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ARTICLE

Animal-to-Human Dose Translation of Obiltoxaximab for Treatment of Inhalational Anthrax Under the US FDA Animal Rule

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Obiltoxaximab, a monoclonal antibody against protective antigen (PA), is approved for treatment of inhalational anthrax under the US Food and Drug Administration's (FDA) Animal Rule. The human dose was selected and justified by comparing observed obiltoxaximab exposures in healthy and infected New Zealand White rabbits and cynomolgus macaques to observed exposures in healthy humans, to simulated exposures in healthy and infected humans, and to serum PA levels in infected animals. In humans, at 16 mg/kg intravenous, obiltoxaximab AUC was >2 times that in animals, while maximum serum concentrations were comparable to those in animals and were maintained in excess of the concentration required for PA neutralization in infected animals for 2–3 weeks. Obiltoxaximab 16 mg/kg in humans provided exposure beyond that of 16 mg/kg in animals, ensuring a sufficient duration of PA neutralization to allow for adaptive immunity development. Our approach to dose translation may be applicable to other agents being developed under the Animal Rule.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ Drug approval under the FDA's "Animal Rule" is rare and obiltoxaximab is the second monoclonal antibody developed under this pathway. There is a paucity of knowledge on the approaches to human dose selection for products whose efficacy cannot be tested in human clinical trials.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ Selection of the human dose of obiltoxaximab for the treatment of inhalational anthrax.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
✔ Our study shows an approach and provides sound justification that a 16 mg/kg dose of obiltoxaximab will produce clinical benefit in the treatment of humans with inhalational anthrax.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
✔ Our approach could help guide human dose selection for other products being developed under the "Animal Rule."

Anthrax is an acute infectious disease caused by *Bacillus anthracis,* a Gram-positive, aerobic, encapsulated, endospore-forming, toxin-producing, rod-shaped bacterial pathogen.[1,2] The incidence of naturally acquired anthrax is rare; however, *B. anthracis* spores are readily bioweaponized.[3,4] *Bacillus anthracis* has been identified as a top-priority, Category A biowarfare threat by the US Department of Homeland Security because it can be easily spread and causes severe illness or death.[5] In 2001, the intentional delivery of *B. anthracis* spores through the US Postal Service resulted in 22 cases of anthrax disease (11 inhalational, 11 cutaneous). Of those who developed inhalational anthrax, five (45%) died despite appropriate, aggressive care.[6]

Obiltoxaximab is a chimeric immunoglobulin G1(κ) monoclonal antibody (mAb) that binds and neutralizes protective antigen (PA), which plays a central role in anthrax toxin assembly and target cell intoxication.[7,8] Since definitive human efficacy studies with *B. anthracis* are not ethical and field trials to study effectiveness have not been feasible, obiltoxaximab was developed under the US Food and Drug Administration's (FDA) Animal Rule regulations (21 CFR 601.90), under which a drug can be approved using efficacy studies in animals and safety studies in healthy humans. For the Animal Rule to apply, four criteria need to be met: i) a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product; ii) the effect is demonstrated in more than one animal species expected to react with a response predictive for humans; iii) the animal study end point is clearly related to the desired benefit in humans; and iv) the data on the pharmacokinetics (PK) and pharmacodynamics (PD) of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.[9,10]

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Obiltoxaximab has been extensively studied in both humans and animals. Human PK data from five controlled studies in over 500 healthy volunteers demonstrated that obiltoxaximab exposure increased proportionally with dose, had a half-life of 17–23 days, and that it was safe and generally well-tolerated.[11] The animal models (New Zealand White (NZW) rabbits and cynomolgus macaques) for investigating anthrax disease progression after inhalational exposure to anthrax spores are well established and correlate well with the pathophysiology of the disease manifested in humans.[12–14] A series of randomized, placebo-controlled, parallel-group, dose-ranging, trigger-to-treat studies in which obiltoxaximab was administered as an intravenous (i.v.) bolus to NZW rabbits and cynomolgus macaques were conducted to describe the PD and efficacy of obiltoxaximab. The results demonstrated the survival efficacy of obiltoxaximab at 16 mg/kg.[15]

Obiltoxaximab pharmacological activity is based on neutralization of circulating PA.[8,16,17] During the course of inhalational anthrax, bacteremia and toxemia correlate[18] and are reasonable measures of disease progression.[19,20] In animal treatment studies, obiltoxaximab administration resulted in serum PA concentrations below the lower limit of assay quantification (<9.68 ng/mL) at the earliest posttreatment time point measured (as early as 15 min in some studies) in both surviving and nonsurviving animals.[15]

This report describes our approach for the selection and justification of the clinical dose of obiltoxaximab for treatment of inhalational anthrax in humans based on translation of efficacy, PK, and PD data from healthy and infected animal studies to humans using observed data and simulations based on validated population PK and survival models.[11,15]

MATERIALS AND METHODS

Study designs and treatments

Animal pharmacokinetic studies

Two NZW rabbit studies (one each in unexposed and *B. anthracis*-infected animals) and five cynomolgus macaque studies (one in unexposed and four in infected animals) were included (**Table 1**). In the healthy animal studies, cynomolgus macaques younger than 5 years (Covance, Alice, TX) and pathogen-free NZW rabbits (Covance, Denver, PA) were administered single doses of obiltoxaximab (3, 10, or 30 mg/kg i.v. or 10 mg/kg intramuscular (i.m.)). Similarly, in treatment studies cynomolgus macaques younger than 5 years weighing 2.7–7.3 kg prior to challenge and pathogen-free NZW rabbits weighing 2.9–4.0 kg prior to challenge were utilized in randomized, blinded, parallel-group, placebo-controlled studies conducted in accordance with Good Laboratory Practice regulations (21 CFR Part 58).[12,15,21] Animals were exposed to aerosolized *B. anthracis* (Ames) spores (target 200 LD_{50}), and obiltoxaximab was initiated at doses of 1–32 mg/kg i.v. based on the appearance of a significant increase in body temperature (rabbits only),[21] or a positive serum PA signal. Body temperature and PA via electrochemiluminescence (ECL) assay were assessed as previously described.[15] A significant increase in body temperature was defined as a ≥2 standard deviation (SD) increase from baseline either three consecutive times or two consecutive times twice. Serum samples were tested for the presence of PA every 6 h starting 18–24 h after spore exposure. Positive results in a PA-ECL assay (≥2 ng/mL for macaques and ≥1 ng/mL for rabbits) were used as a trigger threshold. If neither was observed, obiltoxaximab was administered at a predetermined time point after spore challenge (rabbits 72 h, macaques 54 h).[15]

Studies in spore-exposed animals were conducted at Battelle Biomedical Research Center (BBRC, West Jefferson, OH) in accordance with the Animal Welfare Act (7 U.S.C. §2131) and the Guide for the Care and Use of Laboratory Animals.[22] BBRC is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International, and each study protocol was reviewed and approved by the facility Institutional Animal Care and Use Committee. All procedures in infected animals were performed in a Biosafety Level 3 laboratory registered with the Centers for Disease Control and Prevention and inspected by the US Departments of Defense and Agriculture. Studies in unexposed rabbits were conducted at Ricerca Biosciences (Concord, OH); studies in unexposed macaques were conducted at MPI Research (Mattawan, MI).

Sample collection in animals for pharmacokinetic assessments

Blood samples were obtained from a femoral artery or vein, saphenous vein, or other appropriate vein in macaques and from the venous access port (VAP) or medial ear artery if VAP was not patent in rabbits over a predetermined time course. Additional blood samples were collected for analysis of free serum PA concentrations in infected animals in rabbit Study 2 and macaque Studies 2–5 following challenge with anthrax spores and after treatment with obiltoxaximab or placebo as described elsewhere.[15] Serum was separated, filtered to sterilize, and kept frozen (–80°C to –70°C) until analyzed for obiltoxaximab or PA concentrations.

Human pharmacokinetic studies

The study protocols, including all amendments, were approved by the investigational review boards at each study site, and the studies were conducted in accordance with Good Clinical Practice, the ethical principles that have their origin in the Declaration of Helsinki, The Belmont Report, Title 21 of the Code of Federal Regulations (CFR) (Parts 50, 56, and 312), Title 45 of the CFR (Part 46), the International Conference on Harmonisation (ICH) (E6), and any applicable regulatory requirements. Written informed consent was obtained from each subject prior to performing any evaluations. Obiltoxaximab doses from 120 mg to 16 mg/kg i.v. were investigated in four clinical studies in healthy human subjects (**Table 1**). The PK results from three studies (Studies 2–4) in which healthy human subjects received obiltoxaximab by i.v. infusion over 90 min (4, 8, or 16 mg/kg) were used in comparative exposure analyses (**Table 1**). The study methods have been described in detail previously.[11]

Assays

Serum samples were assayed for free obiltoxaximab concentrations using validated enzyme-linked immunosorbent assay (ELISA) methods for macaques and humans and an

Table 1 Overview of studies included in pharmacokinetic analyses

	Healthy or infected	Obiltoxaximab doses	Pharmacokinetic sampling times
NZW Rabbit Studies			
Study 1 ($N = 50$)	Healthy	Placebo i.m., 10 mg/kg i.m., 3, 10, or 30 mg/kg i.v. (ratio 1:4)	Predose, end of injection, 4, 8, 16, 24, 48, 72, 96, 120, 168, 216, 264, and 336 h postdose
Study 2 ($N = 70$)	Infected	Placebo, 1, 4, 8, or 16 mg/kg i.v. (ratio 1:4)	Pre-challenge, 8, 12, 16, 20, and 28 days postchallenge; 15 min, 4 h, 8 h, and 1, 2, 3 days postdose
Cynomolgus Macaque Studies			
Study 1 ($N = 24$)	Healthy	3, 10, or 30 mg/kg i.v., or 10 mg/kg i.m.	Predose and at 15 min, 2, 6, and 24 h postdose and once daily on Days 3, 5, 6, 8, 15, 22, and 29 Additional samples obtained on days 36, 43, 50, and 57 in the 30 mg/kg group
Study 2 ($N = 44$)	Infected	Placebo, 4 or 8 mg/kg i.v. (ratio ~1:2)	Prechallenge, 7, 14, 21, and 28 days postchallenge; 5 min, 6 h, 1 and 4 days postdose
Study 3 ($N = 48$)	Infected	Placebo, 8 or 32 mg/kg i.v. (ratio 1:2)	Prechallenge, 16, 23, and 28 days postchallenge; 15 min, 2 and 6 h, and 1, 2, 4, 5, 7 days postdose
Study 4 ($N = 48$)	Infected	Placebo, 4 or 16 mg/kg i.v. (ratio 1:2)	Prechallenge, 16, 23, and 28 days postchallenge; 15 min, 2 and 6 h, and 1, 2, 4, 5, 7 days postdose
Study 5 ($N = 51$)	Infected	Placebo, 16 mg/kg i.v. (study formulation), or 16 mg/kg i.v. (commercial formulation) (ratio 1:2)	Prechallenge and 28 days postchallenge; 15 min, and 4, and 7 days postdose
Human Studies			
Study 1 ($N = 45$)	Healthy	Placebo or 120, 240, or 360 mg i.v. (ratio 1:4)	Predose, 1, 3, 6, 12, 24 and 48 h and on Days 7, 14, 21, 42, 56, and 70 postdose
Study 2 ($N = 108$)	Healthy	Placebo or, 4, 8, or 16 mg/kg i.v. (ratio 1:5)	Predose, 4, 8, 24, 36, 48 h and on Days 8, 15, 29, 43, and 71 postdose
Study 3 ($N = 280$)	Healthy	Placebo ($N = 70$) or 16 mg/kg i.v. ($N = 210$)	Predose, end of infusion, 3, 8, and 24 h and on Days 8, 15, 29, 43, and 71
Study 4 ($N = 40$)	Healthy	16 mg/kg i.v. with ($N = 20$) or without ($N = 20$) ciprofloxacin. Ciprofloxacin administered 400 mg i.v. immediately after obiltoxaximab dose on Day 1, followed by 750 mg orally every 12 h from Days 2–8, with a final dose in the morning of Day 9	Predose, end of infusion, and 2.5, 4.5, 7.5, and 24 h after the start of infusion, and on Days 9, 16, 29, 43, and 71

ECL method for rabbits. The ELISA methods for macaques and humans have been described previously,[11,23] and the ECL method for rabbits is detailed in the **Appendix**. Assays for PA have been previously described.[15]

Population pharmacokinetic modeling and simulation and survival modeling

Population PK and survival analyses were conducted via nonlinear mixed-effects modeling using NONMEM software, v. 7, Level 2.0 (ICON Development Solutions, Hanover, MD). PK data from 10 studies (two studies in rabbits (Studies 1 and 2), five studies in macaques (Studies 1–5), and three studies in healthy humans (Studies 1–3)) were used for population PK model fitting (**Table 1**). The combined data set consisted of 791, 929, and 2,830 observations for rabbits, macaques, and humans, respectively. For nonclinical modeling, PK models were simultaneously fit to healthy and infected animal data to describe disease effects on PK. Visual predictive checks were performed to evaluate the suitability of the population PK models for predicting obiltoxaximab exposures.

Survival analyses using data from infected animal studies (rabbit Study 2 and cynomolgus macaque Studies 2–5) were performed to characterize the obiltoxaximab dose–response relationship. It was determined that a parametric cure-rate model, which explicitly incorporates a fraction of animals that survive beyond the end of the study, was preferable to a standard parametric time-to-event model. To explain differences in survival across studies and between animals in the same study, the effects of dose (mg/kg), prior-to-treatment (PTT) quantitative bacteremia, and species (rabbit vs. macaque) on the survival function were investigated.

Following i.v. administration in animals, variability in obiltoxaximab exposure was low within dose groups. Therefore, obiltoxaximab doses did not yield a wide range of exposures within dose groups. An exposure–response model relating area under the concentration-time curve (AUC) to survival was developed as part of the initial modeling analysis. The results were consistent with the dose–response analysis, leading to identical inferences regarding obiltoxaximab dosing in humans.

To account for target-mediated drug disposition (TMDD), which was not included in the human population PK model

since the PA binding target only exists in infected subjects, and predict obiltoxaximab exposures in infected humans, the nonlinear clearance model component from the cynomolgus macaque model was added to the human population PK model, allometrically scaled to human body size.

Simulations were performed for 500 typical human subjects (weight = 75 kg) administered a single 16 mg/kg obiltoxaximab dose for healthy and infected populations, for comparative analysis of obiltoxaximab disposition in different groups and across species.

Noncompartmental pharmacokinetic analyses in animal studies

PK parameters were derived by noncompartmental methods using Phoenix WinNonlin v. 5.2 or higher (Pharsight, Mountain View, CA). Maximum concentration (C_{max}) and time of maximum concentration (T_{max}) were observed values. The terminal-phase rate constant (k) was determined by log-linear regression and half-life ($t_{1/2}$) was calculated as ln(2)/k. AUC was calculated using the trapezoidal method over the first 48 h postdose ($AUC_{0\text{-}48hr}$) and from time 0 to T_{last}, the time of the last measurable serum obiltoxaximab concentration ($AUC_{0\text{-}last}$); AUC from time 0 to infinity ($AUC_{0\text{-}inf}$) was calculated as $AUC_{0\text{-}last} + C_{last}/k$, where C_{last} is the concentration at T_{last}. Systemic clearance (CL) was calculated as dose divided by $AUC_{0\text{-}inf}$. Volume of distribution at steady state (V_{ss}) was calculated as CL*MRT (mean residence time). Infected animals that were sacrificed prior to scheduled termination were designated as having "died" or as "nonsurvivors." Obiltoxaximab PK parameters were determined for infected animals, including nonsurvivors, where the data permitted, and only reported for animals with measurable obiltoxaximab serum concentrations at ≥3 postdose time points. Obiltoxaximab C_{max} and AUC values were compared in unexposed animals, infected animals that survived, and infected animals that did not survive.

Neutralization of protective antigen

Obiltoxaximab concentrations required to ensure 99% or 99.9% neutralization of PA in both simulated healthy and infected human subjects were determined, based on the following equation (using Kd = 0.33 nM and MW = 148 kDa) (24):

$$\%\text{Bound} = 100 * (\text{Kd}^{-1}\text{Conc}_{\text{Obil}})/(1 + \text{Kd}^{-1}\text{Conc}_{\text{Obil}}).$$

Statistical analyses

Quantitative variables were summarized using descriptive statistics, including *n*, mean, SD, percent coefficient of variation (CV%), median, minimum, and maximum values. Geometric mean was included for PK parameters, where applicable.

RESULTS

Animal and human population pharmacokinetic modeling

The final macaque and rabbit structural PK models consisted of a two-compartment model parameterized in terms of CL, central volume of distribution (V_c), peripheral volume of distribution (V_p), and intercompartmental clearance (Q). Absorption kinetics following i.m. administration were described by a first-order absorption rate (k_a) and bioavailability (F1). TMDD in infected NZW rabbits and cynomolgus macaques was approximated via parallel nonlinear elimination for infected animals only, parameterized in terms of maximum velocity (V_{max}) and obiltoxaximab concentration to reach half-maximum velocity (K_m). Volume and clearance parameters were allometrically scaled, normalized to a reference weight of 3.165 kg for NZW rabbits and 2.88 kg for cynomolgus macaques. The final human structural model consisted of a two-compartment model parameterized in terms of CL, V_c, V_p, and Q. The typical estimates for NZW rabbits, macaques, and humans are presented in **Supplementary Table S1**.

The final population PK models provided reasonable descriptions of the obiltoxaximab data. Goodness-of-fit criteria revealed that the final models were consistent with the observed data and that no systematic bias remained. The model evaluation results provided evidence that both the fixed and random effects components of the final models were reflective of the observed data as well (**Supplementary Figure S1**).

Animal survival modeling

The Weibull cure rate model was simultaneously fit to infected rabbit and macaque data. The survivor function for this model is given by:

$$P(T > t) = p_{surv} + (1 - p_{surv}) \exp[-(\lambda t)]^{\alpha}$$

where T is the time to death. The parameter p_{surv} is the probability that an animal survives to the end of the study (Day 28), and λ is the rate at which animals die. The shape of the survival curve is determined by parameter α.

The survival model also included an E_{max} dose–response and an exponential effect of $\log_{10}$(PTT bacteremia) on logit(p_{surv}). The parameters p_{surv} and λ were modeled as functions of dose and PTT quantitative bacteremia in the following manner:

$$logit\ (p_{surv}) = \theta_0 - (\theta_1 \times log_{10}PTT)^{\theta_2} + \frac{E_{max} \times dose}{ED_{50} + dose}$$

$$\log(\lambda) = \lambda_0 + \lambda_1 \times log_{10}PTT$$

where θ_0 is the baseline logit for P(cure), E_{max} is the maximum effect of obiltoxaximab on p_{surv} on the logit scale, ED_{50} is the obiltoxaximab dose (mg/kg) that is needed to achieve half of the maximum effect of drug on p_{surv}, θ_1 is the rate for $\log_{10}$(PTT) on P(cure), θ_2 is the exponent for quantitative bacteremia effect, λ_0 is the P(cure) for placebo, and λ_1 is the slope effect of $\log_{10}$(PTT) on λ (**Supplementary Table S2**).

Obiltoxaximab 8 mg/kg provided ~90% of the maximal possible response; however, the maximum p_{surv} level ranged from nearly 0 with PTT bacteremia levels above 5 $\log_{10}$ colony-forming units (CFU)/mL to nearly 1.0 with low PTT bacteremia levels. At lower PTT bacteremia levels (<3 $\log_{10}$ CFU/mL) a 4 mg/kg dose provided near maximum survival. At higher PTT bacteremia levels (3–4 $\log_{10}$ CFU/mL), an 8 mg/kg dose provided maximum survival. At a PTT bacteremia level

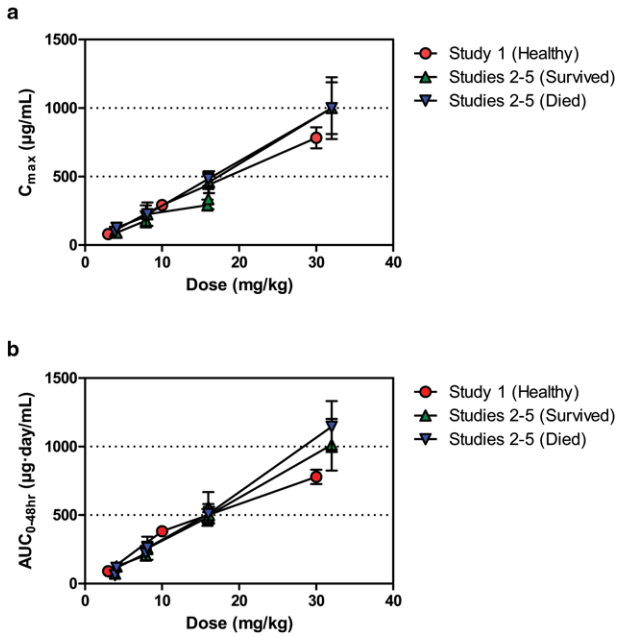

Figure 1 Obiltoxaximab mean (SD) C_{max} (**a**) and $AUC_{(0-48)}$ (**b**) as a function of dose in healthy and infected monkeys. AUC over the first 48 h (AUC_{0-48}) was chosen so as to effect a meaningful evaluation across all of the animal groups (healthy; infected survivors; infected nonsurvivors). There were too few animals with a valid estimate of AUC from time 0 extrapolated to infinity (AUC_{0-inf}), particularly in nonsurviving infected animals, to make a useful comparison with this parameter.

of ~5 log_{10} CFU/mL, there was almost zero probability of survival, indicating that animals at this level were beyond a "point of no return" for survival. The estimated ED_{50} was 1.64 mg/kg (95% confidence interval (CI): 0.515– 5.22), and ED_{90} was 14.8 mg/kg (95% CI: 4.6–47.0 mg/kg). Although there is considerable uncertainty in the 95% CI, the probability that a dose of 16 mg/kg exceeds the ED_{90} is greater than 80%. A 16 mg/kg dose was chosen as the efficacious dose for nonclinical species, as it exceeded the ED_{90} and provided a margin of assurance that the maximum probability of survival would be achieved (**Supplementary Figure S2**).

Noncompartmental pharmacokinetic analyses

Obiltoxaximab 16 mg/kg was administered i.v. to infected NZW rabbits and infected cynomolgus macaques in three studies (rabbit Study 2, macaque Studies 4 and 5). In macaques, mean $t_{1/2}$ values of 4.1 to 7.4 days were observed, and mean V_{ss} was 47.7 to 59.0 mL/kg. Mean CL was 7.8 to 9.0 mL/d/kg. In rabbits, mean $t_{1/2}$ was 1.0 day, V_{ss} was 36.6 mL/kg, and CL was 17.3 mL/d/kg. Across all doses in macaque studies, obiltoxaximab C_{max} and AUC_{0-48hr} values increased linearly with dose, and were not substantively different between healthy animals, infected animals that died, and infected animals that survived (**Figure 1**). Comparable results were observed in infected and uninfected rabbits. This indicates that the pathological processes leading to lethality had no impact on obiltoxaximab disposition. Detailed animal PK results are included in **Supplementary Table S3**.

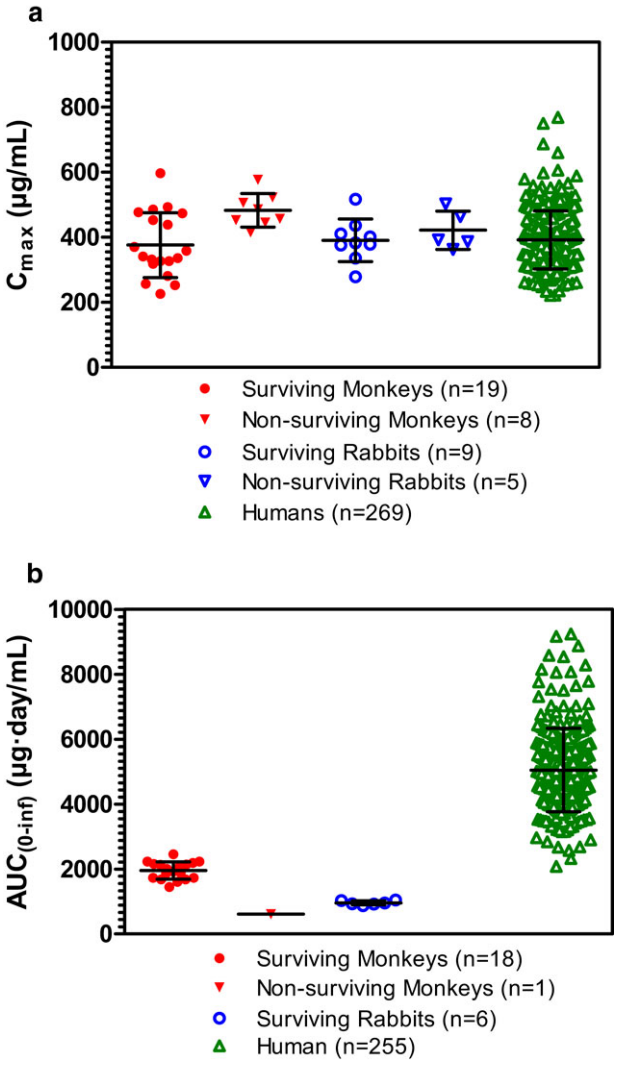

Figure 2 Comparison of individual (and mean ±SD) obiltoxaximab C_{max} **(a)** and AUC_{0-inf} **(b)** in infected animals and healthy humans after a 16 mg/kg i.v. dose.

Human PK data across a 4 to 16 mg/kg dose range from Studies 2 to 4 are summarized in **Supplementary Table S4**. Accounting for body size differences, obiltoxaximab V_{ss} and CL were similar, while $t_{1/2}$ was longer (15–23 days), in humans than in animals.[11] Coadministration of 16 mg/kg obiltoxaximab i.v. with i.v. or oral ciprofloxacin in human subjects did not alter the PK of either ciprofloxacin or obiltoxaximab.[11]

Translation of animal dosing to human dosing

Based on the results of the animal survival analysis and survival modeling results,[15] the dose of 16 mg/kg was chosen as a therapeutic dose and for testing in clinical trials in healthy humans. A comparison of obiltoxaximab systemic exposures (mean and individual C_{max} and AUC_{0-inf} values) observed in healthy adult humans following a single i.v. dose of 16 mg/kg to those at the efficacious dose of 16 mg/kg i.v. in rabbits and macaques, is shown in **Figure 2**.

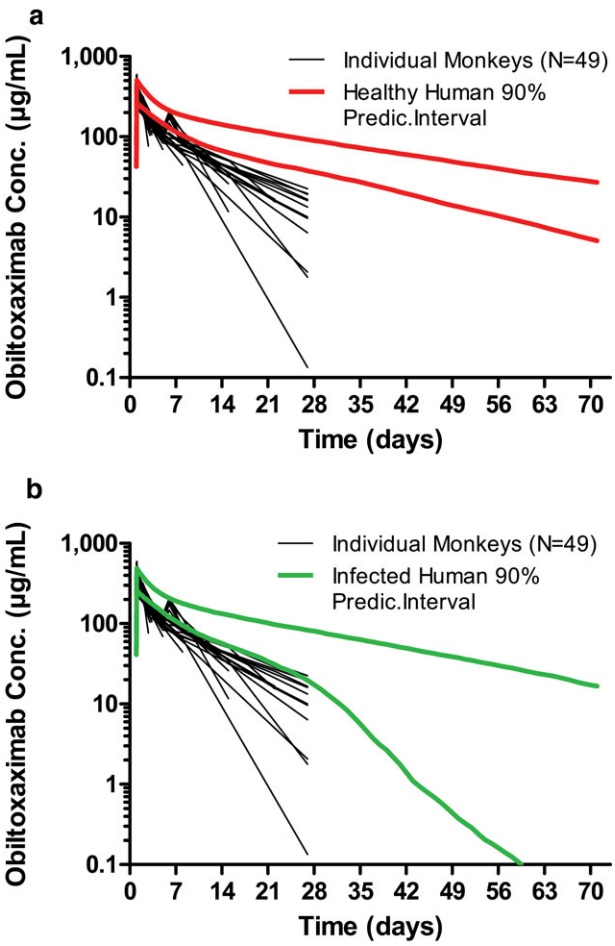

Figure 3 Comparison of obiltoxaximab concentration vs. time profiles at 16 mg/kg in infected monkeys with a simulated population of healthy (**a**) and infected humans (**b**). Monkey obiltoxaximab concentration data are from Studies 3 and 4; human simulations present the 90% prediction intervals for populations of 500 healthy and infected humans.

Obiltoxaximab C_{max} values in healthy humans were comparable to those in infected rabbits and macaques, with overlapping individual values and SD estimates among macaques that survived, macaques that died, rabbits that survived, rabbits that died, and human subjects. $AUC_{0\text{-inf}}$ values were notably greater in healthy humans than in infected rabbits and macaques; only 2 of 255 individual human $AUC_{0\text{-inf}}$ values fell within the range of individual animal values (macaques only), and there was no overlap in SD estimates. Similarly, at 16 mg/kg i.v., maximum obiltoxaximab concentrations for simulated populations of healthy and infected humans were comparable to and overall serum exposures exceeded those observed in individual infected macaques following a 16 mg/kg i.v. dose of obiltoxaximab, as shown in overlaid concentration vs. time profiles in **Figure 3a,b,** respectively. Similar results were observed in rabbit experiments (**Supplementary Figure S3**).

These results were supported by a comparison of obiltoxaximab C_{max} and AUC values obtained for the simulated healthy and infected human populations to those observed in animals at 16 mg/kg i.v. (**Table 2**). C_{max} in simulated healthy and infected humans was similar to those in infected macaques and rabbits (mean C_{max}: 363, 357, 408, and 402 μg/mL, respectively). The overall obiltoxaximab systemic exposures (AUC) for the simulated human populations were at least twice those in macaques and rabbits (mean $AUC_{0\text{-inf}}$,: 4,980, 4,070, 1,870, and 958μg·day/mL in healthy humans, infected humans, infected macaques, and infected rabbits, respectively), which is consistent with the longer terminal half-life in humans. From **Table 2** it can be ascertained that human $AUC_{0\text{-inf}}$ is 18% lower when the effects of TMDD are included in the simulation. The considerable difference in predicted human exposures is the reason that the effects of TMDD were considered when comparing human exposures to preclinical species. However, we believe that TMDD effects on efficacy will be minimal, since the majority of TMDD effects are observed at low obiltoxaximab concentrations occurring long after the dose administration.

Table 2 Comparison of obiltoxaximab PK parameters after a 16 mg/kg i.v. dose in simulated human populations to observed values in infected monkeys and rabbits

	C_{max}, μg/mL	$AUC_{0\text{-inf}}$, μg·day/mL
Infected Monkeys[a]	408 (237, 589)[b]	1,870 (613, 2,458)[c]
Infected Rabbits[a]	402 (279, 517)[d]	958 (867, 1,042)[e]
Healthy Humans	363 (265, 503)	4,980 (3,240, 6,960)
Infected Humans	357 (257, 486)	4,070 (2,370, 6,040)

Values are mean (5^{th} and 95^{th} percentile). Human data are from simulated populations of 500 healthy and infected subjects (75 kg); monkey data are from Studies 4 and 5; rabbit data are from Study 2.
[a]All animals at 16 mg/kg i.v. (survivors and nonsurvivors).
[b]$N = 27$
[c]$N = 19$
[d]$N = 14$
[e]$N = 6$.

Justification of the recommended human dose

Maximum obiltoxaximab serum concentrations in simulated populations of healthy and infected humans after a 16 mg/kg i.v. dose were 1 and 2 orders of magnitude greater than the levels required for 99.9% (48 μg/mL) and 99% (4.8 μg/mL) PA neutralization, respectively **(Figure 4**). Moreover, the time at which the lower end of the 90% prediction interval for obiltoxaximab concentration declines to the 99.9% PA binding concentration is approximately Day 20 and Day 16 for healthy and infected humans, respectively. This time frame would allow for the development of endogenous adaptive immunity in humans; anti-PA IgG was detected in 16 of 17 patients with confirmed or suspected bioterrorism-related clinical anthrax within 11 days after the onset of symptoms (15 days after likely exposure),[25] and within 5 to 7 days after onset of symptoms in a patient with naturally acquired inhalational anthrax.[26]

Observed obiltoxaximab mean C_{max} in humans (~390 μg /mL or 2,600 nM; see **Figure 2a**) was an order of magnitude greater than the maximum individual PTT PA concentration observed across all infected animals in the treatment studies (9.67 μg/mL or 153 nM).[15] Additionally, obiltoxaximab concentrations persisted above these levels for more than 3 weeks (**Supplementary Figure S4**).

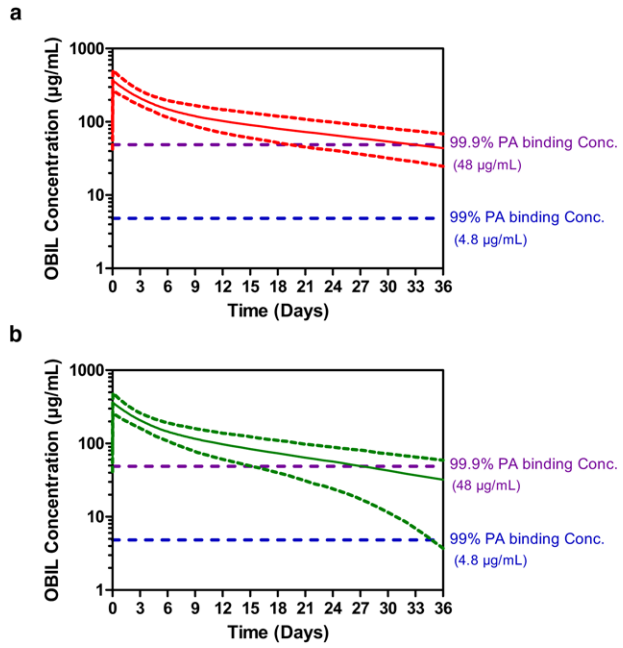

Figure 4 Obiltoxaximab concentration vs. time profiles (mean and 90% prediction interval) for a simulated population of healthy (**a**) and infected (**b**) humans compared with concentrations required for 99% and 99.9% neutralization of protective antigen. Semilogarithmic scale; human simulations are for 500 healthy and infected humans. Maximum obiltoxaximab serum concentrations in simulated human populations are 1 and 2 orders of magnitude greater than the levels required for 99.9% (48 μg/mL) and 99% (4.8 μg/mL) PA neutralization, respectively. Obiltoxaximab concentrations are maintained above these neutralization levels in serum for 2 to 3 weeks. The lower end of the 90% prediction interval declines to the 99.9% PA binding concentration at approximately Day 20 and Day 16 for healthy and infected subjects, respectively.

DISCUSSION

In accordance with the FDA's Animal Rule regulations (21 CFR 601.90), obiltoxaximab demonstrated efficacy in well-controlled animal studies,[15] and has been shown to be safe, with well-described pharmacokinetics in humans.[11] In the absence of robust PK/PD models, a human dose that achieves obiltoxaximab exposures that are comparable to or exceed those in animals was selected. The data support that, relative to animals, a superior human AUC and non-inferior human C_{max} should be sufficient to maximize survival with obiltoxaximab and show that the human experimental dose of 16 mg/kg i.v. meets the tenets of the Animal Rule Guidance.[9,10] The effect of body weight on obiltoxaximab disposition was evaluated in the population PK analysis across a range of 50–125 kg. This analysis demonstrated that a body weight adjusted dose yields obiltoxaximab exposures that are more consistent across the weight range when compared with a flat obiltoxaximab dose.

Using the modeling results from the infected animal studies that demonstrated that 16 mg/kg was maximally efficacious, we constructed a time-to-event model describing observed survival across doses and disease severity. This model included nonlinear effects of dose and PTT bacteremia on survival and the effect of bacteremia on the rate of death. Qualitatively, the model implied that higher PTT bacteremia levels were associated with lower survival rates and faster rates of death, while higher doses and low PTT bacteremia levels were associated with higher survival rates. The obiltoxaximab ED_{90} was 14.8 mg/kg, and a 16-mg/kg dose exceeded the ED_{90} with a probability of 80%. Modeling projections are based on the results of monotherapy efficacy studies where animals received a targeted spore dose of 200 LD_{50}. However, obiltoxaximab is indicated for the treatment of inhalational anthrax in combination with appropriate antibacterial drugs. Thus, our modeling projections represent a conservative approach for estimating the probability of survival. For a 16 mg/kg dose, in the absence of PTT bacteremia, the model predicted a 0.98 probability of cure. At PTT bacteremia of 3.5 $\log_{10}$ CFU/mL, the baseline survival probability (no treatment) was 0.06 and obiltoxaximab 16 mg/kg increased the survival probability to 0.73; however, doses $>$16 mg/kg yielded little increased survival benefit. Our findings of an inverse relationship between disease severity and monotherapy efficacy were reproduced in two animal models and at different dose levels.[15]

Human obiltoxaximab C_{max} values after a 16-mg/kg i.v. infusion of obiltoxaximab were comparable to those in both rabbits and macaques, while $AUC_{0\text{-inf}}$ values in humans were at least twice those in infected animals, and obiltoxaximab serum concentrations were sustained at higher levels over a longer period of time in humans than in animals, consistent with the longer half-life in humans.

Population simulations evaluating the effect of potential covariates on obiltoxaximab PK demonstrated that, compared with simulations of the primary population, age (up to 75 years), gender, body weight (50–125 kg), and non-white race were projected to have minimal ($<$25%) effect on C_{max}, and that $AUC_{0\text{-inf}}$ values might decrease by up to 18%, but exposure margins relative to animals at the efficacious dose would still be substantial ($\geq$1.8x). These findings confirmed that dose adjustment based on gender, age, race, or body size is not required. In addition, concomitant antibacterials and diphenhydramine pretreatment had no effect on obiltoxaximab PK.

PA concentrations rise rapidly in animal models of anthrax infection, generally within 1 to 3 days after spore challenge.[12,20,21,24] The mechanism of obiltoxaximab pharmacological activity is through its binding to PA and subsequent inhibition of PA binding to its membrane receptors.[8,16,17] Animal treatment studies showed that obiltoxaximab rapidly reduced concentrations of circulating PA.[15] A 16 mg/kg human dose is projected to achieve more than sufficient initial obiltoxaximab concentrations to effect the same degree of PA neutralization. Additionally, persistence of the high molar excess of obiltoxaximab during the first 2 to 3 weeks of infection would ensure that all newly secreted PA would be continuously neutralized until bacteremia was resolved. This time frame exceeds the 10-day postchallenge period in animal studies within which all animals that succumbed to infection ultimately died.[1] Moreover, anti-PA IgG was detected in rabbits within 10 to 14 days postchallenge.[8] This same time frame would allow for the development of endogenous adaptive immunity in humans.

Together, these data and analyses, including modeling and simulations, provide sound justification of a high probability for a 16 mg/kg i.v. obiltoxaximab dose to produce clinical benefit for the treatment of humans with inhalational anthrax.

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Conflict of Interest. C.F.N., N.S., L.S.C., and S.E.C. are employees of Elusys Therapeutics, Inc.; R.G. is an employee of Aclairo Pharmaceutical Development Group, Inc.; J.M. and J.F. are employees of Metrum Research Group LLC. L.S.C. is a shareholder of Elusys Therapeutics, Inc., the manufacturer of obiltoxaximab, and a named inventor on a patent application related to obiltoxaximab (Patent No.: US 8,093,360 B2).

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