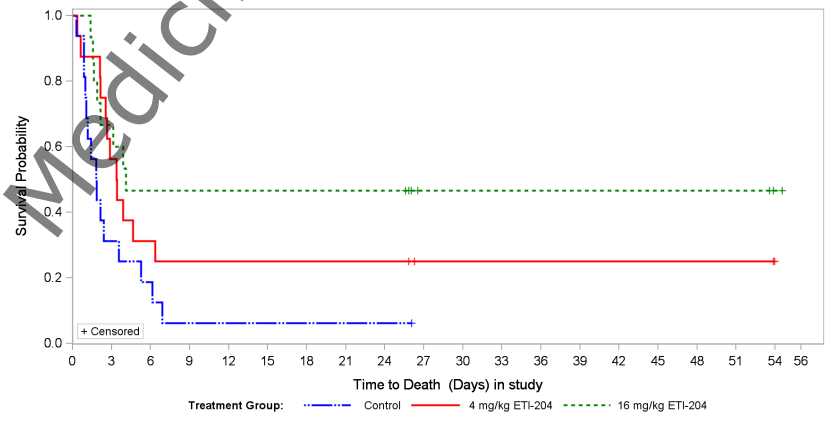


Figure 15 Survival time in IV ETI-204 monotherapy studies in monkeys (AP202)

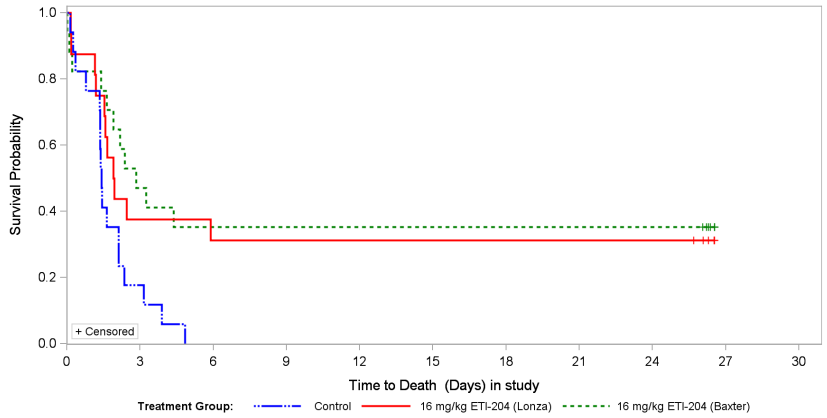
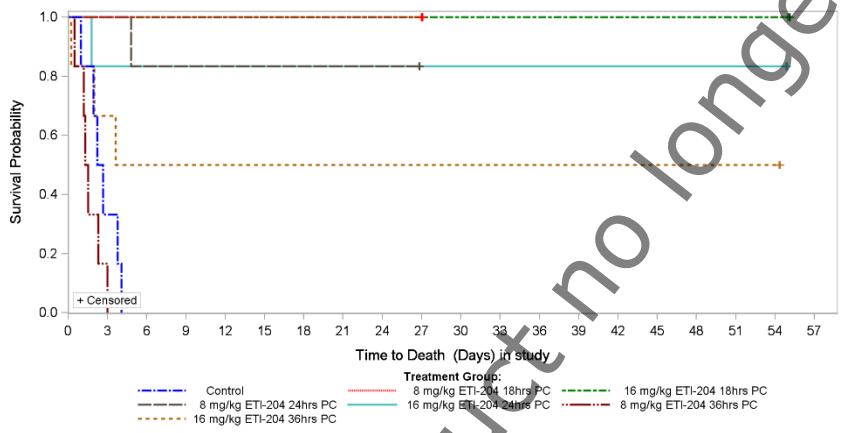


Figure 16 Survival time in IM ETI-204 monotherapy studies in monkeys (AP301)



Bacteraemia

A comparison of the bacteraemia concentrations in the IV ETI-204 monotherapy treatment studies in cynomolgus monkeys as well as to a BARDA meta-analysis is provided in table below. The BARDA meta-analysis was performed to demonstrate the ability of the NZW rabbit and nonhuman primate models to show an added benefit of adjunct therapies over antibiotics for therapeutic treatment of anthrax and to achieve a better understanding of these models. Data from 14 separate studies were included in the meta-analysis. It should however be kept in mind that only AP204, AP202 and AP301 were considered as main studies since they were blinded and had GLP-status.

Animals in AP204, AP203, and AP202 had higher bacteraemia concentrations immediately prior to treatment than animals in AP201. Geometric mean CFU/mL in each group in AP203 and AP202 were almost a log10 higher than in the groups in AP204 and the geometric mean CFU/mL in each group in AP204 were almost a log10 higher than AP201. Additionally, there were more animals with bacteraemia concentrations ≥1 x 10^5 CFU/mL immediately prior to treatment in AP203 and AP202 than in AP204 and AP201. AP201 had the smallest number of animals with bacteraemia concentrations ≥1 x 10^5 CFU/mL.

Treatment was triggered for all animals in study AP203 and for most of the animals in AP201 and AP202 by a positive result in the PA-ECL assay. One animal (7.1%) in the placebo group and 2 animals (14.3%) in the 4 mg/kg group in AP201, 2 animals (12.5%) in the 16 mg/kg group in AP204, and 1

animal (6.3%) in AP202 (16 mg/kg commercial batch) were triggered by time. The median time to trigger and median time to positive bacteraemia were similar across the groups including monkeys (the average time from challenge until treatment ranged from 36.2 hr to 37.5 hr (Study AP203), 34.1 hr to 35.1 hr (Study AP202), 41.3 hr to 44.5 hr (Study AP203), respectively.

Table 18. Comparison of IV ETI-204 monotherapy treatment studies AP201, AP204, AP203, AP202 in Cynomolgus monkeys

	Study Number				
	AP202[a]	**AP203**	**AP204**	**AP201**	**BARDA Meta-analysis**
Median time to death, controls (95% CI), all animals (hours)	75.3 (59.3, 87.0)	74.08 (59.53, 96.23)	81.93 (62.38, 126.42)	134.48 (73.32, 209.70)	91.94 (NC)
Geometric mean time to death, controls (95% CI), nonsurvivors only (hours)	75.68 (62.16, 92.14)	70.08 (55.86, 87.92)	86.75 (66.53, 113.1)	110.40 (78.85, 158.50)	91.58 (85.67, 97.90)
Geometric mean bacteraemia at PTT (95% CI) (CFU/mL)	1.50e+05 (6.26e+04, 3.59e+05)	5.57e+04 (2.2e+04, 1.39e+05)	8.27e+03 (3.89e+03, 1.76e+04)	1.67e+03 (8.22e+02, 3.38e+03)	NA
Geometric mean time to abnormal PA-ECL (95% CI) (hours)	35.08 (33.69, 36.47)	32.73 (31.41, 34.11)	36.77 (34.94, 38.70)	37.81 (35.31, 40.49)	35.97 (34.68, 37.31)
Geometric mean time to abnormal bacteraemia (95% CI) (hours)	28.59[b] (27.13, 30.05)	29.21 (27.79, 30.70)	30.91 (29.14, 32.80)	35.07 (33.17, 37.07)	35.51 (34.21, 36.85)
Fraction CFU/mL $>10^5$ at PTT, % (n/N)	45% (23/51)	42% (20/48)	19% (9/48)	7% (3/44)	NA

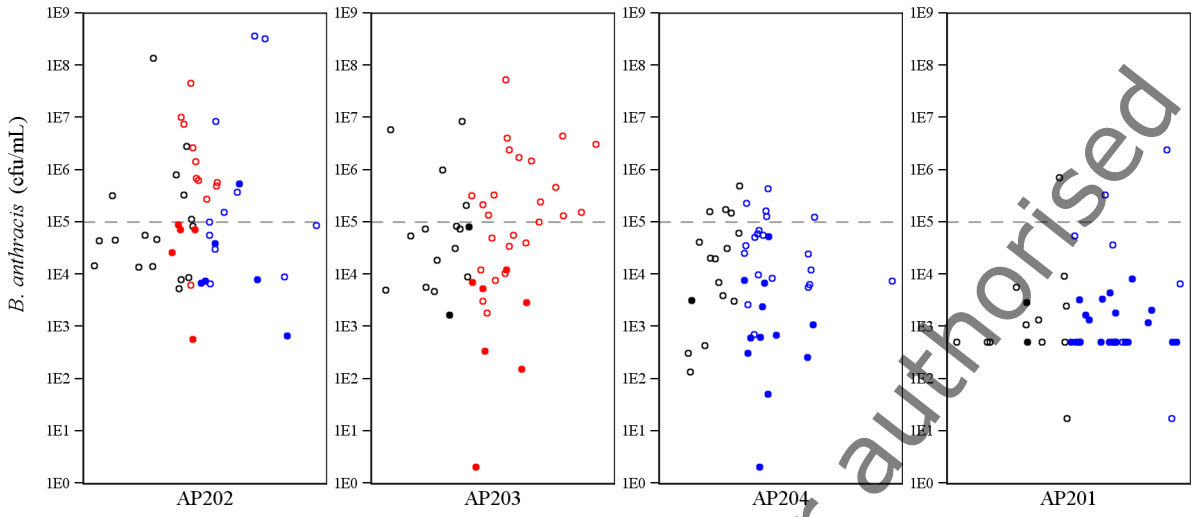
[a]Includes 1 challenged animal that died after PTT sample collection and prior to treatment administration. This animal was not randomised to a treatment group.
[b]By enriched bacteraemia.
IV: intravenous; CI: confidence interval; CFU/mL: colony forming units/milliliter; PA-ECL: protective antigen electrochemiluminescence; PTT: prior to treatment; NA: not available.

Table 19. Bacterial level (CFU/mL) before treatment in IV ETI-204 monotherapy treatment studies in Cynomolgus monkeys

	AP201		AP204		AP203		AP202			
	$<10^5$ CFU/mL n (%)	$\geq10^5$ CFU/mL n (%)	$<10^5$ CFU/mL n (%)	$\geq10^5$ CFU/mL n (%)	$<10^5$ CFU/mL n (%)	$\geq10^5$ CFU/mL n (%)	$<10^5$ CFU/mL Lonza n (%)	$\geq10^5$ CFU/mL Lonza n (%)	$<10^5$ CFU/mL Baxter n (%)	$\geq10^5$ CFU/mL Baxter n (%)
Placebo	13 (92.9)	1 (7.1)	12 (75.0)	4 (25.0)	12 (75.0)	4 (25.0)	11 (64.7)	6 (35.3)	11 (64.7)	6 (35.3)
4 mg/kg IV ETI-204	13 (92.9)	1 (7.1)	13 (81.3)	3 (18.8)						
8 mg/kg IV ETI-204	14 (93.3)	1 (6.7)			6 (37.5)	10 (62.5)				
16 mg/kg IV ETI-204			14 (87.5)	2 (12.5)			6 (37.5)	10 (62.5)	11 (64.7)	6 (35.3)
32 mg/kg IV ETI-204					10 (62.5)	6 (37.5)				

CFU/mL: colony forming units/milliliter; IV: intravenous; kg: kilogram; mg: milligram.

Figure 17. Prior-to-Treatment bacteraemia values in survivors and non-survivors in IV ETI-204 monotherapy treatment studies AP201, AP204, AP203, and AP202 in monkeys



cfu = colony forming unit. Shown are bacteraemia levels for individual animals at PTT (Prior to Treatment). Terminal samples are not included. Filled symbols represent the survivors and open symbols represent the non-survivors; Blue symbols represent ETI-204 (pilot); Red symbols represent ETI-204 (commercial); Black symbols represent Placebo. Dotted line is drawn at 105(1E5) cfu/ml to visually indicate animals with PTT bacteraemia above or below that level. Limit of detection (LOD) and limit of quantification (LOQ) value in each study were as follows:
AP201: LOD = 33 cfu/mL and LOQ = 1000 cfu/mL, respectively; for computational purposes, values below LOD are replaced with 17 cfu/mL (1/2 LOD) and values below LOQ are replaced with 500 cfu/mL (1/2 LOQ). AP204 and AP203: LOD = 3 cfu/mL and LOQ = 100 cfu/mL, respectively; for computational purposes, values below LOD are replaced with 2 cfu/mL (1/2 LOD rounded to the nearest integer); values below LOQ are replaced with 50 cfu/mL (1/2 LOQ). AP202: LOD = 3 cfu/mL; for computational purposes, values below LOD are replaced with 2 cfu/mL (1/2 LOD rounded to the nearest integer) and values reported as positive are replaced with 50 cfu/mL.

In the rabbit study AR033, geometric mean *B. anthracis* and PA concentrations before treatment were similar across the groups. Most animals in each group had *B. anthracis* concentrations < 1 x 105 CFU/mL before treatment (see table below).

Table 20. Time from challenge until a positive bacteraemia result and quantitative bacteraemia in rabbits (AR033)

Group	Treatment	N[a]	Geometric Mean (Hours)[b] (95% Confidence Interval)	PTT Geometric Mean for Quantitative Bacteremia (% CV)
4	Saline	14/14	24.29 (21.99, 26.83)	7.06×10^2 (1.07×10^5)
3	1 mg/kg ETI-204	12/14	25.28 (23.45, 27.25)	1.31×10^3 (2.82×10^5)
1	4 mg/kg ETI-204	14/14	27.99 (21.98, 35.66)	1.94×10^3 (5.60×10^4)
5	8 mg/kg ETI-204	14/14	24.58 (22.48, 26.87)	2.05×10^3 (3.75×10^4)
2	16 mg/kg ETI-204	14/14	25.80 (23.63, 28.17)	1.36×10^3 (3.01×10^4)

[a] Number of animals positive (including quantitative, qualitative, and enriched bacteremia methods) / Number of animals per group
[b] Calculations included quantitative, qualitative, and enriched bacteremia methods

ETI-204 as concomitant treatment with antibiotic

Four studies of ETI-204 as concomitant treatment with antibiotics were conducted in the NZW rabbit model of inhalational anthrax, using human equivalent doses of antibiotics. These studies were open-label (unblinded) trials, and only one was executed in GLP-compliance.

An overview of ETI-204 studies with concomitant administration of antibiotics at human equivalent dose is provided in the table below. Four studies were also provided using significant lower doses of antibiotic; thus, they are considered supportive only.

Table 21. Overview of studies including ETI-204 concomitant administered with antibiotics (human equivalent dose) for the treatment of inhalational anthrax

Study Number GLP status Product (batch)	Species/Strain	Study Design	Treatment Regimen and Dose	Total No. Animals[1]
Human Equivalent Dosing				
NIAID Study 1030 Non-GLP Pilot	Rabbit/NZW	Randomised, controlled, open-label, parallel-group, factorial design study; dose received at 96 hours after anthrax exposure	ETI-204 IV: Untreated 8 mg/kg 8 mg/kg + levo 50 mg/kg/day x 3 days po levo 50 mg/kg/day x 3 days po	 6 16 16 16
NIAID Study 1045 Non-GLP Pilot	Rabbit/NZW	Randomised, controlled, open-label, parallel-group, factorial design study; dose received at 72 hours after anthrax exposure	ETI-204 IV: Untreated 8 mg/kg 8 mg/kg + levo 50 mg/kg/day x 3 days po levo 50 mg/kg/day x 3 days po	 6 16 16 16
AR034 (Phase 1) non-GLP Commercial	Rabbit/NZW	Randomised, controlled, open-label, parallel-group, factorial design study; dose received at 30 hours after anthrax exposure	ETI-204 IV: 0 mg/kg plus no levo 0 mg/kg + levo 50 mg/kg/day po 16 mg/kg 16 mg/kg + levo 50 mg/kg/day x 3 d po	 8 20 20 20
AR007 GLP Commercial	Rabbit/NZW	Randomised, controlled, open-label, parallel-group, factorial design study; dose received at 9 hours after anthrax exposure	ETI-204[2]: 0 mg/kg ~4 mg/kg IV ~4 mg/kg IV + levo50 mg/kg/day x 5 days po ~8 mg/kg IM ~8 mg/kg IM+ levo50 mg/kg/day x 5 days po levo 50 mg/kg/day x 5 days po	 9 9 9 9 9 12

[1]Total number of animals randomised to treatment.

[2]ETI-204 was given at fixed dose. The body weights of rabbits in this study ranged from 2.2 to 2.7 kg. The actual doses administered were 10 and 20 mg/rabbit, which were approximately equal to 4 and 8 mg/kg.

GLP: Good Laboratory Practices, IM: intramuscular; IV: intravenous; kg: kilogram; levo: levofloxacin; mg: milligram; NIAID: National Institute of Allergy and Infectious Disease; NZW: New Zealand White; po: oral.

Study design and objectives

Human Equivalent Antibiotic Dose Studies

The human equivalent antibiotic dose studies were randomised, controlled, open-label, parallel-group, factorial design studies that were conducted in NZW rabbits with levofloxacin. Only one of them had GLP status (AR007).

NIAID Studies 1030 and 1045 were exploratory studies that were conducted for development of an animal model to assess the additive benefit of an antitoxin in combination with an antimicrobial in the treatment of inhalational anthrax. Because antimicrobials alone achieve high survival rates in animal anthrax models when administered early, a delayed-treatment design was used in NIAID Studies 1030 and 1045. In the delayed-treatment design, the delay in treatment was intended by the applicant to result in rabbits with advanced inhalational anthrax and therefore, a reduced probability of survival.

NIAID Study 1030 was the first model development study including ETI-204 to be conducted with a delayed treatment regimen and a human equivalent antibiotic dose. The objective of NIAID Study 1030 was to investigate the feasibility of a 96-hour delayed-treatment model for an added benefit assessment with a human equivalent antibiotic dose.

NIAID Study 1045 was conducted after NIAID Study 1030 to investigate the feasibility of a 72-hour delayed treatment model for an added benefit assessment of ETI-204 with a human equivalent antibiotic dose.

AR034 was a *B. anthracis* re-challenge study conducted to investigate whether ETI-204 prevents the development of protective endogenous immunity to PA. The first phase of this study provides data on the effect of co-administration of ETI-204 and a human equivalent antibiotic dose at 30 hours following exposure to *B. anthracis* spores and thus, the results are presented in this section of combination studies with antibiotics.

AR007 was conducted to investigate the efficacy of ETI-204 administered IV or IM, alone or as concomitant treatment with a human equivalent antibiotic dose, in preventing inhalational anthrax when administered 9 hours following exposure to *B. anthracis* spores. The rationale for co-administration of an anti-toxin and antimicrobials in the post-exposure prophylaxis setting (9 hours after spore challenge) is based on findings in experimental animal models indicating that antibiotic treatment early after *B. anthracis* challenge may lead to persistence of spores and disease development following antibiotic cessation.

Lower than Human Equivalent Antibiotic Dose Studies

According to the applicant, the studies were randomised, controlled, open-label, parallel group, factorial design studies that were conducted as exploratory studies to develop an animal model that allowed for the detection of an interaction (additive, synergistic, antagonistic) between an antitoxin and antibiotics in the treatment of inhalational anthrax. Lower than human equivalent doses were selected for these studies with the rationale that this would allow for any possible interaction between ETI-204 and antibiotics to be more easily detected. A delayed treatment design was also used in these studies to reflect the projected clinical scenario in which antibiotics are not 100% effective when administered for the treatment of inhalational anthrax.

AR028 was an exploratory, model development study conducted with ETI-204 and a lower than human equivalent dose of oral levofloxacin in NZW rabbits. AR028 was conducted to investigate the feasibility of using a treatment delay of 72 hours after exposure to *B. anthracis* spores for detecting an interaction between ETI-204 and antibiotics.

NIAID Study AP-10-055 was conducted to develop a partial protection NZW rabbit model to evaluate the protective efficacy of adjunct antitoxin therapy with an additional antibiotic (i.e., IV doxycycline).

NIAID Study 1056 was an exploratory model development study in cynomolgus monkeys that was conducted to investigate the feasibility of delaying treatment relative to the onset of toxemia rather than exposure to *B. anthracis* spores. Delayed treatment with ETI-204 in combination with an antibiotic or an antibiotic alone relative to toxemia onset was used to determine if this synchronised the time of treatment with disease severity.

NIAID Study 1056 was conducted with oral ciprofloxacin.

NIAID Study 2469 was conducted after NIAID Study 1056 to continue model development in cynomolgus monkeys and to confirm that the results seen in NIAID Study 1056 were reproducible. Delayed treatment relative to the onset of toxemia was also used in this study. The study included two different doses of oral ciprofloxacin.

Randomisation and blinding

Animals in the antibiotic combination studies, except for NIAID Study AP-10-055, were weighed at the time of quarantine and then, within each gender, grouped into tertiles based upon their weight. Separate randomisations were conducted within each of the six strata defined by gender (male, female) and weight.

Animals were then randomised to a challenge day such that there was approximately an equal number of animals from each group on each challenge day. A randomisation was then conducted within each challenge day to randomise the order of challenge.

Randomisation was not performed in NIAID Study AP-10-055.

All the antibiotic combination studies were open-label studies and thus, blinding was not used.

Conduct

Spore challenge

Spore challenge occurred before the administration of study treatment in all the antibiotic combination studies.

Animals in all antibiotic combination studies were aerosol challenged on Day 0 with a targeted 200 LD50 dose of *B. anthracis* spores (Ames strain) aerosolised by a Collison nebuliser and delivered via a nose-only (rabbits) or head-only (monkeys) inhalation exposure chamber.

Minimum inhibitory concentration (MIC) testing was conducted in several of the antibiotic combination studies to confirm sensitivity of the challenge spore lot to either levofloxacin (AR028 and NIAID Studies 1030 and 1045) or ciprofloxacin (NIAID Studies 1056 and 2469). Additionally, bacteria isolated from the blood of animals that died on study were subjected to MIC testing to confirm susceptibility to either levofloxacin or ciprofloxacin.

The challenge spore lot used in NIAID Study AP-10-055 was also prepared using a standardised process for spore lot production and characterisation. The spore lot used in this study had a spore titre of 1.72 x 1010 spores/mL without significant vegetative cells or debris.

Monitoring Period

Human Equivalent Antibiotic Dose Studies

In NIAID Studies 1030 and 1045, animals were observed every 6 hours beginning 18 hours after the median challenge time for all animals and ending at 72 hours after the median challenge time for mortality and outward clinical signs of disease. After study Day 3, animals were observed twice daily until Day 28 when they were observed once immediately prior to scheduled euthanasia. In AR007, animals were observed twice daily for 34 days regarding mortality and outward clinical signs of anthrax disease until the day of euthanasia when they were observed once immediately prior to scheduled euthanasia.

In AR034 (Phase 1), animals were observed twice daily for mortality and outward clinical signs of anthrax disease. Survival in Phase 1 was assessed at 9 months.

Rabbits who exhibited any of the following during the monitoring period in NIAID Study 1030, NIAID Study 1045, AR007, or AR034 were immediately euthanised:

- Presence of any hyperactivity or seizure denoting primary central nervous system disease (ie, meningitis or encephalitis)
- Respiratory distress, dyspnea, or forced abdominal respirations
- Unresponsive to touch or external stimuli
- Moribundity

Lower than Human Equivalent Antibiotic Dose Studies

Rabbits were observed twice daily in AR028 for mortality and outward clinical signs of anthrax disease until Day 28 when they were observed once immediately prior to scheduled euthanasia.

In NIAID Study AP-10-055, appearance, natural behaviour, provoked behaviour, and neurological behaviour of the rabbits were observed and scored twice daily until Day 28 or Day 29 (scores of 0 were considered normal and scores > 0 indicated worsening). Rabbits with an elevated score (ie, score of 4-6) were monitored with increased frequency (3 times per day).

In NIAID Studies 1056 and 2469, monkeys were observed every 6 hours beginning 24 hours after the median challenge time for all animals and ending at 192 hours (8 days) after the median challenge time for mortality and outward clinical signs of anthrax disease. After study Day 8, monkeys were observed twice daily until Day 28 when they were observed once immediately prior to scheduled euthanasia.

Animals who exhibited any of the following criteria during the monitoring period in AR028, NIAID Study 1056, and NIAID Study 2469 were immediately euthanised:

- Presence of any hyperactivity or seizure denoting primary central nervous system disease
- Respiratory distress, dyspnoea, or forced abdominal respirations
- Unresponsive to touch or external stimuli
- Moribundity

In NIAID Study AP-10-055, animals with a score indicative of a very poor health status (ie, score ≥ 7) during the monitoring period were euthanised.

If all following clinical signs listed below were observed in either NIAID Study 1056 or NIAID Study 2469, illness was considered irreversible and the animal was euthanised:

- Recumbency and weakness
- ≥ 20% body weight loss
- Body temperature < 97°F, indicative of shock
- Total anorexia with a duration > 48 hours

Treatment Administration

In the antibiotic combination studies, treatment was administered at varying times following exposure to *B. anthracis* spores to evaluate the effect of ETI-204 in combination with antibiotics from early to late in the disease course.

Human Equivalent Antibiotic Dose Studies

In the human equivalent antibiotic dose studies, a single IV ETI-204 dose, ranging from 4 mg/kg to 16 mg/kg, was administered in sterile 0.9% sodium chloride for injection, USP either alone or in combination with the first dose of multiple oral doses of 50 mg/kg levofloxacin (once daily x 3 days [NIAID Study 1030] or x 5 days [AR007]) or placebo (water). In AR007, ETI-204 was given at a fixed dose of either 10 or 20 mg/rabbit. The body weights of rabbits in this study ranged from 2.2 to 2.7 kg;

thus, the actual doses administered were approximately 4 mg/kg IV and 8 mg/kg IM. These ETI-204 doses were administered either alone or in combination with the first dose of multiple oral doses of 50 mg/kg levofloxacin (once daily x 5 days). The control group in NIAID Studies 1030 and 1045 was untreated whereas the control group in AR007 and AR034 was placebo treated (i.e. sterile 0.9% sodium chloride for injection). IV ETI-204 and IV placebo were administered using a vascular access port (except in AR034) and levofloxacin and its placebo (i.e. water) were administered by oral gavage. In AR034, IV ETI-204 and IV placebo were administered using the marginal ear vein. IM ETI-204 in AR007 was administered in the hind leg.

ETI-204 alone, levofloxacin alone, and ETI-204 in combination with the first dose of levofloxacin was administered at 9 hours, 30 hours, and 72 hours following exposure to *B. anthracis* spores in AR007, AR034, and NIAID Study 1045, respectively. In NIAID Study 1030, ETI-204 alone was administered upon a SIBT (significant increased body temperature) whereas ETI-204 in combination with the first dose of levofloxacin and the first dose of levofloxacin alone were administered at 96 hours following spore challenge.

The levofloxacin dose of 50 mg/kg was based on comparability of the C_{max} values with those obtained from PK studies in human adults who received antibiotics at the dosages recommended for post-exposure prophylaxis.

Lower than Human Equivalent Antibiotic Dose Studies

In the lower than human equivalent antibiotic dose studies, a single IV ETI-204 dose of either 8 or 16 mg/kg was administered in sterile 0.9% sodium chloride for injection, USP either alone (NIAID Study 1056) or with the first dose of multiple doses of either levofloxacin (AR028), doxycycline (NIAID Study AP-10-055), or ciprofloxacin (NIAID Studies 1056 and 2469). The control group in NIAID Studies 1056 and 2469 was untreated whereas the control group in AR028 and NIAID Study AP-10-055 was placebo treated (i.e. sterile 0.9% sodium chloride for injection). Treatment was administered using a vascular access port in rabbits and via the saphenous vein to monkeys.

Treatment was administered at 72 hours following *B. anthracis* spore exposure in AR028 and at first positive PA-ECL or 30 hours after spore challenge in NIAID Study AP-10-055. In NIAID Study 1056, treatment was administered relative to disease progression, with ETI-204 administered at positive results in a PA-ECL assay and ETI-204 plus ciprofloxacin and ciprofloxacin alone administered at 24±12 hours after detection of PA. Treatment was also administered relative to disease progression in NIAID Study 2469 (i.e. 24±12 hours following positive results in PA-ECL assay).

Selection of a lower than human equivalent levofloxacin dose of 6.5 mg/kg (3 days by oral gavage) in AR028 was based on the results of two delayed-treatment studies with levofloxacin in anthrax-infected rabbits, AR023 and AR027, that were designed to identify a levofloxacin dose resulting in ~50% survival.

Selection of the lower than human equivalent ciprofloxacin dose of 10 mg/kg/day (x 4 days by oral gavage) for NIAID Studies 1056 and 2469 was based on the results of the antibiotic therapy study, Study 931, in which 63% of animals treated with a 10 mg/kg ciprofloxacin regimen at 24±12 hours after disease confirmation by positive results in a PA-ECL assay survived.

The doxycycline dose of 2 mg/kg (twice daily x 3 days IV) used in NIAID Study AP-10-055 was based on the results of previous studies conducted at USAMRIID in which this dose resulted in 40-60% survival.

Statistical methods

Sample Size

Sample size calculations were based upon both the ability to detect a statistically significant difference in survival rates between the control and treatment groups and between the antibiotic group and the combination group. The table below summarises the hypothesised survival rates for each group, the number of animals per group, and the resultant power based upon a specific statistical test at a predetermined significance level. Sample size calculations were not performed for NIAID Study AP-10-055 and AR034.

Table 22. Hypothesised survival rates for the ETI-204 antibiotic combination studies

Study	Statistical Test	Hypothesised Survival Rate	Number per Group	Power
Human Equivalent Antibiotic Dose Studies				
AR007	Fisher exact test one-sided	Control: 10% Levo: 30% Treated groups: 80%	9 12 9	- > 80%, comparisons not specified
1030	Fisher exact test one-sided, α=0.05	Additive effect: Levo: 40% Levo + ETI-204: 87% Versus Control: Control: 7% Levo: 65% Levo + ETI-204: 65%	 16 16 6 16 16	 - 81.6% - 80.8% 80.8%
1045	Fisher exact test one-sided, α=0.05	Additive effect: Levo: 50% Levo + ETI-204: 95% Versus Control: Control: 1% Levo + ETI-204: 95% Levo: 50% ETI-204: 75%	 16 16 6 16 16 16	 - 85% - 99% 56% 98%
Lower than Human Equivalent Antibiotic Dose Studies				
AR028	Fisher exact test one-sided, α=0.05	Additive effect: Levo: 60% Levo + ETI-204: 90% Interference Levo: 60% Levo + ETI-204: 90%	 30 30 30 30	 - 80.1% - 80.6%
1056	Fisher exact test one-sided, α=0.05	Additive effect: Cipro: 40% Cipro + ETI-204: 87% Versus Control: Control: 10% Cipro: 70% Cipro + ETI-204: 70% ETI-204: 80%	 16 16 8 16 16 8	 - 81.6% - 81.8% 81.8% 81.2%
2469	Fisher exact test one-sided, α=0.05	Additive effect: Cipro: 40% Cipro + ETI-204: 87% Versus Control: Control: 7% Cipro: 65% Cipro + ETI-204: 65%	 16 16 8 16 16	 - 81.6% - 80.8% 80.8%

Cipro: ciprofloxacin; levo: levofloxacin.

Calculations were conducted using the statistical package StatXact. Sample size calculations did not include adjustments for multiple comparisons.

Analysis populations

Statistical analysis for the combination studies were conducted using a modified ITT population, defined as all animals that survived to treatment, regardless of bacteraemia status, with the exception of AR034 Phase 1 in which survival rates were summarised for all challenged animals regardless of bacteraemia status.

Additional analyses of survival rates were also performed using all challenged animals and bacteraemic only animals.

Demographics and Pre-treatment Disease Characteristics

Demographic and pre-treatment disease characteristics were summarised using descriptive statistics. Because treatment was delayed in these studies and it was anticipated that a number of animals might die before treatment, demographics were summarised for the groups at spore challenge and at treatment for all studies except for AR034. For AR034, demographics were summarised at challenge only.

Demographic variables included gender, weight, and age. Pre-treatment disease characteristics included challenge spore dose, quantitative bacteraemia, quantitative PA, time to positive bacteraemia, and time to positive PA.

Time to positive bacteraemia was defined as the time from spore challenge to the first measurement greater than the LOD in either a qualitative or quantitative assay of bacteraemia. Time to positive PA was defined as the time from spore challenge to the first positive measurement for PA in either an ECL assay or ELISA.

Primary analysis

The intended statistical method for the primary endpoint was Fisher's exact test; the test was one-sided and statistical significance was declared at the 0.05 level. However, the applicant chose to present "a unified set of results across all the studies", both the results from the Boschloo's exact test as well as the Fisher's exact test are presented. The applicant assessed significance at the 0.025 level consistent with the application of one-tailed test and with the approach used for other studies.

Two statistical procedures were utilised to compare survival rates: Fisher's exact test and Boschloo's exact test with a Berger-Boos correction of gamma = 0.001 [McDonald et al, 1977; Mehrotra et al, 2003]. The resultant p-values for both procedures are presented as one-sided with statistical significance declared at the 0.025 level. In the human equivalent antibiotic dose studies, Fisher's exact test was conducted using Proc FREQ of SAS® version 9.3 for AR034, Proc FREQ of SAS® version 9.1 for NIAID Studies 1030 and 1045, and Proc FREQ of SAS® version 9.1.3 for AR007.

In the lower than human equivalent antibiotic dose studies, Fisher's exact test was conducted using Stata®version 12.1 for AR028, Proc Freq of SAS® version 9.4 for NIAID Study AP-10-055, Proc FREQ of SAS® version 9.1 for NIAID Study 1056, and SAS® version 9.3 for NIAID Study 2469. Boschloo's exact test was conducted using the program developed at N.C. State University by Berger and Boos.

An exact 95% CI, based on the score statistic using Proc Freq of SAS® version 9.4, is presented for the difference in survival rates. P-values for differences in survival rates of treated groups compared to the control group and the combination group compared to the antibiotic alone that are included on survival plots, the survival results are based upon Boschloo's exact test. Survival results are discussed for the mITT population.

Results [ETI-204 administered concomitant with antibiotic (human equivalent dose)]

Disposition and Analysis Populations

Overall, 57 rabbits were randomised in AR007: 9 to placebo; 9 to ~4 mg/kg ETI-204 IV; 9 to ~8 mg/kg ETI-204 IM; 12 to levofloxacin alone; 9 to ~4 mg/kg ETI-204 IV plus levofloxacin; and 9 to ~8 mg/kg ETI-204 IM plus levofloxacin. All randomised animals were challenged with *B. anthracis* spores and survived to treatment at 9 hours following spore challenge. Levofloxacin was administered orally (50 mg/kg, once daily, PO) for five days (5 total doses) alone or in combination with ETI-204.

Table 23. Disposition in the IV ETI-204 Human Equivalent Antibiotic Dose Study AR007 in NZW Rabbits in the Post-exposure Prophylaxis/Early Treatment Setting (All Animals)

Study AR007	Total	Placebo	Levo[1]	ETI-204 ~4 mg/kg IV[2]	ETI-204 ~4 mg/kg IV plus levo[1, 2]	ETI-204 ~8 mg/kg IM[2]	ETI-204 ~8 mg/kg IM plus levo[1, 2]
Randomised	57	9	12	9	9	9	9
Challenged	57	9	12	9	9	9	9
Survived to treatment[3]	57	9	12	9	9	9	9

[1]50 mg/kg/day orally for 5 days.
[2]ETI-204 was given at fixed dose. The body weights of rabbits in this study ranged from 2.2 to 2.7 kg. The actual doses administered were 10 and 20 mg/rabbit, which were approximately equal to 4 and 8 mg/kg.
[4]Treatment was administered at 9 hours after spore challenge.
IM: intramuscular; IV: intravenous; kg: kilogram; levo: levofloxacin; mg: milligram.

Overall, 68 rabbits were randomised in AR034: 8 to placebo; 20 to 16 mg/kg ETI-204 IV; 20 to levofloxacin PO alone; and 20 to 16 mg/kg ETI-204 IV plus levofloxacin PO. All randomised animals were challenged with *B. anthracis* spores and survived to treatment at 30 hours following spore challenge.

Table 24. Disposition in the IV ETI-204 Human Equivalent Antibiotic Dose Combination Study AR034 (Phase 1) in NZW Rabbits

Study AR034	Total	Placebo	ETI-204 16 mg/kg IV	Levo[1]	ETI-204 16 mg/kg IV + Levo[1]
Randomised	68	8	20	20	20
Challenged	68	8	20	20	20
Survived to treatment	68	8	20	20	20

[1]50 mg/kg/day orally for 3 days.
IV: intravenous; kg: kilogram; levo: levofloxacin; mg: milligram.

Overall, 54 rabbits were randomised in NIAID Study 1030: 6 to no treatment; 16 to 8 mg/kg ETI-204 IV; 16 to levofloxacin alone; and 16 to 8 mg/kg ETI-204 IV plus levofloxacin. All randomised animals were challenged with *B. anthracis* spores. All animals in the ETI-204 alone group survived to treatment (i.e. at SIBT) whereas only 31% and 25% of animals in the levofloxacin alone and ETI-204 plus levofloxacin groups, respectively, survived to treatment (ie, 96 hours following spore challenge). Of the rabbits that survived to treatment, 3 in the ETI-204 alone group, 1 in the levofloxacin PO alone group, and 1 in the ETI-204 plus levofloxacin PO group were not bacteraemic prior to treatment.

Table 25. Disposition in the IV ETI-204 Human Equivalent Antibiotic Dose Combination Study NIAID 1030 in NZW Rabbits

NIAID Study 1030	Total	Untreated	ETI-204 8 mg/kg IV	Levo[1]	ETI-204 8 mg/kg IV + Levo
Randomised	54	6	16	16	16
Challenged	54	6	16	16	16
Survived to treatment		-	16	5	4
Bacteraemic prior to treatment	20	-	13	4	3

[1]50 mg/kg twice daily x 3 days po.
IV: intravenous; kg: kilogram; levo: levofloxacin; mg: milligram.

Overall, 54 rabbits were randomised in NIAID Study 1045: 6 to no treatment; 16 to 8 mg/kg ETI-204 IV; 16 to levofloxacin only; and 16 to 8 mg/kg ETI-204 IV plus levofloxacin (table below). All of the animals were challenged with *B. anthracis* spores and 69%, 56%, and 69% of animals in the ETI-204 alone, levofloxacin alone, and ETI-204 plus levofloxacin groups, respectively, survived to the time of treatment (ie, 72 hours after spore challenge). All rabbits in the levofloxacin alone and ETI-204 plus levofloxacin groups were bacteraemic at the time of treatment; 2 rabbits in the ETI-204 alone group were not bacteraemic at the time of treatment.

Table 26. Disposition in the IV ETI-204 Human Equivalent Antibiotic Dose Combination Study NIAID 1045 in NZW Rabbits

NIAID Study1045	Total	Untreated	ETI-204 8 mg/kg IV	Levo[1]	ETI-204 8 mg/kg IV + Levo[1]
Randomised	54	6	16	16	16
Challenged	54	6	16	16	16
Survived to treatment	31	-	11	9	11
Bacteraemic prior to treatment	29	-	9	9	11

[1]50 mg/kg twice daily x 3 days po.
IV: intravenous; kg: kilogram; levo: levofloxacin; mg: milligram.

Outcomes

The applicant concludes that ETI-204 did not have a negative impact on the rate of survival when co-administered with a human equivalent dose of levofloxacin in rabbits. The only statistically significant difference observed between treatment with antibiotic vs concomitant treatment with full dose of antibiotics was in study AR007, where 33% (n=12) of the subjects treated with levofloxacin (per os) survived compared to 100% (n=9) treated with ETI-204 and levofloxacin, the treatment was administered 9 h post challenge. The applicant considers this study supports both concomitant treatment with antibiotics as well as post-exposure prophylaxis and emphasise that ETI-204 did not have a negative impact on the rate of survival. All treatments administered in the study AR007 statistically significantly increased the survival rate compared to placebo.

The results (survival rates) from the studies where concomitant administration of antibiotics and ETI-204 were conducted with a human equivalent antibiotic dose (i.e., yields similar exposure to that achieved by the recommended doses in humans) and ETI-204 doses from 4 to 16 mg/kg are presented in table below. The antibiotic used in those studies was levofloxacin.

A summary of the survival rates in the ETI-204 studies with concomitant administration of antibiotics at human equivalent dose is provided in the table below.

Table 27. Summary of survival rates in studies of ETI-204 in combination with antibiotics (human equivalent dose)

Study Product (batch)	Species/Strain	Dose and Route (ETI-204 + antibiotic)	Time of Dosing	Survival % (proportion)	p-value[1] Fisher[2] Boschloo[3]
Human-Equivalent Dose Studies					
NIAID Study 1030 (Pilot)	Rabbit/NZW	Untreated	NA	0 (0/6)	
		8 mg/kg IV	Significant increase body temperature	75 (12/16)	0.0028*[2] 0.0018*
		levo 50 mg/kg/day PO x 3 days	96 hrs post-challenge	40 (2/5)	0.1818[2] 0.0867
		8 mg/kg IV + levo 50 mg/kg/day PO x 3 days	96 hrs post-challenge	100 (4/4)	0.0048*[2] 0.0022*
NIAID Study 1045 (Pilot)	Rabbit/NZW	Untreated	NA	0 (0/6)	
		8 mg/kg IV	72 hrs post-challenge	64 (7/11)	0.170[2] 0.0061*
		Untreated + levo 50 mg/kg/day PO x 3 days	72 hrs post-challenge	78 (7/9)	0.0056*[2] 0.0026*
		8 mg/kg IV + levo 50 mg/kg/day PO x 3 days	72 hrs post-challenge	82 (9/11)	0.0023*[2] 0.0016*
AR007 (Pilot)	Rabbit/NZW	0 mg/kg IV	9 hrs post-challenge	0 (0/9)	
		~4 mg/kg IV	9 hrs post-challenge	100 (9/9)	<0.0001* 0.0010*
		Levo 50 mg/kg/day PO x 5 days	9 hrs post-challenge	33 (4/12)	0.0827 0.0480
		~4 mg/kg IV + levo 50 mg/kg/day PO x 5 days	9 hrs post-challenge	89 (8/9)	0.0002* 0.0011*
		~8 mg/kg IM	9 hrs post-challenge	100 (9/9)	<0.0001* 0.0010*
		~8 mg/kg IM + levo 50 mg/kg/day PO x 5 days	9 hrs post-challenge	100 (9/9)	<0.0001* 0.0010*
AR034 (Phase 1) (Commercial)	Rabbit/NZW	0 mg/kg IV	30 hrs post-challenge	0 (0/8)	
		16 mg/kg IV	30 hrs post-challenge	65 (13/20)	0.0021* 0.0018*
		Untreated + levo 50 mg/kg/day PO x 3 days	30 hrs post-challenge	100 (20/20)	<0.0001* 0.0010*
		16 mg/kg IV + levo 50 mg/kg/day PO x 3 days	30 hrs post-challenge	95 (19/20)	<0.0001* 0.0010*

[1]Total number of animals randomised to treatment. NZW: New Zealand White; IV: intravenous; mg/kg: milligram/kilogram; levo: levofloxacin; po: oral; NIAID: National Institute of Allergy and Infectious Disease; PA: protective antigen; doxy: doxycycline; bid: twice daily; PA-ECL: Protective antigen-electrochemiluminescence.

Summary of main efficacy results

Monotherapy of ETI-204 (Studies AR033, AP202, AP204, AP301)

The activity of ETI-204 in neutralizing the anthrax toxin was evaluated in the monotherapy studies. Four studies are considered as pivotal efficacy monotherapy studies since they were blinded and had GLP-status. In study AR033 a statistically significant difference in survival rate compared to placebo was observed when 4, 8 and 16 mg/kg was administered IV with a numerical survival rate of 33-69%. In the three studies including monkeys, high survival rate (83-100%) was observed in study AP301 when ETI-204 was administered IM at the doses 8 or 16 mg/kg up to 24 h post exposure, however no difference was seen when the treatment was initiated 36 h post exposure. In study AP204 a statistically significant difference in survival rate was observed between placebo (6%) and 16 mg/kg IV administered ETI-204 (47%), however no difference was seen between placebo (6%) and 4 mg/kg IV administered ETI-204 (25%). In study AP202 a statistically significant difference in survival rate compared to placebo (0%) was observed for 16 mg/kg ETI-204 (two formulations; pilot and commercial) administered IV, the survival rate, however was modest (31-35%).

The secondary efficacy endpoint, survival time from spore challenge, was achieved in all four main studies. Significant increases in survival time from spore challenge compared to the placebo group were seen for the 4 mg/kg, 8 mg/kg and 16 mg/kg IV ETI-204 groups in study AR033 and 16 mg/kg IV groups in AP202 and AP204; the 4 mg/kg ETI-204 group in AP204 did not have an increased survival time relative to the placebo group. Following IM administration of ETI-204 (8 and 16 mg/kg, IM), an increased survival time compared to the placebo group was seen in monkeys in study AP301 at all ETI-204 groups except for the 8 and 16 mg/kg group at 36 hours.

It was noted that the response rate was time dependent, where the earlier the treatment was initiated the higher survival rate, and a lower bacterial count was associated with a higher survival rate.

Concomitant use of ETI-204 and antibiotics

No add-on benefit of ETI-204 was demonstrated in the studies using human equivalent doses of antibiotic in rabbits. No studies using the human equivalent dose was executed in monkeys. All antibiotic studies were unblinded and some of them lacked GLP-status. In study AR034 where the concomitant treatment of ETI-204 and antibiotic was administered IV 30 hours post exposure, the animals were highly sensitive to monotherapy of a human equivalent dose of levofloxacin which resulted in a 100% survival rate. If the treatment, (1045 and 1030) was delayed 72 – 96 hours, the efficacy of levofloxacin was lower whereas the number of animals surviving the antibiotic treatment was markedly reduced (28% survived when treatment was delayed to 96 hours). However, a high number of animals died before treatment was initiated. Only one out of four combination studies (AR007, ETI-204 4 mg/kg, IV) showed a statistically significant effect of ETI-204 (non-commercial formulation) concomitantly administered with human equivalent dose of levofloxacin compared to levofloxacin alone. In this study the survival rate with levofloxacin alone was low, 33%, however, the rabbits were dosed for 5 days with levofloxacin administered orally with an early treatment onset at 9 hours post challenge, since the mean day of euthanise after exposure was 14 days it is suggested that the levofloxacin animals died after the treatment was stopped.

Supportive studies

Supportive ETI-204 monotherapy studies in monkeys and rabbits.

The efficacy of ETI-204 in monotherapy studies for the treatment of inhalational anthrax was examined in supportive efficacy studies in cynomolgus monkeys (Studies AP203, AP107, AP107, NIAID 1056 and AP307), and in NZW rabbits (Studies AR007, AR004, AR012, AR021, AR034, AR035, AR037, AR0315, NIAID 1030 and NIAID 1045)). In these studies, NZW rabbits and cynomolgus monkeys were exposed to a target dose of 200 LD50 equivalents of *B. anthracis* (Ames) spores via inhalation and then were treated with a single IV or IM dose of ETI-204 at time ranging from 9 to 48 hours following spore challenge.

Table 28. Overview of the supportive ETI-204 monotherapy studies

Study Number GLP status Product (batch)	Species/Strain	Study Design	Treatment Regimen and Dose	Total No. Animals[1]
AP203 GLP Commercial	Monkey/cynomolgus	Randomised, blinded, placebo-controlled, parallel-group, trigger-to-treat (dosing upon positive PA-ECL or time), dose-ranging study in anthrax-challenged animals. ETI-204 was administered 36.2 hrs to 37.5 hrs post-challenge among the groups. Follow-up period was 28 days.	ETI-204 IV: 0 mg/kg 8 mg/kg 32 mg/kg	 16 16 16
AP201 GLP Pilot	Monkey/cynomolgus	Randomised, blinded-to-group, placebo-controlled, parallel-group, trigger-to-treat (dosing upon positive PA-ECL or time), dose-ranging study in anthrax-challenged animals. ETI-204 was administered 41.3 hrs to 44.5 hrs post-challenge among the groups. Follow-up period was 30 days	ETI-204 IV: 0 mg/kg 4 mg/kg 8 mg/kg	 14 14 15
AP107 GLP Pilot	Monkey/cynomolgus	Randomised, open-label, placebo-controlled, parallel group, IV and IM ETI-204 dose-ranging study (dosing at 24 hrs following *B. anthracis* spore exposure). Follow-up period was 30 days.	ETI-204: 0 mg/kg IV 2 mg/kg IV 8 mg/kg IV 4 mg/kg IM 8 mg/kg IM	 6 9 9 8 9
AP307 Non-GLP Commercial	Monkey/cynomolgus	Randomised, open-label, placebo-controlled, parallel group, IM ETI-204 study (dosing at 24, 36, and 48 hrs following *B. anthracis* spore exposure). Follow-up period was 28 days.	ETI-204 IM: 0 mg/kg (24 hrs) 16 mg/kg (24 hrs) 16 mg/kg (36 hrs) 16 mg/kg (48 hrs)	 10 14 14 16
AR021 GLP Pilot	Rabbit/NZW	Randomised, open-label, placebo- and positive-controlled (levofloxacin), parallel group, trigger-to-treat (dosing upon positive PA-ECL, elevated body temperature), dose-ranging study in anthrax-challenged animals. Treatment was initiated 26.9 hrs to 30.7 hrs post-challenge. Follow-up period was 28 days.	ETI-204 IV: 0 mg/kg 1 mg/kg 4 mg/kg 16 mg/kg	 9 9 17 17
AR004 Non-GLP Commercial	Rabbit/NZW	Randomised, open-label, placebo-controlled, parallel group, IV ETI-204 study (dosing at 24, 36, and 48 hrs following *B. anthracis* spore exposure). Follow-up period was 28 days.	ETI-204 IV[2]: 0 mg/kg (24 hrs) ~4 mg/kg (24 hrs) ~4 mg/kg (36 hrs) ~4 mg/kg (48 hrs)	 10 10 10 10
AR007 GLP Elusys	Rabbit/NZW	Randomised, open-label, placebo-controlled, parallel group, IV and IM ETI-204 study in combination with a human equivalent dose of antibiotic (dosing at 9 hrs following *B. anthracis* spore exposure). Follow-up period was 34 days.	ETI-204[3]: 0 mg/kg IV 0 mg/kg IV+ levo[4] ~4 mg/kg IV ~4 mg/kg IV + levo[3], ~8 mg/kg IM	 9 12 9 9 9

Study Number GLP status Product (batch)	Species/Strain	Study Design	Treatment Regimen and Dose	Total No. Animals[1]
			~8 mg/kg IM + levo[3]	9
AR012 GLP Elusys	Rabbit/NZW	Randomised, open-label, placebo-controlled, parallel group, IV and IM ETI-204 dose-ranging study (dosing at 24 hrs following *B. anthracis* spore exposure). Follow-up period was 14 days.	ETI-204[5]: 0 mg/kg IM ~1 mg/kg IV ~4 mg/kg IV ~8 mg/kg IV ~2 mg/kg IM ~4 mg/kg IM ~8 mg/kg IM ~15 mg/kg IM	 9 9 12 12 9 9 12 12
AR0315 Non-GLP Pilot	Rabbit/NZW	Randomised, open-label, placebo-controlled, parallel group, IM ETI-204 dose-ranging study (dosing at 18 and 24 hrs following *B. anthracis* spore exposure). Follow-up period was 28 days.	ETI-204 IM: 0 mg/kg (24 hrs) 4 mg/kg (18 hrs) 16 mg/kg (18 hrs) 4 mg/kg (24 hrs) 16 mg/kg (24 hrs)	 10 12 12 12 12
AR035 GLP Commercial	Rabbit/NZW	Randomised, open-label, placebo-controlled, parallel group, IM ETI-204 study (dosing at 18, 24, and 30 hrs following *B. anthracis* spore exposure). Follow-up period was 28 days.	ETI-204 IM: 0 mg/kg (18 hrs) 16 mg/kg (18 hrs) 16 mg/kg (24 hrs) 16 mg/kg (30 hrs)	 10 10 10 10
AR037 GLP Commercial	Rabbit/NZW	Randomised, open-label, placebo-controlled, parallel group, IM ETI-204 dose-ranging study (dosing at 24 hrs following *B. anthracis* spore exposure). Follow-up period was 28 days.	ETI-204 IM: 0 mg/kg 8 mg/kg 16 mg/kg 32 mg/kg	 10 16 16 16

[1]Total number of animals randomised to treatment. BDS: bulk drug substance; GLP: Good Laboratory Practices; IV: intravenous; kg: kilogram; mg: milligram; NZW: New Zealand White; PA-ECL: protective antigen electrochemiluminescence; po=oral.
[2]ETI-204 was given at fixed dose. The body weights of rabbits in this study ranged from 2 to 2.8 kg at the time of randomisation. The actual dose administered was 10 mg/rabbit, which was approximately equal to 4 mg/kg.
[3]ETI-204 was given at fixed dose. The body weights of rabbits in this study ranged from 2.2 to 2.7 kg. The actual dose administered were 10 and 20 mg/rabbit, which were approximately equal to 4 and 8 mg/kg.
[4]Levofloxacin dose was 50 mg/kg/day oral gavage x 5 days.
[5]ETI-204 was given at fixed dose. The body weights of rabbits in this study ranged from 2.3-3.0 kg. The actual dose administered was 2.5, 5, 10, 20 or 40 mg/rabbit, which was approximately equal to 1, 2, 4, 8. or 15 mg/kg; HED = human equivalent dose.

Results ***[Supportive ETI-204 monotherapy studies in monkeys and rabbits]***

In rabbits and monkeys, ETI-204 increased survival when administered either IV or IM following exposure to *B. anthracis* spores. The survival benefit was time dependent with statistically significantly higher survival observed with earlier treatment after spore exposure (≤ 24 hrs) (see table below).

Table 29. Survival data for ETI-204 in supportive monotherapy studies

Study Number	Species/Strain	Dose and Route[1]	Time of Administration Post Anthrax Exposure	Survival % (proportion)	p-value[2] Fisher Boschloo[3]
AR004	Rabbit/NZW	0 mg/kg IV	24 hrs	0 (0/9)	
		~4 mg/kg IV	24 hrs	80 (8/10)	0.0006* 0.0011*
AR007	Rabbit/NZW	0 mg/kg IV	9 hrs	0 (0/9)	
		~4 mg/kg IV	9 hrs	100 (9/9)	<0.0001* 0.0010*
		~4 mg/kg IV + Levo[4]	9 hrs	89 (8/9)	0.0002* 0.0011*
		~8 mg/kg IM	9 hrs	100 (9/9)	<0.0001* 0.0010*
		~8 mg/kg IV + Levo[4]	9 hrs	100 (9/9)	<0.0001* 0.0010*
		Levo[4]	9 hrs	33 (4/12)	0.0827 0.0480
AR012	Rabbit/NZW	0 mg/kg IM	24 hrs	0 (0/9)	
		~1 mg/kg IV	24 hrs	11 (1/9)	0.5000 0.4074
		~4 mg/kg IV	24 hrs	50 (6/12)	0.0170* 0.0084*
		~8 mg/kg IV	24 hrs	58 (7/12)	0.0068* 0.0036*
		~2 mg/kg IM	24 hrs	11 (1/9)	0.500 0.4074
		~4 mg/kg IM	24 hrs	33 (3/9)	0.1029 0.0498
		~8 mg/kg IM	24 hrs	42 (5/12)	0.0389 0.0196*
		~15 mg/kg IM	24 hrs	33 (4/12)	0.0827 0.0480
AR0315	Rabbit/NZW	0 mg/kg IM	24 hrs	0 (0/10)	
		4 mg/kg IM	18 hrs	92% (11/12)	<0.0001* 0.0010*
		4 mg/kg IM	24 hrs	42 (5/12)	0.0396 0.0141*
		16 mg/kg IM	18 hrs	92% (11/12)	<0.0001* 0.0010*
		16 mg/kg IM	24 hrs	67 (8/12)	0.0017* 0.0015*
AR037	Rabbit/NZW	0 mg/kg IM	24 hrs	0 (0/10)	
		8 mg/kg IM	24 hrs	31 (5/16)	0.07 0.0355
		16 mg/kg IM	24 hrs	31 (5/16)	0.07 0.0355
		32 mg/kg IM	24 hrs	31 (5/16)	0.07 0.0355

Study Number	Species/Strain	Dose and Route[1]	Time of Administration Post Anthrax Exposure	Survival % (proportion)	p-value[2] Fisher Boschloo[3]
AP107	Monkey/cynomolgus	0 mg/kg IV	24 hrs	17 (1/6)	
		2 mg/kg IV	24 hrs	44 (4/9)	0.2902 0.1735
		8 mg/kg IV	24 hrs	67 (6/9)	0.0594 0.0378
		4 mg/kg IM	24 hrs	75 (6/8)	0.0513 0.0211*
		8 mg/kg IM	24 hrs	56 (5/9)	0.1434 0.0879
AP307	Monkey/cynomolgus	0 mg/kg IM	24 hrs	10 (1/10)	
		16 mg/kg IM	24 hrs	93 (13/14)	<0.0001* 0.001*
		16 mg/kg IM	36 hrs	43 (6/14)	0.0967 0.0536
		16 mg/kg IM	48 hrs	25 (4/16)	0.3431 0.2196

IM: intramuscular; IV: intravenous; Levo: levofloxacin; NZW: New Zealand white
[1] Each group included approximately equal numbers of males and females with the exception of Study AR035, which included males only.
[2] Compared to control.
[3] Boschloo testing was performed by Sponsor
[4] Levofloxacin was administered by the oral route at a dose of 50 mg/kg/day x 5 days.
* Denotes statistical significance at the 0.025 level.
Note: For Studies AR004, AR007, and AR012, ETI-204 was given to rabbits at fixed doses. The body weights of rabbits in these studies ranged from 2.0-2.9 kg. The actual doses administered were 2.5, 5, 10, 20, and 40 mg/rabbit, which were approximately equal to 1, 2, 4, 8, and 15 mg/kg.
Only animals that survived to receive treatment are included regardless of bacteraemia status in the analysis for Study AR004; all challenged animals were included in the analysis for Studies AR012, AR0315, AR035, and AR037.
Note: All challenged animals were included in the analysis of Studies AP107 and AP307; all animals that survived to receive treatment were included regardless of bacteraemia status in the analysis of Study AP301

Table 30. Survival data for ETI-204 in supportive monotherapy studies (contd)

Study Number	Species/Strain	Dose and Route[1]	Survival %[2] (proportion)	p-value[3] Fisher Boschloo[4]
AR021	Rabbit/NZW	0 mg/kg IV	0 (0/9)	
		1 mg/kg IV	37.5 (3/8)	0.0824 0.0327
		4 mg/kg IV	73.3 (11/15)	0.0005* 0.0012*
		16 mg/kg IV	92.9 (13/14)	<0.0001* 0.0010*
		Levofloxacin 50 mg/kg[5]	88.9 (8/9)	0.0002* 0.0011*
AP201	Monkey/cynomolgus	0 mg/kg IV	14.3 (2/14)	
		4 mg/kg IV	78.6 (11/14)	0.0009* 0.0015*
		8 mg/kg IV	73.3 (11/15)	0.0019* 0.0017*
AP203	Monkey/cynomolgus	0 mg/kg IV	12.5 (2/16)	
		8 mg/kg IV	6.3 (1/16)	0.50 0.8038
		32 mg/kg IV	37.5 (6/16)	0.11 0.0599

[1] Each group included approximately equal numbers of male and female rabbits.
[2] Only animals that were bacteraemic prior to treatment were included in the analysis.
[3] Compared to placebo

[4] Boschloo test was performed by Sponsor
[5] Once daily for 3 days
* Denotes statistical significance at the 0.025 level

Supportive ETI-204 studies in combination with antibiotics (low dose)

Four studies of ETI-204 in combination with antibiotics lower than the human equivalent dose were conducted in either the NZW rabbit or cynomolgus monkey model of inhalational anthrax. These studies were open-label (unblinded) trials that may lack GLP-compliance. An overview of the performed ETI-204 studies with concomitant administration of antibiotics is provided in the table below.

Table 31. Overview of ETI-204 studies in combination with antibiotics for the treatment of inhalational anthrax (lower than human equivalent dose)

Study Number GLP status Product (batch)	Species/Strain	Study Design	Treatment Regimen and Dose	Total No. Animals[1]
Lower than Human Equivalent Dosing – Exploratory studies				
AR028 non-GLP Pilot	Rabbit/NZW	Randomised, controlled, open-label, parallel-group, factorial design study; dose received 72 hours after anthrax exposure	ETI-204 IV: 0 mg/kg 16 mg/kg + levo 6.5 mg/kg x 3 days po levo 6.5 mg/kg/day x 3 days po	 12 34 38
NIAID Study AP-10-055 non-GLP Pilot	Rabbit/NZW	Randomised, controlled, open-label, parallel-group, factorial design study; dose received at detection of elevated PA	Saline control Doxy 2 mg/kg/day x 3 days bid IV ETI-204 8 mg/kg + doxy 2 mg/kg/day x 3 days bid IV	4 10 10
NIAID Study 1056 non-GLP Pilot	Monkey/ cynomolgus	Randomised, controlled, open-label, parallel-group, factorial design study; dose received upon positive PA-ECL (ETI-204 only treatment) or 24±12 hours after positive PA-ECL (ETI-204 + cipro; cipro alone)	ETI-204 IV: Untreated 8 mg/kg 8 mg/kg + cipro 10 mg/kg/day x 4 days po cipro 10 mg/kg x 4 days po	 8 8 16 16
NIAID Study 2469 non-GLP Pilot	Monkey/ cynomolgus	Randomised, controlled, open-label, parallel-group, factorial design study; dose received 24±12 hours after positive PA-ECL. Included both ETI-204 and another Sponsor's antitoxin	Untreated ETI-204 8 mg/kg IV + cipro 10 mg/kg/day x 4 days Antitoxin "A" 10 mg/kg/day + cipro 10 mg/kg/ day x 4 days Cipro po 10 mg/kg/day x 4 days Cipro po 26 mg/kg/day x 4 days	8 16 16 16 16

[1]Total number of animals randomised to treatment. GLP: Good Laboratory Practices; NZW: New Zealand White; IV: intravenous; mg/kg: milligram/kilogram; levo: levofloxacin; po: oral; NIAID: National Institute of Allergy and Infectious Disease; PA: protective antigen; doxy: doxycycline; bid: twice daily; PA-ECL: Protective antigen-electrochemiluminescence; cipro: ciprofloxacin

Results – *[ETI-204 in combination with antibiotics (low dose) for the treatment of inhalational anthrax]*

The results (survival rates) from the studies where concomitant administration of antibiotics and ETI-204 were conducted with a less than human equivalent antibiotic dose (i.e., yields lower exposure to that achieved by the recommended doses in humans) and ETI-204 doses from 8 to 16 mg/kg are presented in the table below. The antibiotics used in those studies were either levofloxacin, ciprofloxacin or doxycycline.

Table 32. Survival rates in studies of ETI-204 in combination with antibiotics (low dose)

Study	Species/ Strain	Dose and Route (ETI-204 + antibiotic)	Time of Dosing	Survival % (proportion)	p-value[1] Fisher[2] Boschloo[3]
Below Human-Equivalent Dose Studies					
1056[4,5]	Monkey/ cynomolgus	Untreated	NA	0 (0/8)	
		8 mg/kg IV	Positive PA-ECL	50 (4/8)	0.0385[2] 0.0149*
		Untreated + cipro 10 mg/kg/day PO x 4 days	Positive PA-ECL + 24 hrs	15 (2/13)	0.3714[2] 0.2257
		8 mg/kg IV + cipro 10 mg/kg/day PO x 4 days	Positive PA-ECL + 24 hrs	62 (8/13)	0.0063*[2] 0.0028*
2469[4,8]	Monkey/ cynomolgus	Untreated	NA	25 (2/8)	
		Untreated + cipro 10 mg/kg/day PO x 4 days	Positive PA-ECL + 24 hrs	31 (4/13)	0.5903 0.4306
		Untreated + cipro 26 mg/kg/day PO x 4 days	Positive PA-ECL + 24 hrs	50 (7/14)	0.2455 0.1516
		8 mg/kg IV + cipro 10 mg/kg/day PO x 4 days	Positive PA-ECL + 24 hrs	57 (8/14)	0.1563 0.0886
AR028[8,9]	Rabbit/ NZW	0 mg/kg IV	72 hrs post-challenge	0 (0/12)	
		0 mg/kg + levo 6.5 mg/kg/day PO x 3 days	72 hrs post-challenge	58 (22/38)	0.0004* 0.00002*
		16 mg/kg IV + levo 6.5 mg/kg/day PO x 3 days	72 hrs post-challenge	68 (23/34)	<0.0001* 0.0010*
AP-10-055[9]	Rabbit/ NZW	Saline Control	Positive PA	0 (0/4)	
		Doxy 2 mg/kg bid x3 days IV	Positive PA	50 (5/10)	NA[10] 0.0547
		Doxy 2 mg/kg bid x 3 days, IV + ETI-204 8 mg/kg, IV	Positive PA	90 (9/10)	NA[10] 0.0023*

Cipro: ciprofloxacin; Doxy: doxycycline; IV: intravenous; Levo: levofloxacin; NZW: New Zealand white; PO: oral
1 Compared to placebo/untreated.
2 Fisher's exact testing was not reported in Studies 1030, 1045, and 1056. Values calucalated by Sponsor.
3 Boschloo testing was performed by Sponsor.
4 Study conducted by National Institute of Allergy and Infectious Disease [NIAID].
5 All challenged animals that survived to receive at least one treatment are included in the analysis.
6 ETI-204 was given at a fixed dose. The body weights of the rabbits in this study ranged from 2.2 to 2.7 kg. The actual doses administered were 10 and 20 mg/rabbit, which were approximately equal to 4 and 8 mg/kg.
7 Study AR034 was conducted in two phases: Phase 1 and Phase 2. Animals were treated as indicated at 30 hours post spore challenge in Phase 1 and were rechallenged with *B.anthracis* spores without treatment 9 months later.
8 Includes only animals that received treatment.
9 All animals that survived to receive treatment are included regardless of bacteraemia status for both Studies AP-10-055 and AR028.
10 Study report presents results for exact permutation Cochran-Armitage trend test stratified by experimental iteration with p-values corrected by permutation to correct for multiple comparison. Fisher p-values were not computed.
* Denotes statistical significance at the 0.025 level

Other supportive studies

Pre-Exposure Prophylaxis Studies

The efficacy of ETI-204 monotherapy for the pre-exposure prevention of inhalational anthrax was evaluated in three studies: AR001 and AR003 in NZW rabbits, and AP305 in cynomolgus monkeys. Two routes of ETI-204 administration were examined: IV and IM.

In cynomolgus monkeys, a 16 mg/kg IM dose administered 24-72 hours before spore exposure statistically significantly increased survival rate and survival time and prevented the development of bacteraemia for 56 days after spore challenge. All monkeys administered a single dose of 16 mg/kg IM ETI-204 at 24, 48, and 72 hours before spore exposure survived compared to 10% survival in placebo-treated animals. In NZW rabbits, 4 mg/kg IV and 8 mg/kg IM administered 30-45 minutes before spore exposure also statistically significantly increased survival rates and survival time compared to placebo and prevented the development of bacteraemia for 28 days after spore challenge.

The applicant concluded that ETI-204 prevents the development of inhalational anthrax when administered as pre-exposure prophylaxis to animals that were subsequently exposed to a high inoculum of *B. anthracis* spores. Since pre-exposure prophylaxis treatment is out of scope for the present application, these studies are not assessed herein.

Re-challenge Study

An anthrax re-challenge study was conducted to provide evidence that a single IV dose of ETI-204, either as monotherapy or in combination with multiple doses of an antibiotic, did not interfere with the development of protective endogenous immunity to PA. This study included two phases: Phase 1 and Phase 2. Results from Phase 1 are relevant to the treatment of inhalational anthrax in combination with an antibiotic and results from Phase 2 are relevant to generation of a protective endogenous immune response to PA.

ETI-204 administered either alone or in combination with levofloxacin following a primary challenge with *B. anthracis* spores does not interfere with the development of protective endogenous immunity as shown by increased survival and the prevention of inhalational anthrax in the absence of treatment following a secondary *B. anthracis* spore challenge in AR034.

Table 33. Survival Rates in Phase 2 of the IV Rechallenge Study AR034

	AR034 (Phase 2)	
	% (proportion)	p-value[1]
Naïve control	0 (0/12)	
16 mg/kg ETI-204 IV[2]	100 (13/13)	0.0010*
levofloxacin[2, 3]	95 (19/20)	0.0010*
16 mg/kg ETI-204 IV + levofloxacin[2, 3]	89 (17/19)	0.0010*

[1]p-value is from 1-sided Boschloo Test (with Berger-Boos modification of gamma=0.001) compared to placebo.
[2]Phase 2 included animals from the treated groups (Groups 1 through 3) of Phase 1 that survived 9 months following the primary challenge. Twelve additional naive animals were assigned as the Phase 2 control group.
[3]In Phase 1, animals received 16 mg/kg IV ETI-204 alone or in combination with levofloxacin (50 mg/kg/day once daily for 3 days) or levofloxacin alone 30 hours post challenge; in Phase 2, no additional treatment was administered.
*Denotes statistical significance at the 0.025 level.
mg/kg: milligram/kilogram; IV: intravenous.

2.5.3. Discussion on clinical efficacy

Efficacy is bridged from data obtained in the animal disease model, via healthy animals to humans based on similar or higher exposure in humans. Since PK data are generated in healthy humans, the impact of physiological consequences of disease, as well as target mediated clearance, remains an uncertainty.

The selected animal models including NZW rabbits and non-human primates are considered acceptable models of inhalational anthrax and are widely used for assessing the efficacy of anthrax countermeasures such as vaccines and antibodies. The artificial nature of the model is recognised, e.g., in terms of a standardised inoculum as well as different disease kinetics compared to the human situation. A residual uncertainty is how time of treatment initiation in relation to infection and initiation of symptoms impacts efficacy in humans, especially since the disease progression is more rapid in herbivores such as rabbit.

Four monotherapy efficacy studies were GLP-compliant and blinded (AP204, AP202, AP301 and AR033). These are considered pivotal for the efficacy demonstration. In addition, two earlier studies investigating other dose levels (AP201 and AP203), are considered supportive.

Monotherapy efficacy studies of obiltoxaximab

The activity of obiltoxaximab in neutralizing the anthrax toxin was evaluated in four pivotal monotherapy studies. In study AR033 a statistically significant difference in survival rate compared to placebo was observed when 4, 8 and 16 mg/kg was administered IV with a numerical survival rate of 33-69%. In the three studies including monkeys, high survival rate (83-100%) was observed in study AP301 when obiltoxaximab was administered IM at the doses 8 or 16 mg/kg up to 24 h post exposure, however no difference was seen when the treatment was initiated 36 h post exposure. In study AP204 a statistically significant difference in survival rate was observed between placebo (6%) and 16 mg/kg IV administered ETI-204 (47%), however no difference was seen between placebo (6%) and 4 mg/kg IV administered ETI-204 (25%).

In study AP202 a statistically significant difference in survival rate compared to placebo (0%) was observed for 16 mg/kg obiltoxaximab (two formulations; pilot and commercial) administered IV, the survival rate was however modest (31-35%). Despite the inclusion of two formulations, the study cannot be used to demonstrate comparability between the two formulations since in-vivo models would not provide sufficient sensitivity and power for that purpose. However, efficacy studies including both the commercial formulation and the investigational formulation are acceptable given that comparability has been demonstrated.

It can be concluded that the provided data from the main monotherapy studies are sufficient to support proof of concept for obiltoxaximab, since a substantially higher survival rate in general was observed compared to placebo in both monkey and rabbit, when treatment was initiated based on clinical symptoms or PA antigen levels. In addition, the secondary efficacy endpoint, survival time from spore challenge, was increased in ETI-204 treated animals in the four main studies.

It was noted that the response rate was time dependent, where the earlier the treatment was initiated the higher the survival rate. Furthermore, a lower bacterial count was associated with a higher survival rate. This suggests that, as would be anticipated, the magnitude of efficacy will depend on the time from infection or onset of symptoms and treatment initiation.

The results from the supportive monotherapy studies with obiltoxaximab show that, in general, a dose of 16 mg/kg was active in the treatment of inhalation anthrax in both monkeys and rabbits after IV and IM administration of obiltoxaximab up to 24 hrs post-challenge. Thus, the data from these

exploratory studies are relevant as they show an increased survival rate similar to the blinded and GLP compliant proof-of-concept studies for obiltoxaximab in monotherapy.

The SmPC section 5.1 includes data from the blinded and GLP compliant monotherapy treatment studies (AR033, AP301, AP202 and AP204). The applicant had submitted data supporting that the blinded to group design for study AP204 did not impact the survival rate of the control group. It was therefore considered acceptable to include results from study AP204 in SmPC section 5.1. It has been made clear in the SmPC that this study was blinded by group. Study AP203 is not described in the SmPC as it did not investigate the 16 mg/kg dose level.

Concomitant use of obiltoxaximab and antibiotics

No add-on benefit of obiltoxaximab was demonstrated in the studies where human equivalent doses of antibiotic were administered to rabbits. None of the studies using the human equivalent dose was executed in monkeys. All antibiotic studies were unblinded and some of them lacked GLP-status. In study AR034 where the concomitant treatment of obiltoxaximab (commercial batch) and antibiotic was administered IV 30 hours post exposure, the infection was highly sensitive to monotherapy levofloxacin administered as a human equivalent dose which resulted in a 100% survival rate. If the treatment, (1045 and 1030) was delayed 72 – 96 hours, the efficacy of levofloxacin was lower whereas the number of animals surviving the antibiotic treatment was markedly reduced (28% survived when treatment was delayed to 96 hours). Since a high number of animals died before treatment was initiated the results must be interpreted with caution. Only one of the four combination studies (AR007, ETI-204 4 mg/kg, IV) showed a statistically significant effect of obiltoxaximab (pilot batch) concomitantly administered with human equivalent dose of levofloxacin compared to levofloxacin alone. In this study the survival rate with levofloxacin alone was low (33%). However, the rabbits were dosed for 5 days with levofloxacin administered orally with an early treatment onset at 9 hours post challenge and since the mean day of euthanise after exposure was approximately 14 days, it is suggested that the levofloxacin animals died after the treatment of levofloxacin was stopped.

It appears that the combination studies were not sensitive to demonstrate potential additive or synergistic effects of obiltoxaximab and antibiotics. Due to the rapid progress of anthrax disease in herbivores such as rabbit, a large number of animals died before treatment was initiated and no clear results were provided from those studies.

It is recognised that obiltoxaximab will be administered to patients in combination with antibiotics. Still, given the high lethality of inhalational anthrax once symptoms have occurred, it does not appear that a demonstration of additive effects in combination is necessary, as it would be reasonable to add an intervention with a different mechanism of action to antibiotics, provided that there is reasonable evidence of the efficacy of that intervention. Duly recognising the limitations of an efficacy demonstration based on animal models, it is considered that the efficacy of obiltoxaximab has been sufficiently substantiated, given the limitations of possible studies.

While the precise time-window after infection or onset of symptoms where obiltoxaximab therapy is beneficial cannot be defined based on the animal models, data support the anticipation that efficacy will be greater the earlier therapy is initiated. Therefore, the prophylaxis indication for the rare patient that is not eligible for antibiotics is considered supported by the data from the monotherapy studies.

The conclusions above are contingent on the clarification that exposure in humans is therefore likely to be sufficient.

Additional efficacy data needed in the context of a MA under exceptional circumstances

It is not feasible to evaluate efficacy of medical countermeasures against inhalational anthrax in clinical studies, because the incidence of naturally occurring disease is too low, and it is unethical to intentionally expose humans to anthrax.

An open-label field study AH501, which is also part of the agreed PIP for Obiltoxaximab SFL, is planned to be conducted upon the occurrence of an anthrax outbreak in a country where obiltoxaximab is authorised and available. To gather as much data on the efficacy (and safety) of obiltoxaximab as possible, the protocol for study AH501 foresees data collection on any use of obiltoxaximab in the approved indication, including use of obiltoxaximab in the context of large and small anthrax incidents, including single cases. In addition, the protocol for study AH501 allows for retrospective data collection.

The primary objective of study AH501 is to evaluate the clinical response, including the course of illness and survival in subjects with suspected, probable, or confirmed cases of inhalational anthrax treated with obiltoxaximab.

2.5.4. Conclusions on the clinical efficacy

Proof-of-concept is considered reasonably established for obiltoxaximab in monotherapy studies for the treatment of inhalational anthrax. An add-on benefit of administering obiltoxaximab in concomitant use with antibiotics has not been demonstrated in the provided studies. However, it would be logical to add an intervention with a different mechanism of action to antibiotics, since there is reasonable evidence of efficacy in terms of survival rate for obiltoxaximab administered as monotherapy.

The CHMP considers the following measures necessary to address the missing efficacy data in the context of a MA under exceptional circumstances:

A post-authorisation efficacy study (PAES), i.e. the phase 4 open-label field study AH501 is to be conducted upon an anthrax outbreak in a country where obiltoxaximab is authorised and available (study protocol to be re-submitted for assessment by 1Q2021).

2.6. Clinical safety

Exposure

The Primary Safety Population includes pooled safety data from subjects who received a single IV dose of 16 mg/kg obiltoxaximab (commercial formulation) or placebo in AH104 and subjects who received a single IV dose of 16 mg/kg obiltoxaximab either with or without ciprofloxacin in AH110. In addition, repeat dose date from subjects who received a repeat-dose of 16 mg/kg obiltoxaximab (commercial formulation) in Study AH109 either within 2 weeks or ≥4 months after the first dose are included in the Primary Safety Population.

Supportive data include the Expanded Safety Population which includes pooled data for all subjects who received a single IV dose of 16 mg/kg obiltoxaximab (either the commercial formulation or the investigational material) or placebo in AH104, AH110, and AH105. Further data from subjects who received other IV ETI-204 obiltoxaximab doses in study AH105, AH102, and AH101 are also considered supportive.

Overall, 497 healthy adults received obiltoxaximab (all doses; both commercial formulation and investigational material; both IV and IM routes of administration; of these 250 healthy subjects (single dose pool study AH104 and AH110) were treated with a single 16 mg/kg dose of the commercial obiltoxaximab formulation.

Adverse events

In the single-dose pool of the Primary Safety Population, 46.0% and 38.6% of subjects in the obiltoxaximab and placebo groups, respectively, had at least one AE. The majority of the AEs in the single-dose were mild or moderate. Two severe AEs, urticaria and pruritus, were reported in 1 subject.

The most frequent AEs (reported in >2% of subjects) in the obiltoxaximab group were headache, upper respiratory tract infection, somnolence, pruritus, vessel puncture site bruise, cough, nausea, and urticaria. Out of these AEs, the rates of headache, somnolence, pruritus, cough, and urticaria were ≥2% higher in the obiltoxaximab group compared to the placebo group.

In the single-dose pool, 14.4% and 12.9% of subjects in the obiltoxaximab and placebo groups, respectively, had an AE that was judged by the investigator as related to study drug. Related AEs that occurred in ≥1% of subjects in the obiltoxaximab group were pruritus, headache, urticaria, rash, and cough and infusion site pain.

In the repeat-dose groups, 34.3% and 25.7% of subjects in the 2 weeks apart and ≥4 months apart groups, respectively, had an AE that was judged by the investigator as related to study drug. The most frequently reported related AEs in the 2 weeks and ≥4 months apart repeat-dose groups were infusion site swelling (8.6% and 11.4%, respectively), infusion site erythema (8.6% and 2.9%, respectively), and infusion site pain (5.7% and 2.9%, respectively). The majority of AEs were mild and moderate.

Four subjects in the repeat-dose groups had severe AEs, including 1 subject in the 2 weeks apart and 3 subjects in the ≥4 months apart groups. One of these AEs (back pain occurred in conjunction with mild to moderate flushing, chills, dyspnoea, cyanosis, pallor, rash, restlessness, and myalgia) was rated related to study drug by the investigator.

In the single-dose pool of the Expanded Safety Population, 47.9% and 46.6% of subjects in the obiltoxaximab and placebo groups, respectively, had at least one AE; one subject in the obiltoxaximab group permanently discontinued study drug due to an AE.

Frequent AEs that occurred in a higher percentage (≥2% difference) of subjects in the obiltoxaximab group compared to the placebo group were headache (obiltoxaximab , 9.2%; placebo, 5.7%), somnolence (obiltoxaximab, 4.4%; placebo, no subjects), pruritus (obiltoxaximab, 4.0%; placebo, 1.4%), cough (obiltoxaximab, 2.8%; placebo, no subjects), and urticaria (obiltoxaximab , 2.4%; placebo, no subjects).

- **Events of special interest (most salient findings)**

Events of interest for obiltoxaximab were defined by the applicant based on the known safety profile for mAbs, non-clinical data with obiltoxaximab and published data with an anti-PA mAb. They include infusion-related reactions (defined as AEs occurring within 24 hours of the start of study drug infusion), hypersensitivity (including rash), rash, neurologic AEs, gastrointestinal disorders, infections and infestations, and respiratory, thoracic, and mediastinal disorders.

Infusion-related reactions (IRRs) were defined as any AE timely related to drug administration, i.e. any AE occurring within 24 hours of administration of IV obiltoxaximab. The infusion site reactions were assessed by the investigator according to a 4-grade scale.

In the single-dose pool of the Primary Safety Population, 15.6% and 4.3% of subjects in the obiltoxaximab and placebo groups had an AE within the first 3 hours of the start of study drug infusion. Within 3 to 24 hours of the start of study drug infusion, 13.6% and 7.1% of subjects in the obiltoxaximab and placebo groups in the single-dose pool had an AE. The rate of hypersensitivity was 8.8% (22/250) in the obiltoxaximab group compared to 5.7% (4/70) in the placebo group. The rate of rash in the single-dose pool was 5.6% and 2.9% of subjects in the obiltoxaximab and placebo groups, respectively. All except two hypersensitivity AEs were mild or moderate in severity (independent of pre-treatment with diphenhydramine) One subject not pre-treated with diphenhydramine had severe urticaria and severe pruritus reported within 3 hours of the start of the infusion. Both AEs decreased to mild in severity at 6 hours post start of infusion. One case of anaphylactic reaction was reported based on investigator's assessment. The subject was pre-treated with diphenhydramine and had an acute anaphylactic reaction during the first (and only) obiltoxaximab infusion. This was characterised by shortness of breath, coughing and a diffuse, pruritic urticarial rash covering most of the body, including neck, chest, back, abdomen, arms, and legs. This event was categorised as moderate in severity and non-serious by the investigator.

The majority of subjects in the obiltoxaximab and placebo groups in the single-dose pool of the Primary Safety Population received diphenhydramine pre-treatment before study drug infusion (obiltoxaximab, n=184 [73.6%]; placebo, n=48 [68.6%]). In the single-dose pool, the incidence of hypersensitivity was slightly higher in subjects who received obiltoxaximab and pre-medication with diphenhydramine compared to those who did not (17/184 [9.2%] vs. 5/66 [7.6%], respectively). Conversely, the incidence of rash was slightly lower in subjects who received pre-medication with diphenhydramine compared to those who did not (10/184 [5.4%] vs. 4/66 [6.1%], respectively). The severity of hypersensitivity AEs was similar with and without diphenhydramine pre-treatment.

In the repeat-dose groups, hypersensitivity (including rash) was only reported following obiltoxaximab administration. The percentage of subjects with hypersensitivity (including rash) was 8.6% and 5.9% after the first and second doses of obiltoxaximab in the 2 weeks apart repeat-dose group, respectively, and 5.7% and 3.2% after the first and second doses of obiltoxaximab in the ≥4 months apart repeat-dose group, respectively. The percentage of subjects with rash was 2.9% and 5.9% of subjects after the first and second doses of obiltoxaximab in the 2 weeks apart repeat-dose group, respectively, and 2.9% and 3.2% after the first and second doses of obiltoxaximab in the ≥4 months apart repeat-dose group, respectively. No hypersensitivity (including rash) or rash AEs occurred in more than 1 subject in either of the repeat-dose groups. Thus, repeat-dosing with obiltoxaximab, either 2 weeks apart or ≥4 months apart, does not appear to be associated with an increase in hypersensitivity (including rash) or rash.

In the single-dose pool of the Expanded Safety Population, AEs within the first 3 hours of the start of study drug infusion consistent with a possible hypersensitivity reaction in the obiltoxaximab group were identical to those in the Primary Safety Population. Compared to the Primary Safety Population, there were 2 additional cases of urticaria in the obiltoxaximab group of the Expanded Safety Population within the first 3 hours of the start of study drug infusion.

Nervous system disorders were reported in 15.2% of subjects in the obiltoxaximab group and 5.7% of subjects in the placebo group in the single-dose pool of the Primary Safety Population. The higher frequency of nervous system disorders in the obiltoxaximab group compared to the placebo group was largely driven by the PTs of headache, somnolence, and dizziness. Other PTs in the nervous system disorders SOC were only reported in single subjects without a clear pattern. Headache and somnolence were reported for a higher percentage of subjects in the obiltoxaximab group (9.2% and 4.4%, respectively) compared to the placebo group (5.7% and 0.0%, respectively). In the repeat-dose group, the frequencies of headache and somnolence were identical regardless of whether an obiltoxaximab or

a placebo infusion had most recently been administered. Somnolence was only seen in the obiltoxaximab group in subjects who were pre-treated with diphenhydramine.

Psychiatric AEs in the Expanded Safety Pool were consistent with those reported in the Primary Safety Pool.

Headache, somnolence, and dizziness were also the most frequently reported nervous system disorders in the repeat dose groups, however the frequencies were similar regardless of whether an obiltoxaximab or a placebo infusion had most recently been administered.

In the single-dose pool, gastrointestinal AEs were reported in 6.4% and 2.9% of subjects in the ETI 204 and placebo groups, respectively. A subset of gastrointestinal AEs combined (toothache, vomiting, dry mouth, anal fissure, eructation, hiatus hernia and lip pain) accounted for a higher incidence of gastrointestinal AEs in the obiltoxaximab group compared to the placebo group (11% vs. 0% of subjects, respectively).

In the repeat-dose group, gastrointestinal AEs, including toothache and vomiting, did not occur more frequently following obiltoxaximab infusion compared to placebo infusion

Serious adverse event/deaths/other significant events

No deaths occurred in the obiltoxaximab clinical development program.

Three serious adverse events (SAEs) were reported in the obiltoxaximab clinical development program: an ovarian cyst in the placebo group of Study AH104, an ankle fracture in the obiltoxaximab group in Study AH109, and a herniated disc in a subject treated with 4 mg/kg obiltoxaximab in Study AH105. All of these events were judged as unrelated to study medication by the investigator.

Laboratory findings

Haematological parameters were in general similar and stable over time in subjects who received single doses of obiltoxaximab or placebo, and in subjects who received repeat doses of obiltoxaximab either within 2 weeks or ≥4 months after the first dose.

For each serum biochemistry parameter, the majority of subjects in the obiltoxaximab and placebo groups in the single-dose pool of the Primary Safety Population had a toxicity grade 0 at Baseline and no change in toxicity grade post-baseline. Increased cholesterol (obiltoxaximab, 16.9%; placebo, 18.6%) and increased creatine kinase (obiltoxaximab, 10.0%; placebo, 15.7%) were the most frequent ≥2 grade shifts in the obiltoxaximab and placebo groups in the single-dose pool of the Primary Safety Population.

There were no differences between the Expanded and Primary Safety Populations in the most frequent ≥2-grade shifts in serum biochemistry parameters.

No subjects in the single-dose pools of the Primary and Expanded Safety Populations met the criteria for Hy's law.

In the repeat-dose groups, the majority of subjects in the 2 weeks apart and ≥4 months apart groups had a toxicity grade 0 at Baseline in each serum biochemistry parameter and no change in toxicity grade post-baseline. Increased cholesterol (2 weeks, 25.7%; ≥4 months, 22.9%), increased creatine kinase (22.9% in each group), and increased potassium (2 weeks, 2.9%; ≥4 months, 20.0%) were the most frequent ≥2-grade shifts in the repeat dose groups

Vital signs, mean systolic and diastolic blood pressures, pulse rate, respiratory rate, and oral body temperature were similar and stable over time in subjects who received single doses of ETI-204 or placebo and in subjects who received repeat doses of obiltoxaximab either within 2 weeks or ≥4 months after the first dose.

Mean values for ECG parameters were stable over time and were similar for subjects who received single doses of obiltoxaximab and placebo and in subjects who received repeat doses of obiltoxaximab either within 2 weeks or ≥4 months after the first dose. QT prolongation was not seen with either single or repeat doses

Safety in special populations

Obiltoxaximab has not been evaluated in pregnant women, nursing mothers, or paediatric subjects. Clinical studies did not include a sufficient number of subjects ≥65 years to determine if the safety profile of obiltoxaximab is different from that seen in younger subjects.

Although the following subgroup analyses of AEs were performed for the Primary and Expanded Safety Populations and the repeat-dose groups: gender (males and females), race (white vs all other races), age (≤ 65 years and > 65 years), baseline BMI (<median and ≥median for each pool), baseline CrCl of < 102 mL/min, ≥ 102 and ≤ 144 mL/min, and > 144 mL/min (based on quartiles) and, diphenhydramine pre-treatment (yes or no)

In the single-dose pool of the Primary Safety Population the incidence of moderate and severe AEs, SAEs, and AEs resulting in study discontinuation were low precluding any meaningful conclusions about the potential differences for these events between subgroups. The limited number of additional subjects (30 subjects) in the single-dose pool of the Expanded Safety Population compared to the Primary Safety Population and in each of the repeat-dose groups (35 subjects each) precludes any meaningful conclusions about potential differences in AEs between subgroups in the Expanded Safety Population and the repeat-dose groups.

Immunological events

Of the 470 subjects who received at least one dose of IV obiltoxaximab in the clinical development program, 14 subjects (3%) developed treatment-emergent ADA. Titres ranged from 1:20 to 1:320. In the single-dose pool, 2.5% (8/320) of subjects who received one dose of IV obiltoxaximab were positive for a treatment-emergent ADA response with titres ranging from 1:20 to 1:320.

In the repeat-dose groups, 8.8% of subjects who received obiltoxaximab doses 2 weeks apart, and 3.2% who received doses ≥4 months apart were positive for a treatment-emergent ADA response. Titres were quantitatively low, ranging from 1:20 to 1:80. No ADA responses were seen with IM administration of obiltoxaximab.

No AEs were reported coincident with the development of ATA in any of these subjects. The presence of ADA did not appear to affect obiltoxaximab disposition (see pharmacology).

Safety related to drug-drug interactions and other interactions

- **Obiltoxaximab in combination with ciprofloxacin**

Part 2 of study AH101 evaluated the safety, tolerability, and PK of a single dose of 114 mg obiltoxaximab IV or placebo administered on Day 1 plus ciprofloxacin (500 mg every 12 hours orally

for 14 days). Six subjects received obiltoxaximab in combination with ciprofloxacin and 6 subjects received placebo in combination with ciprofloxacin.

AEs were reported by 100% of subjects in the obiltoxaximab plus ciprofloxacin group and 66.7% of subjects in the ciprofloxacin alone group. The most frequently reported AEs were headache (obiltoxaximab + ciprofloxacin, 66.7%; ciprofloxacin, 33.3%), nausea (obiltoxaximab + ciprofloxacin, 33.3%; ciprofloxacin, 16.7%) and vomiting (obiltoxaximab + ciprofloxacin, 33.3%; ciprofloxacin, 16.7%).

AH110 was an open-label, randomised, parallel group study of IV obiltoxaximab administered alone on Day 1 and in the presence of IV ciprofloxacin (400 mg on Day 1; following obiltoxaximab infusion) and oral ciprofloxacin (750 mg orally every 12 hours on Days 2 through 8 and a single 750 mg dose of oral ciprofloxacin on the morning of Day 9). The percentage of subjects with AEs was similar in the obiltoxaximab plus ciprofloxacin and obiltoxaximab alone groups. Somnolence (obiltoxaximab + ciprofloxacin, 25.0%; ETI 204 alone, 30.0%) and upper respiratory tract infection (obiltoxaximab + ciprofloxacin, 10.0%; obiltoxaximab alone, 25.0%) were the most frequently reported AEs.

Three severe AEs occurred in Part 2 of AH101: dizziness and upper respiratory tract infection in 1 subject in the ciprofloxacin alone group and nausea in 1 subject in the obiltoxaximab plus ciprofloxacin group. No severe AEs were reported in AH110.

Two subjects in the obiltoxaximab plus ciprofloxacin group in AH110 permanently discontinued study drug before completing the obiltoxaximab infusion due to AEs of urticaria. No premature discontinuations of study drug due to an AE occurred in Part 2 of AH101.

- **Obiltoxaximab in combination with anthrax vaccine**

No studies have been conducted with obiltoxaximab to evaluate the effect of concomitant administration with an anthrax vaccine.

- **Diphenhydramine pre-treatment**

The majority of subjects in the obiltoxaximab and placebo groups in the single-dose pool of the Primary Safety Population received diphenhydramine pre-treatment before study drug infusion (obiltoxaximab 73.6%; placebo, 68.6%). The percentage of subjects who did not receive diphenhydramine pre-treatment before study drug infusion was 26.4% in the obiltoxaximab group and 31.4% in the placebo group.

All subjects in study AH110 were pre-treated with antihistamine (i.e. diphenhydramine), whereas 63 subjects in study AH104 and 8 subjects in study AH109 did not receive the pre-treatment. Overall, a slightly higher frequency of AEs was observed in subjects that were not pre-treated with diphenhydramine in the SOC general disorders and administration site conditions (10.6% vs 17.6%) and in the SOC nervous system disorders (15% vs 21.6%).

In the obiltoxaximab group, subjects who were pre-treated with diphenhydramine prior to the infusion of study drug had a lower overall incidence of AEs than subjects without diphenhydramine pre-treatment (43.5% and 53.0%, respectively). In the placebo group, the overall incidence of AEs was similar with and without diphenhydramine pre-treatment (37.5% and 40.9%, respectively). AEs in the obiltoxaximab group that were decreased with diphenhydramine pre-treatment compared to without pre-treatment included headache (6.0% and 18.2%, respectively) and cough (1.1% and 7.6%, respectively).

AEs that were increased in the obiltoxaximab group with diphenhydramine pre-treatment compared to without pre-treatment included somnolence (6.0% and no subjects, respectively ;), vessel puncture site bruise (3.8% and 1.5%, respectively), and nausea (3.8% and no subjects, respectively).

Discontinuation due to adverse events

Of the 320 subjects who received a single dose of obiltoxaximab in either the single-dose or the repeat-dose groups, 8 subjects (2.5%) permanently discontinued study drug before infusion completion because of one or more AEs.

All 8 subjects who permanently discontinued the obiltoxaximab infusion before completion experienced a possible hypersensitivity reaction, including 5 subjects with urticaria (mild to severe) (3 of these subjects had concomitant pruritus [mild to severe]), 2 subjects with rash (moderate), and 1 subject with an anaphylactic reaction (moderate). The subject who discontinued obiltoxaximab due to severe urticaria and pruritus did not receive diphenhydramine pre-medication.

In study AH104, a slightly higher frequency of discontinuation was observed among the non-pre-treated subjects (4.5%) vs the pre-treated subjects (2.1%).

Post marketing experience

Not applicable

2.6.1. Discussion on clinical safety

Overall, obiltoxaximab dose of 16 mg/kg is well-tolerated when administered IV as a 90-minute infusion. The AEs were usually mild and moderate. Infusion related reactions including hypersensitivity were frequently reported in subjects treated with obiltoxaximab. The data suggest that diphenhydramine pre-treatment did not mitigate the infusion related reactions; however, the database is too small to draw a robust conclusion.

Obiltoxaximab has been evaluated only in healthy adult volunteers, however it was not evaluated in the target population i.e. patients with inhalation anthrax or in healthy people exposed to anthrax spores. There is a theoretical risk that the safety profile of obiltoxaximab could be different in the target population. However, since obiltoxaximab does not have an endogenous human target and does not exhibit unspecific cross-reactivity in human tissues, the theoretical risk of target-mediated toxicity is not a concern for obiltoxaximab treatment. Further, antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) via its Fc region is a theoretical concern of IgG1 antibodies such as obiltoxaximab. Fc-mediated cytotoxicity (ADCC or CDC) requires antibody binding to antigens on the cell surface, since obiltoxaximab only binds to soluble PA and does not bind to cell bound PA, thus Fc-mediated cytotoxicity (ADCC or CDC) is not a concern for obiltoxaximab treatment in the sought indication.

Patients with comorbidities such as hepatic impairment, renal impairment or with cardiovascular impairment were not included in the development program. Considering the mode of action of obiltoxaximab i.e. the ligand is a non-human target, drug-disease interactions are not expected therefore it is not expected that acute and/ or active medical conditions will have clinically meaningful effects on safety and efficacy of obiltoxaximab.

Obiltoxaximab has not been evaluated in pregnant women, nursing mothers, or paediatric subjects. The frequency of AEs such as infusion related reactions, might be influenced by age-related factors, concomitant diseases, concurrent medications, however, clinical studies did not include a sufficient number of subjects ≥65 years to determine if the safety profile of obiltoxaximab is different from that seen in younger subjects. Thus, no safety data in vulnerable population are available are available, however due to the nature of the disease to be treated / prevented this shortcoming is acceptable.

A limited number of subjects received obiltoxaximab in combination with ciprofloxacin; a slightly increase in AEs were reported in the obiltoxaximab + ciprofloxacin groups compared to ciprofloxacin alone. However, the data have to be interpreted with caution due to the small study population.

Additional measures are necessary to address the missing safety data in the context of a under exceptional circumstances (see below)

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

Additional safety data needed in the context of a MA under exceptional circumstances

An open-label field study AH501, which is also part of the agreed PIP for Obiltoxaximab SFL, is planned to be conducted upon the occurrence of an anthrax outbreak in a country where obiltoxaximab is authorised and available. To gather as much data on the (efficacy and) safety of obiltoxaximab as possible, the protocol for study AH501 foresees data collection on any use of obiltoxaximab in the approved indication, including use of obiltoxaximab in the context of large and small anthrax incidents, including single cases. In addition, the protocol for study AH501 allows for retrospective data collection.

The study population may include men and women (including pregnant and lactating women) and children of all ages who received obiltoxaximab as part of their clinical care for anthrax infection

The study will evaluate the safety and tolerability of obiltoxaximab in subjects with suspected, probable, or confirmed cases of inhalational anthrax. Safety evaluations will include TEAEs and SAEs during the study, including infusion and hypersensitivity reactions, vital signs and physical findings, as well as ECG and clinical laboratory tests.

2.6.2. Conclusions on the clinical safety

The safety data of obiltoxaximab show an acceptable safety profile in healthy young adults. The exclusion of vulnerable population in the phase I study program is acceptable. Due to the epidemiology of the disease i.e. very rare incidence of natural inhalation anthrax the generation safety data in the target population for obiltoxaximab i.e. general population including vulnerable population is not feasible at the moment and will be subject to a Post-Marketing Study.

The CHMP considers the following measures necessary to address the missing safety data in the context of a MA under exceptional circumstances:

A post-authorisation study, i.e. the phase 4 open-label field study AH501 is to be conducted upon an anthrax outbreak in a country where obiltoxaximab is authorised and available (study protocol to be re-submitted for assessment by 1Q2021).

2.7. Risk Management Plan

Safety concerns

Important identified risks	Hypersensitivity (including rash) and anaphylaxis
Important potential risks	None
Missing information	Safety profile in patients with inhalational anthrax disease

Pharmacovigilance plan

No specific routine pharmacovigilance activities beyond adverse reactions reporting and signal detection are considered necessary to address the safety concerns. No safety studies are required to monitor the safety of the product.

Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Hypersensitivity (including rash) and anaphylaxis	Routine risk minimisation measures: *SmPC sections 4.2, 4.4 and 4.8* *PL section 2, 3 and 4*	None.
Safety profile in patients with inhalational anthrax disease	Routine risk minimisation measures: *SmPC section 4.8, stating that safety has only been studied in healthy volunteers*	None

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.0 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 18.03.2016. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant declared that obiltoxaximab has not been previously authorised in a medicinal product in the European Union.

The review of data on the quality, non-clinical and clinical properties of the active substance, indicates that the mAb obiltoxaximab in comparison to the known approved mAbs and to other potential treatment options (antibiotics, anthrax vaccines, anthrax immunoglobulins is to be qualified as a new active substance as it differs significantly in properties with regard to safety and efficacy from the previously authorised substance.

Raxibacumab, another mAb with the same mechanism of action as ETI-204 (*B. anthracis* protective-antigen binding and neutralisation) is not authorised in the EU but was granted orphan drug designation (ODD) by the European Commission in 2014.

The CHMP, based on the available data, considers obiltoxaximab to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Labelling exemptions

A request of translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

The Group accepted an English only vial label with a bi-lingual outer carton (EN-DE), including some text simplifications.

Furthermore, the Group agreed with the applicant's request for an English-only package leaflet with the commitment for the applicant to provide patients with a printed version in local language(s). The labelling subject to translation exemption as per the QRD Group decision above will however be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD Group.

2.10.3. Quick Response (QR) code

A request to include a QR code in the labelling and the package leaflet for the purpose of providing statutory information in all EU languages has been submitted by the applicant and has been found acceptable.

The following elements have been agreed to be provided through a QR code: The outer carton (inner flap) and on the package leaflet.

2.10.4. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Obiltoxaximab SFL (obiltoxaximab) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU and also since it is approved under exceptional circumstances [REG Art 14(8), DIR Art (22)].

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

The following indication is claimed for obiltoxaximab:

- Obiltoxaximab SFL is indicated in combination with appropriate antibacterial drugs in all age groups for treatment of inhalational anthrax due to *Bacillus anthracis*.
- Obiltoxaximab SFL is indicated in all age groups for post-exposure prophylaxis of inhalational anthrax when alternative therapies are not appropriate or are not available.

Anthrax is a disease caused by the Gram-positive, encapsulated, spore-forming bacterium *B. anthracis*, which is found in soils all over the world. Inhalational anthrax, the most severe form of anthrax, is caused by exposure to aerosolised *B. anthracis* spores.

Inhalational anthrax is a life-threatening disease, with an overall mortality rate of approximately 50% even with aggressive treatment including multiple antimicrobials. Patients who progress to the fulminant stage of inhalational anthrax have an extreme high mortality rate (~97 %), regardless of the treatment they receive.

Although, naturally occurring inhalational anthrax is a rare condition, occurring sporadically around the world in individuals and small clusters of people exposed to infected animals and animal products, anthrax spores have been used in the past and have the potential to be used in the future as biological weapons. *B. anthracis* spores are readily weaponised and highly virulent.

3.1.2. Available therapies and unmet medical need

Currently, the main treatment for suspected or confirmed inhalational anthrax is antibiotic therapy. Antibiotics are also recommended for post-exposure prophylaxis in individuals with suspected or confirmed exposure *to B. anthracis*. In some cases, immunisation with anthrax vaccines is recommended, and this may reduce the duration of post-exposure prophylactic antibiotic treatment.

- Ciprofloxacin is licensed for post-exposure prophylaxis and curative treatment of inhalational anthrax in children, adolescents and adults. Oral ciprofloxacin 500 mg bid (or 10-15 mg/kg in children) for 60 days is recommended to prevent inhalational anthrax in humans, starting as soon as possible after suspected or confirmed exposure. At least 60 days of prophylaxis after exposure to *B. anthracis* spores is recommended because the spores can potentially remain dormant for up to 60 days before they are activated. For treatment of inhalational anthrax, it is recommended that the treating physician refer to national and /or international consensus documents.
- Levofloxacin is licensed for post-exposure prophylaxis and curative treatment of inhalational anthrax, based on in-vitro susceptibility and animal data, together with limited human data. Dosing recommendations are similar, including reference to national and /or international consensus documents for the treatment of inhalational anthrax.

- Benzylpenicillin is licensed for the treatment of *B. anthracis* infections but no specific recommendations are made in the SmPC.

The main treatment for suspected or confirmed inhalational anthrax in the EU is fluoroquinolone therapy. Ciprofloxacin is recommended as a first line treatment of inhalational anthrax in all age groups [EMA, 2014], and as an alternative for oral follow-up. In addition, the EMA recommends ciprofloxacin as a first line prophylaxis until susceptibility to other agents has been confirmed. Other quinolones (ofloxacin and levofloxacin) can be used as alternative treatment options but dose recommendations are only available for adults. Doxycycline and penicillin provide treatment options when susceptibility has been confirmed although penicillin is not bactericidal against *B. anthracis*.

Natural resistance of *B. anthracis* is known for several antibiotics, including sulfamethoxazole, trimethoprim, cefuroxime, cefotaxime sodium, aztreonam, and ceftazidime. They should not be used for treatment or prophylaxis of anthrax infection [Inglesby et al, 2002].

Two additional approaches for treatment of inhalational anthrax are currently not authorised in the EU: passive immunisation with anthrax immunoglobulins, and antitoxin therapy with mAbs targeting anthrax toxins.

The possible exposure of large numbers of people to air-borne *B. anthracis* spores in the context of a bioterrorist attack raises many issues, not least the availability of antibiotics, compliance of long-term antibiotic administration, safety profile of the antibiotics or resistance development. It is noted that since March 2019 the use of fluoroquinolone antibiotics is restricted in the EU (EMA/175398/2019) due to safety concerns related to disabling and potentially permanent side effects involving tendons, muscles, joints and the nervous system. Special caution is warranted in elderly patients, patients with kidney disease and those who have had organ transplantation because these patients are at a higher risk of tendon injury.

3.1.3. Main clinical studies

It is not feasible to evaluate efficacy of medical countermeasures against inhalational anthrax in clinical studies, because the incidence of naturally occurring disease is too low, and it is unethical to intentionally expose humans to anthrax. The applicant is requesting an authorisation under exceptional circumstances, since human efficacy studies are not possible. Therefore, it is considered acceptable to evaluate efficacy in animal trials. Efficacy studies in treatment and the pre- and post-exposure prophylaxis of inhalational anthrax were conducted in two well-characterised animal models, NZW rabbits and cynomolgus monkeys.

Four monotherapy treatment studies are GLP-compliant as well as blinded (AP204, AP202, AP301 and AR033). These are considered pivotal for the efficacy demonstration. In those studies, obiltoxaximab was administered IV or IM to rabbits or monkeys that were exhibiting clinical signs or symptoms of systemic anthrax. The evaluated doses were 0, 1, 4, 8 and 16 mg/kg IV (rabbit) and 0, 4, 8 and 16 mg/kg, IV or IM (monkey). Two different formulations of obiltoxaximab were used (pilot and commercial). In two monkey studies (AP202 and AP301) animals were treated with the commercial formulation whereas obiltoxaximab in the pilot formulation was administered in rabbits (AR033) as well as in monkeys (Studies AP204 and AP202).

Other monotherapy studies were also provided, however, since investigated other doses than 16 mg/kg, they were unblinded or without GLP-status, they are considered supportive.

Furthermore, four studies of obiltoxaximab as concomitant treatment with antibiotics were conducted in the NZW rabbit model of inhalational anthrax, using human equivalent doses of levofloxacin administered orally for 3-5 days. These studies were open-label (unblinded) exploratory trials, and only one of them was conducted in compliance with GLP (AR007). Obiltoxaximab at the doses of 4, 8 and 16 mg/kg were administered IV concomitant with antibiotics 9-96 hours post exposure. In the studies where treatment was delayed up to 72-96 h post-exposure, several animals died before receiving treatment.

Four studies including monkey and rabbit using significant lower doses of antibiotic were also provided, they were considered exploratory and not relevant to demonstrate an add-on benefit of obiltoxaximab.

3.2. Favourable effects

Four studies were considered as pivotal efficacy monotherapy studies since they were blinded and GLP-compliant. Of these, Study AP204 was only blinded to group but the applicant showed that this did not have any impact on the outcome of the study and this study is therefore considered as pivotal.

In study AR033 including rabbit a statistically significant difference in survival rate (primary endpoint) compared to placebo (0%) was observed when 4, 8 and 16 mg/kg was administered IV with a numerical survival rate of 33-69%.

In the monkey studies, high survival rate (83-100%) compared to placebo (0%) was observed in study AP301 when obiltoxaximab was administered IM at the doses 8 or 16 mg/kg up to 24 h post exposure, however no difference was seen when the treatment was initiated 36 h post exposure. In study AP204 a statistically significant difference in survival rate was observed between placebo (6%) and 16 mg/kg IV administered ETI-204 (47%), however no difference was seen between placebo (6%) and 4 mg/kg IV administered ETI-204 (25%). In study AP202 a statistically significant difference in survival rate compared to placebo (0%) was observed for 16 mg/kg obiltoxaximab (two formulations; pilot and commercial) administered IV, the survival rate was however modest (31-35%). Moreover, survival time from spore challenge (secondary endpoint), was increased for all obiltoxaximab treated animals included in the four pivotal studies.

In addition, the efficacy of obiltoxaximab is supported by several non-blinded, non-GLP compliant studies, executed in both rabbit and monkey.

In the exploratory studies in anthrax-challenged animals, treatment with antibiotic plus obiltoxaximab increased survival rate compared to placebo.

Treatment with obiltoxaximab did not prevent the development of a protective anti-PA immune response. Rabbits that survived an anthrax-challenge upon treatment with obiltoxaximab alone or in combination with antibiotics, developed anti-PA antibodies and showed an increased survival upon re-challenge 9 months later.

3.3. Uncertainties and limitations about favourable effects

The rabbit and non-human primate models used are considered acceptable models of inhalational anthrax, although the artificial nature of the model is recognised, e.g., in terms of a standardised inoculum as well as different disease kinetics compared to the human situation. Therefore, it remains uncertain what would be the time-window from infection or onset of symptoms, where obiltoxaximab would be effective. Furthermore, it is not known what level of bacteraemia that may occur in humans infected with inhalation anthrax since infectious dose and time from exposure will be different from

case to case, which adds a further uncertainty to the anticipated extent of benefit and how long time after exposure the treatment will be effective.

Most of the efficacy studies were exploratory studies and a limited number of the monotherapy studies were GLP-compliant and blinded (four pivotal studies). Two monotherapy studies (AP201 and AP204) included in the exposure response modelling were only partly blinded (i.e. by group). The applicant was asked to clarify whether these limitations have affected the outcome of the studies and thereby their reliability to be included in dose evaluation models. According to the applicant's response, the survival in the control groups across the performed studies in monkey was consistent with expected survival in untreated animals based on published literature and natural history studies. The blinding schema did not appear to impact the overall survival outcomes for the control group. Thus, the inclusion of all four monkey studies (AP201, AP204, AP203 and AP202) in the PK modelling appears acceptable.

The effectiveness of the human dose is not verified in patients. Efficacy is bridged from the data obtained in the animal disease model, via healthy animals to humans based on similar or higher exposure in humans. Since PK data are generated in healthy humans, the impact of physiological consequences of disease, as well as target mediated clearance, remains an uncertainty.

PK data are critical in terms of supporting that the proposed human dose is adequate, i.e. that the exposure in humans after administration of a 16 mg/kg dose is not lower than the exposure in primates dosed at 16 mg/kg. Taking into account the applicant's arguments, the dose in human is considered sufficiently characterised.

No add-on benefit of obiltoxaximab was demonstrated in the studies using human equivalent doses of antibiotic in rabbits. However, since treatment with obiltoxaximab in combination with an antibiotic increased survival compared to placebo, it can be concluded that obiltoxaximab does not have a negative impact on the survival rate in rabbits or cynomolgus monkeys when co-administered with an antibiotic at human-equivalent or lower than human-equivalent dose.

No human data on the PD effects are available. The description of PD effects is based solely on in animal models of inhalation anthrax.

No human data in patients with inhalation anthrax are available. The effectiveness of obiltoxaximab in patients is solely based solely on efficacy data in animal models of inhalation anthrax.

The effectiveness of obiltoxaximab in paediatric patients is solely based on efficacy data in animal models of inhalation anthrax.

3.4. Unfavourable effects

Infusion related reactions including hypersensitivity reactions were frequently reported in subjects who received obiltoxaximab. In single-dose Pool of the Primary Safety Population (subjects who received the commercial formulation) hypersensitivity reactions occurred in 8.8% of subjects in the obiltoxaximab group compared to 5.7% in the placebo group.

3.5. Uncertainties and limitations about unfavourable effects

The number of subjects presented with ADAs was low considered that this is a chimeric mAb.

Obiltoxaximab has been evaluated only in healthy adult volunteers, however it was not evaluated in the target population i.e. patients with inhalation anthrax or in healthy people exposed to anthrax spores. There is a theoretical risk that the safety profile of obiltoxaximab could be different in the

target population. However, since obiltoxaximab does not have an endogenous human target and does not exhibit unspecific cross-reactivity in human tissues, the theoretical risk of target-mediated toxicity is negligible. Fc-mediated cytotoxicity (ADCC or CDC) is not a concern for obiltoxaximab treatment in the sought indication since obiltoxaximab only binds to soluble PA and does not bind to cell bound PA.

Obiltoxaximab has not been evaluated in pregnant women, nursing mothers, or paediatric subjects. The frequency of AEs such as infusion related reactions, might be influenced by age-related factors, concomitant diseases, concurrent medications; however, clinical studies did not include a sufficient number of subjects ≥65 years to determine if the safety profile of obiltoxaximab is different from that seen in younger subjects.

3.6. Effects Table

Table 34 Effects Table for obiltoxaximab SFL

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Survival rate	ETI-204 administered as monotherapy compared to placebo in animals exposed to inhalation anthrax		4 mg/kg IV: 4/16 (25.0%) 16 mg/kg IV:7/15 (46.7%)	1/16 (6.3%)	Commercial product (commercial) Significant difference in survival rate compared to placebo for 16 mg/kg. Non-significant trend for 4 mg/kg. Numerical survival rate ~25%	AP204
			16 mg/kg IV (commercial): 5/16 (31.3%) 16 mg/kg IV (pilot): 6/17 (35.3%)	0/17 (0%)	Two formulations used (pilot and commercial) Significant difference compared to placebo, numerical survival rate ~35%	AP202

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
			8 mg/kg IM 18 h: 6/6 (100%) 16 mg/kg IM 18 h: 6/6 (100%) 8 mg/kg IM 24 h: 5/6 (83%) 16 mg/kg IM 24 h: 5/6 (83%) 8 mg/kg IM 36 h: 0/6 (0%) 16 mg/kg IM 36 h: 3/6 (50%)	0/6 (0%)	Significant difference compared to placebo for administration of ETI-204 ≤ 24 h post exposure.	AP301
			1 mg/kg IV: 2/12 (16.7%) 4 mg/kg IV: 4/12 (33.3%) 8 mg/kg IV: 9/13 (69.2%) 16 mg/kg IV: 8/13 (61.5%)	0/13 (0%)	Significant difference in survival rate compared to placebo	AR033
Unfavourable Effects						
Infusion related AEs Such as swelling, pain and erythema observed <3 h or 3-24 h post infusion	Outcome in the primary safety population		<3 h: ETI-204 2.8% 3–24 h: ETI-204 4.4%	<3 h: Placebo 2.9% 3-24 h: Placebo 2.9%		AH104, AH109, AH110 single administration.
Rash	Outcome primary safety population		Single dose 14/250 (5.6%) Double dose 5/70 (7.1%)	Placebo 3/70 (3.4%)		AH104, AH109, AH110

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Headache	Outcome primary safety population		Single dose 23/250 (9.2%)	Placebo 4/70 (5.7%)		AH104, AH109, AH110 Single administration.
Hypersensitivity reactions (including rash terms)	Outcome primary safety population		Single dose 22/250 (8.8%) Double dose 8/70 (11.4%)	Placebo 4/70 (5.7%)		AH104, AH109, AH110

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Since trials in patients with inhalation anthrax are not possible, either in the real life setting or as challenge studies, the establishment of therapeutic efficacy must be based on animal models and PK bridging. This application is therefore evaluated under exceptional circumstances in accordance with Article 14(8). Available data provide proof-of-concept for the treatment principle and indicate that efficacy in humans is possible, provided that relevant exposure is reached. While the translation of efficacy between species, and from a challenge model to real life, is fraught with uncertainty, the mechanism of action is plausible, since obiltoxaximab targeting a PA subunit of the anthrax toxin which is similar irrespective species. With PK and comparability issues solved, it is considered that proof of concept for obiltoxaximab administered as monotherapy is sufficiently demonstrated to a standard allowing product authorisation.

It is appreciated that obiltoxaximab will be administered to patients in combination with antibiotics. Still, given the high lethality of inhalational anthrax once symptoms have occurred, it does not appear that a demonstration of additive effects in combination is necessary, as it would be reasonable to add an intervention with a different mechanism of action to antibiotics, provided that there is reasonable evidence of the efficacy of that intervention. Duly recognising the limitations of an efficacy demonstration based on animal models, it is considered that the efficacy of obiltoxaximab has been sufficiently substantiated, given the limitations of possible studies for inhalation anthrax.

While the precise time-window after infection or onset of symptoms, as prerequisites for obiltoxaximab therapy to be beneficial, cannot be defined based on the animal models, data support the anticipation that efficacy will be greater the earlier therapy is initiated. Therefore, not only the treatment indication, but also the prophylaxis indication for the rare patient that is not eligible for antibiotics is considered supported by the data.

The conclusions above are contingent on the clarification that exposure in humans is likely to be sufficient. Overall obiltoxaximab dose of 16 mg/kg is well-tolerated when administered IV as a 90-minute infusion. The AEs were usually mild and moderate. Infusion related reactions including hypersensitivity were frequently reported in subjects treated with obiltoxaximab. However, no data in

the target population as well as in vulnerable population i.e. pregnant women, nursing mothers, paediatric subjects or elderly are available. Due to the mode of action target-mediated toxicity is not expected.

3.7.2. Balance of benefits and risks

Inhalational anthrax is a life-threatening disease, with an overall mortality rate of approximately 50% even with aggressive treatment including multiple antimicrobials [Jernigan et al, 2002].

A combination treatment with antibiotics and obiltoxaximab benefits from the complementary mode of action, i.e. targeting the bacteria and targeting the toxin moiety. In the efficacy studies in anthrax-challenged animal, the combination of obiltoxaximab and antibiotic was effective in increasing the survival rate when compared to placebo, even though the added benefit of the combination treatment to antibiotic was not demonstrated.

Although naturally occurring inhalational anthrax is a rare condition, occurring sporadically around the world in individuals and small clusters of people exposed to infected animals and animal products, anthrax spores have been used in the past and have the potential to be used in the future as biological weapons. The possible exposure of large numbers of people to air-borne *B. anthracis* spores in the context of a bioterrorist attack raises many issues, not least the availability of antibiotics, compliance of long-term antibiotic administration, or resistance development. In this situation obiltoxaximab offers an additional option for post-exposure prophylaxis especially in subjects with contraindications for antibiotics or in situations where a shortage of antibiotics is experienced. Such use is supported by the efficacy study using obiltoxaximab as a monotherapy. Given the indication claim to use of obiltoxaximab also in asymptomatic patients that were or may have been exposed to inhalation anthrax when alternative therapies are not appropriate or are not available, is acceptable.

The safety data of obiltoxaximab show an acceptable safety profile in healthy young adults. The exclusion of vulnerable population in the phase I study program is acceptable. Due to the epidemiology of the disease i.e. very rare incidence of natural inhalation anthrax, the generation of safety data in the target population for obiltoxaximab i.e. general population including vulnerable population, is not feasible at the moment and will be subject to a Post-Marketing Study.

3.7.3. Additional considerations on the benefit-risk balance

As inhalational anthrax is encountered very sporadic, it is impossible to predict when and where cases /outbreak will occur. Due to the high mortality rate even with standard of care and the anticipated clinical benefit, it would be highly unethical to conduct randomised controlled clinical studies with obiltoxaximab in patients with inhalational anthrax. It was not feasible to evaluate efficacy of medical countermeasures against inhalational anthrax in clinical studies, because the incidence of naturally occurring disease is too low, and it is unethical to intentionally expose humans to anthrax. Therefore, efficacy is bridged for pre- and post-exposure prophylaxis of inhalational anthrax from two well-characterised animal models, NZW rabbits and cynomolgus monkeys, via healthy animals to humans based on similar or higher exposure in humans. Since PK data are generated in healthy humans, the impact of physiological consequences of disease, as well as target mediated clearance, remain an uncertainty.

Marketing authorisation under exceptional circumstances

As comprehensive data on the product are not available, a marketing authorisation under exceptional circumstances was requested by the applicant in the initial submission.

The CHMP considers that the applicant has sufficiently demonstrated that it is not possible to provide comprehensive data on the efficacy and safety under normal conditions of use, because the applied for indication is encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence, and it would be contrary to generally accepted principles of medical ethics to collect such information.

The following arguments were carefully considered and supported by CHMP:

1. Inability to provide comprehensive efficacy and safety data due to rarity of the indication

Given the extremely rare occurrence of spontaneous cases of inhalational anthrax, it is not feasible to conduct clinical trials in humans with inhalational anthrax disease. Furthermore, while it may be possible to collect clinical data with obiltoxaximab in patients with anthrax disease upon the occurrence of an anthrax outbreak, it remains highly unpredictable if, when and to what extent such an outbreak would occur.

2. Inability to collect such information because it would be contrary to medical ethics

Performing randomised clinical trials actively exposing human subjects to infectious diseases such as *B. anthracis* spores would be against medical ethics.

Performing a randomised controlled trial, in the case of an anthrax outbreak, in which a treatment that is expected to increase the approximately 50% survival rate in patients with inhalational anthrax disease is withheld from the patients in the control arm, would also be against medical ethics.

Conditional approval is not appropriate for obiltoxaximab, as it is very unlikely that a comprehensive data package can be generated for obiltoxaximab in the future. This is because there is no possibility to conduct prospective randomised controlled clinical studies with obiltoxaximab in patients with inhalational anthrax disease and the opportunity for data collections is limited to open-label single-arm field studies with retrospective elements that can only be conducted upon the occurrence of an anthrax outbreak.

The totality of the above limitations prevents generation of comprehensive data for Obiltoxaximab SFL.

Therefore, recommending a marketing authorisation under exceptional circumstances is considered appropriate.

3.8. Conclusions

The overall B/R of Obiltoxaximab SFL is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Obiltoxaximab SFL is favourable in the following indication:

"Obiltoxaximab SFL is indicated in combination with appropriate antibacterial drugs in all age groups for treatment of inhalational anthrax due to *Bacillus anthracis* (see section 5.1).

Obiltoxaximab SFL is indicated in all age groups for post-exposure prophylaxis of inhalational anthrax when alternative therapies are not appropriate or are not available (see section 5.1)."

The CHMP therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Description	Due date
SOB 1. Validation of the obiltoxaximab PK method (GCL-160) in human serum In order to validate the obiltoxaximab PK method (GCL-160) in human serum, the MAH should submit the results from the assay validation for the following aspects prior to use of the assay for sample analysis for clinical study AH501: interference by PA (63 and 83), EF, LF and ADAs, and assay performance in haemolytic and lipemic serum. Parallelism should be performed with incurred samples from the planned open-label field study AH501.	To be submitted together with the final clinical report of study AH501
SOB 2. A phase 4, open-label field study (Study AH501) to evaluate the clinical benefit, safety, and pharmacokinetics of obiltoxaximab when used in the treatment of suspected, probable, or confirmed cases of inhalational anthrax due to *B. anthracis* In order to evaluate the clinical response, safety and tolerability, including the course of illness and survival in subjects with suspected, probable, or confirmed cases of inhalational anthrax treated with obiltoxaximab, the MAH should conduct, according to an agreed protocol, and submit the results of the final report for the phase 4, open-label field study AH501 upon the occurrence of an anthrax outbreak in the countries where obiltoxaximab is authorised and available.	Annual reports to be submitted Final report will be provided no later than 12 months after the last administration of obiltoxaximab or last data collection in case of retrospective data collection

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that obiltoxaximab is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.