Pharmacokinetics and Tolerability of Obiltoxaximab: A Report of 5 Healthy Volunteer Studies

Christa F. Nagy, PhD[1]; Timothy S. Leach, MD, MPH[2]; Joseph H. Hoffman, MD[3]; Arthur Czech, BS[1,*]; Sarah E. Carpenter, PhD[4,†]; and Robert Guttendorf, RPh, PhD[5]

[1]Department of Clinical Operations, Elusys Therapeutics, Inc, Pine Brook, New Jersey; [2]InterimMD, Inc, Groton, Massachusetts; [3]Hoffman Consulting LLC, Park Ridge, New Jersey; [4]Department of Research and Nonclinical Development, Elusys Therapeutics, Inc, Pine Brook, New Jersey; and [5]Aclairo Pharmaceutical Development Group, Inc, Vienna, Virginia

ABSTRACT

Purpose: This report describes the safety, immunogenicity, and pharmacokinetic results of obiltoxaximab treatment in healthy subjects from 5 clinical trials.

Methods: Healthy men and women were enrolled in randomized, double-blind studies of obiltoxaximab versus placebo (studies 1–3), an open-label, parallel-group study of obiltoxaximab alone versus obiltoxaximab and ciprofloxacin (study 4), or a randomized, double-blind, placebo-controlled study involving administration of a second dose of obiltoxaximab 13 or 119 days after an initial dose (study 5). Obiltoxaximab was administered intravenously in all studies. The safety profile was characterized by physical examinations, including focused examinations of the skin and infusion sites; study drug infusion discontinuations; and assessment of adverse events, vital signs, electrocardiographic findings, laboratory parameters, and immunogenicity. Studies 3 to 5 were the primary safety profile studies. Pharmacokinetic parameters were calculated using noncompartmental methods.

Findings: Results of 2 multiple dose studies (studies 1 and 2) revealed that obiltoxaximab exposure increased proportionally. Pharmacokinetic results were consistent across studies. After administration of 16 mg/kg of obiltoxaximab, serum concentrations decreased in a biexponential or multiexponential fashion with a terminal half-life of 17 to 23 days. Mean volume of distribution was approximately 6.3 to 7.5 L, suggesting obiltoxaximab distribution outside the vascular compartment and potentially into tissues. Mean systemic clearance was approximately 0.27 L/d, suggesting that hepatic metabolism and/or renal excretion are not critical to obiltoxaximab elimination. Obiltoxaximab was generally well tolerated. Hypersensitivity reactions were the most common adverse reactions in the safety profile clinical trials, occurring in 34 of 320 subjects (10.6%) receiving obiltoxaximab and 4 of 70 subjects (5.7%) receiving placebo. The most common adverse events were headache, pruritus, upper respiratory tract infection, cough, infusion site swelling, bruising and/or pain, nasal congestion, urticaria, and extremity pain. Of the 320 subjects in the primary safety profile studies who received $\geq$1 dose of 16 mg/kg of obiltoxaximab, 8 (2.5%) tested positive for a exposure-emergent antiobiltoxaximab response; however, quantitative titers were low (1:20–1:320).

Implications: On the basis of consistent results of 5 clinical trials in healthy volunteers, the pharmacokinetic properties of obiltoxaximab after a 16-mg/kg IV infusion can be considered adequately characterized, a criteria of the Animal Rule. Obiltoxaximab appears to be generally well tolerated. ClinicalTrials.gov identifiers: NCT00829582, NCT01453907, NCT01929226, NCT01952444, NCT01932242. (*Clin Ther*. 2016;38:2083–2097) © 2016 The Authors. Published by Elsevier HS Journals, Inc.

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*Current affiliation: Celgene Corporation, Summit, New Jersey.
†Current affiliation: Merck Research Laboratories, Kenilworth, New Jersey.

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INTRODUCTION

There is continued great interest in the development of effective therapeutics for the treatment of anthrax, the highly lethal infection caused by *Bacillus anthracis,* a gram-positive, aerobic, encapsulated, endospore-forming, rod-shaped bacterial pathogen.[1,2] Anthrax can manifest as cutaneous, gastrointestinal, inhalational, or injection-related infections, depending on the route of exposure, with the inhalational form having a fatality rate of approximately 50% even under optimal treatment conditions.[3–6] The incidence of naturally acquired anthrax is rare; however, *B anthracis* spores are readily bioweaponized.[7,8] In 2011, a bioterrorism attack in which *B anthracis* spores were intentionally delivered through the US Postal Service resulted in 22 cases of anthrax disease, 11 of which were inhalational. Of these individuals, 5 died despite appropriate care, including aggressive treatment with multiple antimicrobials.[9]

Anthrax infection begins with a prodromal phase that has a median duration of 3.9 days (range, 3.5–4.4 days) and consists of localized infection with flulike symptoms.[4] After this prodromal phase, there is an abrupt transition to the fulminant phase, which is characterized by fever, dyspnea, diaphoresis, and shock, with a rapid progression (ie, approximately 3 days) to death in the absence of immediate antibiotic therapy. According to the Centers for Disease Control and Prevention,[10] therapy for suspected anthrax with the possibility of anthrax meningitis should include at least 3 antimicrobial drugs active against *B anthracis*. At least 1 agent should have bactericidal activity, with intravenous ciprofloxacin being the preferred agent (levofloxacin and moxifloxacin are considered equivalent). In addition, at least 1 should be a protein synthesis inhibitor, and all 3 agents should have good central nervous system penetration.

The pathogenic virulence of *B anthracis* is in large part due to the anthrax toxins—edema toxin and lethal toxin—that are critical for the later and more terminal stages of the disease. The virulence of *B anthracis* is expressed only when a protective antigen (PA) is combined with a lethal factor (LF), forming the lethal toxin, or an edema factor (EF), forming the edema toxin.[11] PA is a 83 kDa protein that binds to specific membrane receptors (TEM8 and CMG2) found on most mammalian cells. Once bound to receptors, PA is proteolytically cleaved to form active PA (PA_{63}), which, after binding to the cell surface, facilitates binding and subsequent transfer of LF and EF into the host cell cytoplasm.[12] This leads to impairment of intracellular signaling pathways, interference with phagocytosis by macrophages, and disruption of water homeostasis with resulting edema.[13–15] Because of the central role that PA plays in toxin assembly and intoxication of target cells, PA neutralization and blocking of the binding to its receptor have been found in animal models to be an effective therapeutic strategy for preventing the establishment and progression of inhalational anthrax disease.[16]

Obiltoxaximab, a monoclonal antibody (mAb) that binds and neutralizes PA, has been developed under the US Food and Drug Administration's Animal Rule regulation (21 CFR §601.90). This rule is specifically intended for agents for which the conduct of definitive human efficacy studies is not ethical or feasible, as in the case of agents for the treatment of anthrax. For a new therapeutic to be approved under this rule, the pharmacokinetic (PK) properties and safety profile of the product must be well described in humans. Dose selection is based on human PK properties and safety profile in conjunction with animal efficacy and pharmacodynamic studies.[17,18] We report the PK results of obiltoxaximab in humans after intravenous administration to healthy subjects from 5 clinical trials: 4 single-dose studies (1 dose-escalation study using nominal dosing [study 1], 1 dose-escalation study using weight-based doses [study 2], 1 definitive safety profile and PK study [study 3], 1 drug-drug interaction study with ciprofloxacin [study 4]), and 1 repeat-dose study (study 5) and safety profile results from 3 primary safety profile studies (studies 3–5).

SUBJECTS AND METHODS

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 have their origins in the Declaration of Helsinki; the Belmont Report; 21 CFR §50, 56, and 312; 45 CFR §46; the International Conference on Harmonisation (E6); and any applicable regulatory

requirements. Written informed consent was obtained from each subject before any evaluations were performed.

Study Participants

Details of enrollment criteria are presented in Supplemental Table I. In general, healthy adult men and nonpregnant women of any race were included in these studies. There were no restrictions for body weight or body mass index except for study 1, in which body mass index was limited ($>18.5–<35$ kg/m^2), and study 2 ($>18.5–<30$ kg/m^2). In all studies, exclusion criteria included clinically significant comorbidities; contraindications to the use of mAbs, such as history of allergic or hypersensitivity reactions to other therapeutic antibodies or immunoglobulins; clinically significant abnormalities apparent on electrocardiogram; and blood donation or loss of ≥ 1 pints of blood within 30 days or plasma donation within 7 days of day 1 of the study. Subjects with systolic and diastolic blood pressure measurements outside reference ranges were also excluded. Smoking status as an entry criterion varied by study. Prior immunization with any approved or investigational anthrax vaccine or prior treatment with any approved or investigational anthrax treatment (eg, raxibacumab, anthrax immune globulin) was also prohibited. Military personnel deployed in 1990 or later were excluded, unless the subject provided documentation that indicated that he/she had not received any approved or investigational anthrax vaccine previously (studies 3–5).

Studies 1 and 2 excluded regular use of medications (except acetaminophen in study 2) for chronic conditions (subjects who discontinued >1 week before study initiation were allowed in study 1) and receipt of an investigational agent within 3 months of screening in study 1 or 30 days or 5 half-lives (whichever was longer) of screening in study 2. Use of systemic steroids, immunosuppressive agents, anticoagulants, or antiarrhythmics within 1 year before day 1 (a single <14-day course of systemic steroid therapy was permitted if it concluded >6 months before day 1) and use of histamine$_1$-receptor antagonists (ie, antihistamines) within 5 days before day 1 were exclusion criteria in studies 3 to 5. Because study 4 required administration of ciprofloxacin, exclusion criteria specific to ciprofloxacin were included in the protocol (Supplemental Table I).

Study Design

Studies 1, 2, 3, and 5 were randomized, double-blind, and placebo-controlled, and study 4 was an open-label, parallel-group study (Supplemental Table I). The primary objective of these trials was to evaluate the safety and tolerability of obiltoxaximab in healthy volunteers. Secondary objectives were to evaluate PK properties and immunogenicity. Study 1 was a dose-escalation study in which 45 subjects were randomized to receive 120, 240, or 360 mg of obiltoxaximab or placebo in a ratio of 4:1 ($n = 15$ in each group, including at least 4 females), with higher doses administered only after the previous dose was deemed tolerable by the Safety Monitoring Committee after review of clinical and laboratory data collected during 42 days. Study 2 was a dose-escalation study in which 108 subjects were randomly assigned to receive either single doses of obiltoxaximab, 4, 8, or 16 mg/kg, or placebo (ratio 5:1) in 3 sequential cohorts of 30 subjects. Enrollment was controlled so that at least 4 female subjects were enrolled in each cohort. The 16-mg/kg cohort was not initiated until the 8-mg/kg dose was deemed tolerable by the Safety Monitoring Committee.

Study 3 was a primary safety profile study in which 280 subjects were randomized in a 3:1 ratio to receive a single 16-mg/kg dose of obiltoxaximab or placebo. Because obiltoxaximab may be administered with ciprofloxacin in the clinical setting, study 4 was a drug-interaction study in which 40 subjects were randomized in a 1:1 ratio to receive 16 mg/kg of obiltoxaximab alone or 16 mg/kg of obiltoxaximab, followed immediately by 400 mg of IV ciprofloxacin on day 1, then 750 mg twice daily of oral ciprofloxacin, from days 2 to 8 and a single dose on day 9. Study 5 was a repeat-dose study in which 70 subjects were randomized to receive a 16-mg/kg dose of obiltoxaximab on days 1 and 14, with a placebo infusion on day 120 ($n = 35$) or a 16-mg/kg dose of obiltoxaximab on days 1 and 120 with a placebo infusion on day 14 ($n = 35$).

Obiltoxaximab and matching placebo were supplied as a liquid formulation and stored before use in sterile, single-use vials at 2°C to 8°C. Studies 1 and 2 were conducted with investigational material, and studies 3 to 5 were conducted using the commercial formulation. Study drug was prepared by an unblinded pharmacist at each clinical site by diluting the appropriate volume of stock solution from the supplied

single-use vial(s) into 0.9% sterile sodium chloride for infusion based on the assigned dose level and the subject's weight (studies 2–5). All doses were administered by intravenous infusion for 60 (study 1) or 90 minutes (studies 2–5) on study day 1 in studies 1 to 4 and on study day 1 and study day 14 or 120 in study 5.

During the conduct of studies 3 and 5, the protocol was amended to include the administration of 50 mg of oral diphenhydramine 30 minutes before the administration of obiltoxaximab. Study 4 included diphenhydramine pretreatment as part of the original protocol. Studies 1 and 2 were completed before the requirement for pretreatment with diphenhydramine was introduced.

Sample Collection

Blood samples (3.5 mL) for obiltoxaximab PK analysis were collected before dosing (0 hours) and after the start of infusion at specified times (Supplemental Table I). Serum was separated and kept frozen (–80°C to –70°C) until analyzed.

Blood samples (3.5 mL) for screening of antitherapeutic antibodies (ATAs) were collected before dosing on day 1 and at specified times during each of the 5 studies. Serum was separated and kept frozen (–80°C to –70°C) until analyzed.

Determination of Obiltoxaximab Concentrations and ATA Detection

Serum samples for obiltoxaximab analysis and ATAs were batched and shipped to EMD Millipore Corporation (now Eurofins; St. Charles, Missouri) for analysis. Obiltoxaximab concentrations were determined using a validated ELISA method in which PA_{83} is used as the capture reagent, with an assay range of 100 to 5000 ng/mL. Selectivity was found in individual lots of normal human serum in the presence of spiked obiltoxaximab at 300 and 3000 ng/mL. Selectivity was also tested in individual lots of normal human serum spiked with an irrelevant antibody; all results were below the assay lower limit of quantitation of 100 ng/mL. The accuracy (relative error) ranged from –2.4% to 4.7%, and the %CV ranged from 4.4% to 15.2%. There was no interference from ciprofloxacin or diphenhydramine, individually or in combination.

ATA detection followed a tiered approach (screening and confirmatory assays) using a validated electrochemiluminescence method. Briefly, samples were first subjected to a minimum required dilution of 1:10. Samples were then acidified to release ATAs from obiltoxaximab complexes, followed by neutralization and capture of the ATAs with biotinylated obiltoxaximab. The biotinylated obiltoxaximab-ATA complex was subsequently bound to streptavidin-coated plates to immobilize the complex. The samples were washed and reacidified to free captured ATAs. The solution that contained the acidified ATAs was added to a MesoScale Discovery plate, neutralized, incubated, and washed, and the detection antibody, ruthenium-labeled obiltoxaximab, was then added. A signal was generated from ruthenylated-obiltoxaximab antibody on the MesoScale Discovery plate integrated with carbon electrodes after the addition of a MesoScale Discovery read buffer. This qualitative method used rabbit antiobiltoxaximab polyclonal antisera–positive controls spiked at low (1:10,000 dilution) and high (1:1000 dilution) concentrations in pooled human serum to monitor the assay. The negative control was nonspiked pooled human sera. Samples screened positive in this assay were confirmed positive in an additional confirmatory assay. Free nonlabeled obiltoxaximab was added in the detection step to compare with ruthenium-labeled obiltoxaximab for bound ATAs to confirm that the ATAs were truly obiltoxaximab-binding antibodies. Serum samples were assayed for ATAs at an initial dilution of 1:10. Samples that had detectable ATAs in the confirmatory assay at an initial dilution of 1:10 were serially diluted 1:2 and assayed until a negative result was attained. The titer of the most dilute sample that yielded a measurable result was recorded as the titer for that time point. Subjects were considered to have had an immune reaction if the titer of ≥ 1 postobiltoxaximab administration sample(s) was ≥ 4 times higher than baseline in subjects who had detectable ATAs at baseline or $\geq 1{:}20$ in subjects who had undetectable ATAs (titer $< 1{:}10$) at baseline.

PK Analysis

PK parameters were derived using noncompartmental methods with Microsoft Office Excel 2007 using the PK Solutions 2.0 template (Summit Research Services, Montrose, Colorado) (study 1) and WinNonlin Professional version 5.2 (study 2) and version 6.2.1 (studies 3–5). C_{max} and T_{max} were observed values. The terminal-phase rate constant (k) was determined by linear regression of the terminal

phase of the log concentration-time profile, and $t_{1/2}$ was calculated as ln(2)/k. The AUC was calculated using the trapezoidal method to AUC_{0-last}, and $AUC_{0-\infty}$ was calculated as $AUC_{0-last} + C_{last}/k$, where C_{last} is the last quantifiable concentration. CL was calculated as dose divided by $AUC_{0-\infty}$, and V_d was calculated as dose divided by $AUC_{0-\infty} \times k$, and volume of distribution at steady state (V_{ss}) was calculated as CL × mean residence time.

Only subjects from the PK populations in each study were included in the analyses. Any subject who received a partial obiltoxaximab dose or for whom the dosing record was missing was excluded from the PK population.

Safety Profile and Tolerability Assessments

Safety profile and tolerability were assessed throughout the studies. Safety profile assessments included vital signs, clinical laboratory tests (including hematologic testing, serum chemical analysis, urinalysis, free triiodothyronine, free thyroxine, thyrotropin, thyroid antibodies, and creatinine clearance), electrocardiographs, physical examinations, skin assessments, infusion site assessments, and adverse events (AEs). The safety profile was assessed for 71 days after dosing in studies 1 to 4 and during 191 days in study 5. The primary human safety profile studies (studies 3–5) were conducted with the commercial formulation and included 320 subjects who received ≥1 IV doses of 16-mg/kg of obiltoxaximab.

Statistical Analysis

For qualitative variables, the population size (N for sample size and n for available data) and the percentage (of available data) for each class of the variable were presented. Quantitative variables were summarized using descriptive statistics, including n, mean, SD, %CV, median, minimum, and maximum values.

In studies 1 and 2, dose proportionality of the PK parameters, $AUC_{0-\infty}$, AUC_{0-last}, and C_{max} over the administered dose range was investigated using the following power model: log (parameter) = a + b × log (dose), where a was the intercept and b was the slope. Dose proportionality was assessed based on whether the 90% CI constructed for the estimate of b was contained within the interval (0.80–1.25). The hypothesis of dose proportionality was rejected if the 90% CI constructed on the estimate of b was not contained in this interval. The power model parameters were estimated using least-squares regression. A minimum of 3 values per dose had to be available for a given parameter to estimate dose proportionality with the power model. Data analysis was performed using SAS software, version 9.1 or higher (SAS Institute, Cary, North Carolina), or Excel 2013 (Microsoft Corp, Redmond, Washington).

RESULTS

Subject Characteristics and Disposition

In study 1 (N = 45), all subjects received the planned doses of study drug, completed the study, and were included in the PK population. In study 2, 103 of 108 randomized subjects received all planned doses of study drug and completed all study procedures. Of the 90 subjects who received obiltoxaximab, 89 were included in the PK analysis; one subject's infusion (16-mg/kg group) was stopped prematurely because of a urticarial rash. In study 3 (N = 280), 4 subjects in the obiltoxaximab group were lost to follow-up, and 1 subject each in the obiltoxaximab and placebo group withdrew consent. Of the 210 subjects who received obiltoxaximab, 202 had at least 1 valid PK measurement and were included in the PK population. Subjects were excluded from the PK population for reasons of missing dosing record (1 subject), infusion discontinued because of hypersensitivity reactions (6 subjects), or mechanical issues with the infusion pump (1 subject). In study 4 (N = 40), of the 40 subjects who received the study drug, 38 were included in the PK population. The PK population comprised all 20 subjects who received obiltoxaximab alone and 18 subjects who received obiltoxaximab and ciprofloxacin. Two subjects in the obiltoxaximab and ciprofloxacin group had the infusion of obiltoxaximab discontinued because of hypersensitivity reactions and did not receive ciprofloxacin. One subject in the obiltoxaximab and ciprofloxacin group withdrew prematurely on day 1 for personal reasons after having received the intravenous obiltoxaximab and ciprofloxacin doses and did not complete all the study assessments but was included in the PK population. In study 5 (N = 70), all subjects received the first dose of obiltoxaximab on day 1, but 34 and 31 subjects received a second dose after 13 days or ≥119 days, respectively. Two subjects did not receive the second infusion of obiltoxaximab because of a hypersensitivity reaction during the first infusion.

The primary human safety studies were conducted with the commercial formulation at the efficacious dose identified in animals[17] and included 320 healthy volunteers who received ≥1 16-mg/kg IV doses of obiltoxaximab (studies 3, 4, and 5) and 70 subjects who received an intravenous infusion of placebo (study 3). The single-dose safety population consisted of 300 healthy adults who were exposed to a single dose of 16 mg/kg of IV obiltoxaximab and included all subjects in the obiltoxaximab arm of study 3 (n = 210), 20 subjects in the obiltoxaximab-only arm of study 4, and 70 subjects in the first administration period of study 5.

Baseline characteristics were similar between the obiltoxaximab and placebo groups (Table I). Subjects in the single-dose safety population were 18 to 79 years of age, 54% were male, 70% white, 27% black or African American, 1% American Indian/Alaska Native, 1% Asian, and 11 % Hispanic.

Diphenhydramine pretreatment was received by 194 of 280 subjects (69%) in study 3, all subjects (100%) in study 4, and in 62 of 70 subjects (89%) in study 5.

Table I. Demographics and baseline characteristics.

	Study 3		Single-Dose Safety Profile Population*
Characteristic	Placebo (N = 70)	Obiltoxaximab, 16 mg/kg (N = 210)	Obiltoxaximab, 16 mg/kg (N = 300)
Sex, No. (%)			
Male	38 (54.3)	106 (50.5)	162 (54.0)
Female	32 (45.7)	104 (49.5)	138 (46.0)
Age, y			
Mean (SD)	41.5 (13.9)	42.4 (15.6)	42.0 (15.5)
Median	40.0	43	40.5
Range	20–78	18–79	18–79
Race, No. (%)			
White	44 (62.9)	151 (71.9)	210 (70.0)
Black or African American	23 (32.9)	53 (25.2)	80 (26.7)
Asian	2 (2.9)	3 (1.4)	3 (1.0)
American Indian/Alaska Native	0	0	3 (1.0)
Other	1 (1.4)	3 (1.4)	4 (1.3)
Ethnicity, No. (%)			
Hispanic or Latino	9 (12.9)	20 (9.5)	27 (10.8)
Not Hispanic or Latino	61 (87.1)	189 (90.0)	222 (88.8)
Unknown	0	1 (0.5)	1 (0.4)
Weight, kg			
Mean (SD)	77.6 2 (13.6)	81.2 (17.9)	80.50 (17.8)
Median	75.9	79.2	78.9
Range	55.4–110.6	48.4–149.5	48.4–149.5

*Single-dose safety population (n = 300): 210 subjects who received obiltoxaximab in study 3, 20 subjects in the obiltoxaximab alone arm of study 4 (excluding the 20 subjects who received obiltoxaximab and ciprofloxacin), and all 70 subjects who received the first dose of obiltoxaximab in study 5.

PK Parameters

A summary of mean PK results is presented in Table II. Considering the results of all 5 studies, serum concentrations decreased in a biexponential or multiexponential fashion (Figure 1A and 1B) after intravenous administration of obiltoxaximab, with $t_{1/2}$ values of 17 to 23 days. The overall mean V_d and V_{ss} values for obiltoxaximab in humans across studies were approximately 7.5 and 6.3 L, respectively, which exceed plasma volume, but are substantially lower than extracellular fluid volume.[19] This finding suggests that obiltoxaximab distributes outside the vascular compartment but not extensively. This is generally comparable to other mAbs, which have been observed to distribute beyond the vascular space, with potential uptake into tissues.[20] Mean systemic clearance of obiltoxaximab after intravenous administration was approximately 0.27 L/d, which is $\leq$0.03% of hepatic or renal plasma flow (1170 and 1000 L/d, respectively), and $<$0.2% of the glomerular filtration rate (180 L/d) in a 70-kg human,[19] suggesting that, like other mAbs, hepatic metabolism and/or renal excretion is not critical to the elimination of obiltoxaximab.[20]

PK results in studies 1 and 2 revealed that obiltoxaximab exposure increased proportionally with dose. The similarities in mean $t_{1/2}$, CL, and V_d across groups indicated dose proportionality from 120 mg (approximately 1.49 mg/kg) to 360 mg (approximately 4.69 mg/kg) in study 1 and from 4 to 16 mg/kg in study 2. A 3-fold increase in dose from 120 to 360 mg and a 4-fold increase in dose from 4 to 16 mg/kg resulted in commensurate increases in mean AUC_{0-last} and $AUC_{0-\infty}$ values in studies 1 and 2, respectively. Mean C_{max} increased approximately 3.9-fold in study 1 and 3.5-fold in study 2 across these same dose ranges. For C_{max} (study 2), AUC_{0-last} and $AUC_{0-\infty}$, 90% CIs constructed for the estimate of the slope of the linear

Table II. Obiltoxaximab pharmacokinetic parameters following single-dose intravenous administration to healthy humans.[*]

Study	Dose, mg/kg	n	C_{max}, µg/mL	T_{max}, mean (range), d	$AUC_{0-\infty}$, µg•d/mL	$t_{1/2}$, d	CL, L/d	V_d, L	V_{ss}, L
1	1.49[†]	12	39.3 (19)	0.13 (0.04–0.25)	507 (28)	21.9 (41)	0.252 (24)	7.47 (24)	5.90 (19)
	3.04[†]	12	89.5 (36)	0.13 (0.04–1.00)	1090 (21)	20.9 (18)	0.229 (21)	6.95 (32)	5.75 (20)
	4.69[†]	12	154 (37)	0.13 (0.04–1.00)	1680 (23)	16.8 (14)	0.225 (23)	5.43 (26)	4.86 (21)
2	4	30	94.0 (21)	0.17 (0.167–2.00)	1080 (23)	18.2 (4.3)	0.279 (23)	7.08 (18)	6.03 (21)
	8	30	210 (41)	0.17 (0.17–1.50)	2390 (22)	20.8 (4.4)	0.270 (24)	7.89 (20)	6.62 (20)
	16	29	330 (19)	0.17 (0.17–2.00)	4410 (23)	20.4 (5.0)	0.287 (28)	8.05 (18)	7.18 (17)
3	16	202	400 (23)	0.08 (0.07–1.01)	5170 (26)	20.2 (26)	0.270 (33)	7.41 (26)	6.34 (24)
4	16	20	402 (23)	0.10 (0.06–1.00)	4891 (18)	19.5 (21)	0.268 (22)	7.57 (34)	6.28 (27)
	16[‡]	18	397 (16)	0.10 (0.06–1.00)	4990 (19)	19.0 (16)	0.247 (30)	6.59 (20)	5.68 (17)
5	16[§]	35	384 (27)	0.13 (0.06–1.00)	4690 (29)	21.5 (31)	0.300 (35)	8.75 (24)	7.20 (21)
	16[‖]	31	402 (33)	0.07 (0.06–1.00)	4400 (18)	18.6 (19)	0.313 (24)	8.33 (29)	7.01 (33)
	32[¶]	32	NA[#]	NA[#]	10,300 (25)	22.8 (26)	0.274 (31)	8.69 (31)	NA[#]

NA = not applicable; V_{ss} = volume of distribution at steady state.
[*]Values are mean (%CV) unless specified.
[†]Nominal doses were 120, 240, and 360 mg; the milligram per kilogram dose was estimated using mean body weight in each group.
[‡]Coadministered with a single dose of 400 mg of IV ciprofloxacin on day 1 followed by 750 mg orally twice daily from days 2 to 8 and a single dose on day 9.
[§]Day 1 dose.
[‖]Dose was administered 119 days after the day 1 dose (day 120).
[¶]Two 16-mg/kg doses (day 1 and day 14).
[#]Not valid for comparison (split dose).

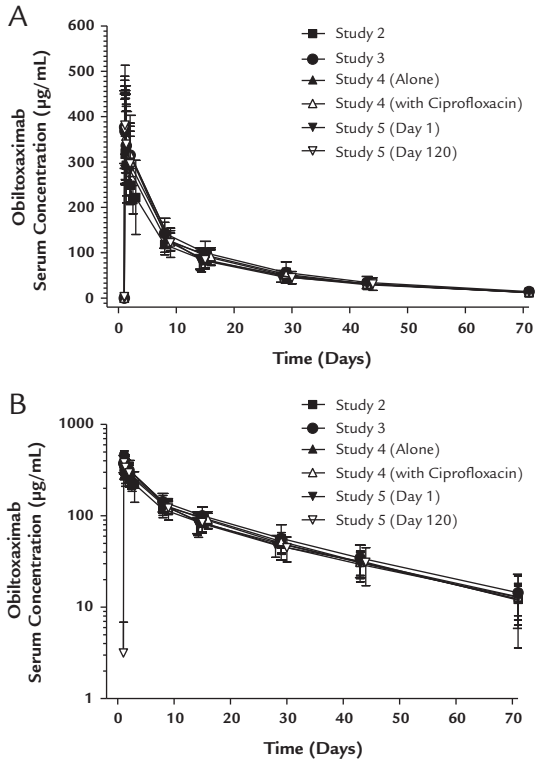

Figure 1. Mean (SD) plasma obiltoxaximab concentration-time profile during 70 days after single-dose IV administration of a 16-mg/kg dose in subjects in studies 2 to 5 (N = 335). A, Linear scale; B, log Scale.

relationship between exposure and dose (after log transformation) were contained within the interval (0.80–1.25), indicating that these parameters increased in a dose-proportional manner from 120 to 360 mg in study 1 (based on doses in milligrams per kilogram) and from 4 to 16 mg/kg in study 2. The 90% CI for the slope for C_{max} (0.97–1.28) in study 1 did not strictly meet the criteria for dose proportionality but only marginally exceeded the upper limit of the target interval, suggesting that this parameter increased in a generally dose-proportional manner as well. Dose-proportional increases in C_{max} and $AUC_{0-\infty}$ values were also observed when mean exposure parameters from all single-dose groups across the 5 studies were combined (Figure 2A and 2B).

In the repeat-dose study (study 5), given the limited sampling during days 1 to 14, the lack of attainment of steady state after the second dose, and the slow rates of distribution and elimination relative to the dosing interval, there were insufficient serum concentration data in the group that received study drug on days 1 and 14 to adequately characterize the PK profile of obiltoxaximab separately after each of the 2 dose administrations. Therefore, the PK profile in this group was treated as a 32-mg/kg dose split into two 16-mg/kg administrations (day 1 and day 14), and PK parameters were calculated based on continuous concentration-time data from day 1 before dosing through day 191 after dosing. The mean concentration-time profiles after the day 1 and day 120 doses in study 5 were virtually superimposable (Figure 3A and 3B), and obiltoxaximab disposition on day 1 was essentially indistinguishable from that on day 120. There were measurable obiltoxaximab concentrations in the predose serum samples of each subject on day 120. However, these concentrations

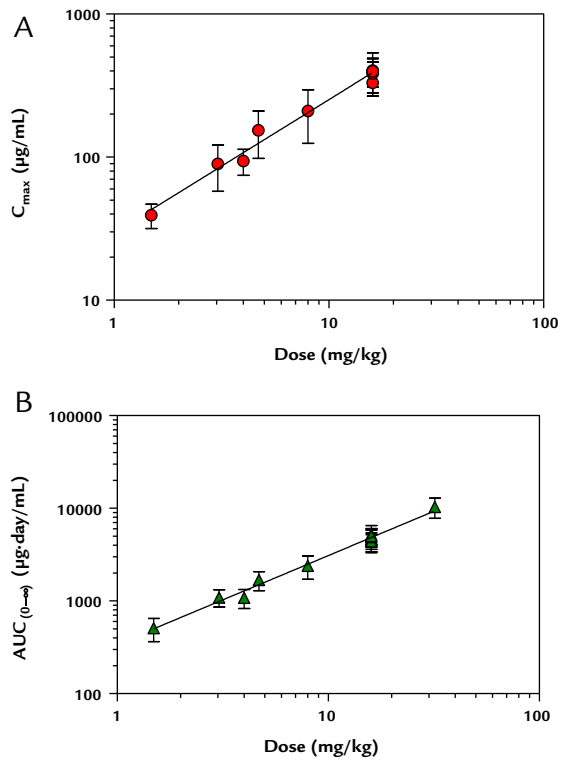

Figure 2. Mean (SD) plasma C_{max} (n = 431) (A) and $AUC_{0-\infty}$ (n = 441) (B) show dose-proportional increases. The slope (90% CI) was 0.93 (0.86–1.00) for C_{max} and 0.96 (0.90–1.01) for $AUC_{0-\infty}$.

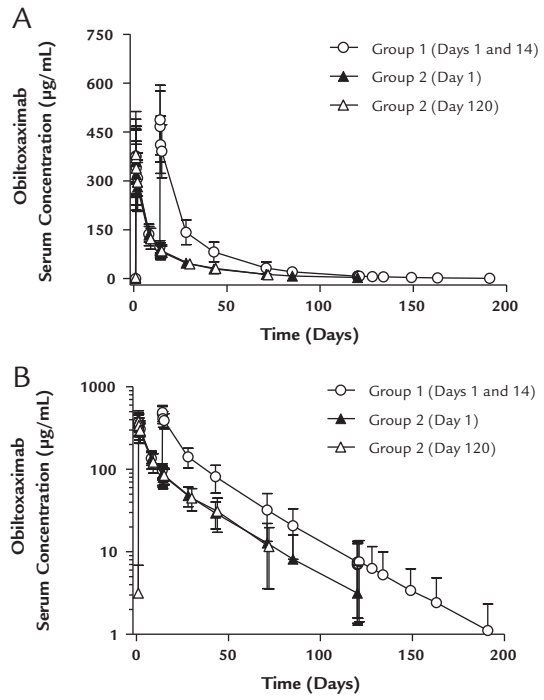

Figure 3. Mean (SD) plasma obiltoxaximab concentration-time profile through 191 days in the pharmacokinetic population (N = 69) of study 5. Subjects received a single 16-mg/kg dose of obiltoxaximab on day 1 and day 14 in group 1 and on day 1 and day 120 in group 2. One subject from group 1 was excluded from concentration-time summary statistics. For group 2, n = 35 for day 1 and n = 31 for day 120. Group 2 concentration-time curves on days 1 and 120 are overlapping to demonstrate that they are superimposable. A, Linear scale; B, log scale.

were so slight relative to exposures later in the profile (0.09%–5.5% of the subsequent end-of-infusion concentration) that their effect on PK parameters determined for the day 120 dose was considered negligible. The obiltoxaximab $AUC_{0-\infty}$ after two 16-mg/kg doses in relatively short succession (days 1 and 14) was approximately twice that after a single 16-mg/kg dose on day 1 or day 120. In addition, $t_{1/2}$, CL, and V_d values in the day 1/day 120 dose group were similar to those in the day 1/day 14 dose group. This finding suggests that overall obiltoxaximab exposure after two 16-mg/kg doses 2 weeks apart increased proportionally relative to a single 16-mg/kg dose and that the disposition of obiltoxaximab after two 16-mg/kg doses in relatively short succession did not differ from that after a single 16-mg/kg dose. Coadministration of 16 mg/kg of IV obiltoxaximab with IV or oral ciprofloxacin in human subjects (study 4) did not alter the PK profile of either ciprofloxacin or obiltoxaximab.

Safety Profile and Tolerability

An obiltoxaximab dose of 16 mg/kg was generally well tolerated when administered intravenously as a 90-minute infusion to healthy volunteers (studies 3–5). The percentage of subjects with AEs in study 3 was similar in the obiltoxaximab and placebo groups (49.1% and 38.6%, respectively). The most frequently reported AEs in the obiltoxaximab arm of the primary safety study (study 3) were headache (10%), pruritus (4.8%), rash (4.3%), upper respiratory tract infection (3.8%), vessel puncture site bruise (3.3%), and cough (2.9%) (Table III). All AEs were mild to moderate in severity, except for severe pruritus and urticaria in 1 subject who was not premedicated with diphenhydramine. Most subjects in the obiltoxaximab arm (96.7%) and all subjects in the placebo arm (100%) completed study drug infusion. Six subjects (2.9%) in the obiltoxaximab arm and no subjects in the placebo arm permanently discontinued the infusion of the study drug because of hypersensitivity reactions, 2 of which met the criteria for anaphylaxis.[21] In the single-dose pooled safety population (n = 300), the most frequently reported adverse reactions that occurred in ≥1.5% of healthy subjects receiving a single 16-mg/kg dose of obiltoxaximab and more frequently than in those receiving placebo were headache (8%), pruritus (4%), upper respiratory tract infection (5%), cough (3%), vessel puncture site bruise (3%), infusion site swelling (3%), nasal congestion (2%), infusion site pain (2%), urticaria (2%), and extremity pain (2%) (Table IV). Clinical laboratory test results, vital signs, and electrocardiography results were unremarkable and stable over time. Tolerability at the infusion site was acceptable.

Subjects in the single-dose safety population who were pretreated with diphenhydramine before the infusion of study drug had a lower overall incidence of adverse reactions than those without diphenhydramine pretreatment (42% vs 58%, respectively) and a lower incidence of headache (5% vs 16%), cough (1%

Table III. Number (percentage) of obiltoxaximab exposure-emergent adverse events occurring in >1 subject in either group with a higher incidence in obiltoxaximab group in study 3.

Adverse Event	Placebo (N = 70)*	Obiltoxaximab, 16 mg/kg (N = 210)
All subjects with adverse events	27 (38.6)	88 (41.9)
Headache	4 (5.7)	21 (10.0)
Pruritus	1 (1.4)	10 (4.8)
Rash	2 (2.9)	9 (4.3)
Upper respiratory tract infection	2 (2.9)	8 (3.8)
Vessel puncture site bruise	1 (1.4)	7 (3.3)
Cough	0	6 (2.9)
Infusion site pain	0	4 (1.9)
Nasal congestion	1 (1.4)	4 (1.9)
Pain in extremity	1 (1.4)	4 (1.9)
Fatigue	0	3 (1.4)
Infusion site discoloration	0	3 (1.4)
Oropharyngeal pain	0	3 (1.4)
Urticaria	0	3 (1.4)
Vomiting	0	3 (1.4)
Application site erythema	0	2 (1.0)
Asthenia	0	2 (1.0)
Dizziness	0	2 (1.0)
Laceration	0	2 (1.0)
Musculoskeletal pain	0	2 (1.0)
Myalgia	0	2 (1.0)
Rhinorrhea	0	2 (1.0)
Toothache	0	2 (1.0)
Vessel puncture site pain	0	2 (1.0)

*Placebo subjects from study 3.

vs 8%), rash (2% vs 7%), pruritus (3% vs 4%) throat irritation (0% vs 3%), rhinorrhea (0% vs 3%), and infusion site erythema (0% vs 4%). In addition, somnolence was only reported in subjects who were pretreated with diphenhydramine.

There was no change in the safety profile, no additional adverse reactions identified, and no change in frequency of adverse reactions when a second 16-mg/kg IV dose of obiltoxaximab was administered 13 days or ≥119 days after the first 16-mg/kg dose.

Hypersensitivity reactions were the most common adverse reactions in the safety profile trials, occurring in 34 of 320 healthy subjects (10.6%) receiving obiltoxaximab and 4 of 70 (5.7%) subjects receiving placebo. Obiltoxaximab infusion was discontinued in 8 subjects (2.5%), and 2 subjects in the repeat-dose study (study 5) did not receive a second dose of obiltoxaximab, 1 because of urticaria and 1 because of anaphylaxis (Table V). The subjects were 28 to 70 years of age, 6 were male and 4 were female, and 9 were white and 1 was American Indian or Alaska

Table IV. Number (percentage) of adverse reactions reported in ≥1.5% of the single-dose pooled safety population.

Adverse Reaction	Placebo* (N = 70)	Single-Dose Obiltoxaximab Safety Profile Population† (N = 300)
Headache	4 (6)	24 (8)
Pruritus	1 (1)	11 (4)
Upper respiratory tract infection	2 (3)	14 (5)
Cough	0	9 (3)
Vessel puncture site bruise	1 (1)	8 (3)
Infusion site swelling	1 (1)	8 (3)
Nasal congestion	1 (1)	5 (2)
Infusion site pain	0	7 (2)
Urticaria	0	5 (2)
Extremity pain	1 (1)	5 (2)

*Placebo subjects from study 3.
†Single-dose safety profile population: 210 subjects in study 3, 20 subjects in the obiltoxaximab-alone arm of study 4, and 70 subjects who received the first dose of obiltoxaximab in study 5.

Table V. Subjects with hypersensitivity reactions leading to premature discontinuation of obiltoxaximab infusion.

Subject No. Sex, Age, y, and Race	Diphenhydramine Pretreatment	AEs Leading to Discontinued Infusion of the Study Drug Preferred Term [Verbatim Term]	Onset Time After Dosing, day:h:min	Severity	Concomitant Medication
Study 3					
1, male, 43, white	No	Pruritus [pruritus]	0:00:34	Severe	Diphenhydramine (IV and oral)
		Urticaria [hives]			
		Throat irritation [scratchy throat]	0:00:34	Mild	
		Cough [dry cough]	0:00:35	Mild	
		Muscle twitching [twitching]	0:00:36	Mild	
		Headache [head pressure]	0:1:50	Mild	
		Urticaria [hives]	0:6:02	Mild	
		Pruritus [pruritus]	0:6:06	Mild	
		Dysphonia [hoarseness]	0:7:20	Mild	
		Pruritus [pruritus]	<1 day	Moderate	
		Urticaria [hives]			
2, female, 54, white	Yes	Ear discomfort [pressure in ears bilaterally]	24 min	Mild	Diphenhydramine (IV and oral)
		Urticaria [hives]	34 min	Moderate	
		Pruritus [pruritus]			
		Dizziness [dizziness]	48 min	Moderate	
		Chills [chills]	53 min	Moderate	
		Urticaria [hives]	1 day	Mild	
3 female, 29, white	Yes	Urticaria [hives]	0:01:06	Moderate	Diphenhydramine (IV)
		Pruritus [itching]	0:01:06	Mild	
		Urticaria [hives]	0:02:00	Mild	
4, male, 28, white	No	Cough [cough]	0:00:58	Mild	Diphenhydramine (IV); famotidine (IV)
		Rash [rash]	0:00:58	Moderate	
		Throat irritation [scratchy throat]	0:00:58	Mild	
		Hypoesthesia [numbness of fingers left hand]	0:01:14	Mild	
		Hypoesthesia [numbness of fingers right hand]	0:01:41	Mild	
		Cough [cough]	0:03:25	Mild	
5, female, 49, white	No	Pruritus [itching]	0:00:23	Mild	Diphenhydramine (IV); famotidine (IV)
		Rash [rash]	0:01:03	Moderate	
		Headache [headache]	0:01:30	Moderate	
		Cough [coughing]	0:02:02	Mild	

(continued)

Table V. (continued).

Subject No. Sex, Age, y, and Race	Diphenhydramine Pretreatment	AEs Leading to Discontinued Infusion of the Study Drug: Preferred Term [Verbatim Term]	Onset Time After Dosing, day:h:min	Severity	Concomitant Medication
6, male, 62, white	Yes	Acute anaphylactic allergic reaction [diffuse pruritic urticarial rash on most of the body, including neck, chest, back, abdomen, arms, and legs; shortness of breath; and coughing]	0:00:55	Moderate	Diphenhydramine (IV and oral); famotidine (IV); salbutamol; methylprednisolone (IV); epinephrine (IM)
Study 4[*]					
7, male, 59, white		Urticaria [generalized urticarial rash]	0:00:28	Moderate	Diphenhydramine (IV); famotidine (IV); methylprednisolone (IV)
		Dysarthria [dysarthria]	0:00:35	Mild	
		Pain in Jaw [jaw pain]	0:00:48	Mild	
		Chest Discomfort [chest discomfort]	0:01:02	Mild	
		Dizziness postural [postural lightheadedness]	0:01:05	Moderate	
		Palpitations [palpitations]	0:07:24	Mild	
		Fatigue [tired feeling]	0:23:20	Mild	
8,male, 58, white	Yes	Urticaria [generalized urticarial reaction]	0:00:50	Moderate	None
Study 5[†]					
9, female, 66, American Indian or Alaska Native	No	Back pain [back pain]	0:01:24	Severe	Acetaminophen (oral); diphenhydramine (oral); sodium chloride (IV); oxygen
		Chills [shaking chills]	0:01:32	Moderate	
		Dyspnea [dyspnea]	0:01:32	Moderate	
		Flushing [flushing]	0:01:32	Mild	
		Cyanosis [acrocyanosis]	0:01:35	Moderate	
		Pallor [pallor]	0:01:35	Moderate	
		Restlessness [restlessness]	0:01:35	Moderate	
		Myalgia [myalgias, generalized]	0:01:40	Moderate	
		Rash [rash – trunk]	0:02:20	Mild	
10, male, 70, white	Yes	Urticaria [urticaria]	0:04:00	Moderate	Diphenhydramine (IV)

AE = adverse event; IM = intramuscular; IV = intravenous.

[*]Both subjects were randomized to receive obiltoxaximab and ciprofloxacin but discontinued the obiltoxaximab infusion because of hypersensitivity reactions and did not receive IV or oral ciprofloxacin.

[†]Both subjects completed the first infusion of obiltoxaximab but did not receive a second dose.

Native. Six subjects were premedicated with diphenhydramine before the infusion of obiltoxaximab, and 4 were not because they were enrolled before the protocol amendment requiring premedication. The adverse reactions reported in these 10 subjects included urticaria, rash, cough, pruritus, postural dizziness, throat irritation, dysphonia, dyspnea, chest discomfort, and cyanosis. The symptoms in 3 of the 10 subjects [3 of 320 (0.9%)] met the criteria for anaphylaxis (subjects 6, 7, and 9) (Table V).[21] Manifestations of anaphylaxis were rash/urticaria (n = 2), cough (n = 1), dyspnea (n = 2), cyanosis (n = 1), postural dizziness (n = 1), and chest discomfort (n = 1). The vital signs of these 3 subjects were stable throughout the event, and hypotension and hypoxia were not noted. There was also no evidence of bronchospasm or upper airway angioedema. All but 1 of the 10 subjects with hypersensitivity reactions required additional therapy, including intravenous diphenhydramine (n = 9), famotidine (n = 3), methylprednisolone (n = 2), normal saline (n = 1), intramuscular epinephrine (n = 1), and oxygen (n = 1). The remaining subjects with hypersensitivity had predominantly skin-related symptoms, such as pruritus and rash, and 6 subjects reported cough.

The development of antiobiltoxaximab antibodies was evaluated in all subjects receiving one or two 16-mg/kg doses of the commercial formulation of obiltoxaximab in studies 3, 4, and 5. Eight of 320 subjects (2.5%) who received at least 1 dose of intravenous obiltoxaximab tested positive for a administration-emergent ATA response. Quantitative titers were low, however, ranging from 1:20 to 1:320. Assays for neutralizing antibodies were not performed because of residual obiltoxaximab concentrations in postobiltoxaximab administration sera, which interfered with the assay, but there was no evidence of altered PK or toxicity profile in subjects with ATA development. There was also no evidence of increased immunogenicity after a second exposure to obiltoxaximab compared with a single exposure.

DISCUSSION

The PK results from 5 studies in more than 450 healthy volunteers were consistent. Obiltoxaximab had dose-proportional increases in exposure, and serum concentrations decreased in a biexponential fashion after intravenous administration, with $t_{1/2}$ values that approached 3 weeks. The volume of distribution was approximately 6.3 to 7.5 L, a volume that may suggest tissue penetration, as has been noted with other mAbs.[15] Mean clearance was only 0.27 L/d, suggesting that, like other mAbs, obiltoxaximab is not extensively metabolized by the liver or eliminated by the kidneys.[20] In addition, study 5 found that obiltoxaximab exposure after two 16-mg/kg doses approximately 2 weeks apart increased proportionally relative to a single 16-mg/kg dose and that the disposition of obiltoxaximab after two 16-mg/kg doses in relatively short succession did not differ from that after a single 16-mg/kg dose. The disposition of obiltoxaximab when a second dose was administered on day 120 was virtually identical to that after the first dose on day 1. As the human pharmacokinetic studies progressed, it was determined that the 16-mg/kg dose produced systemic exposures similar to or greater than at the maximally efficacious dose in cynomolgus macaques and New Zealand White rabbits.[17]

The 16-mg/kg dose of obiltoxaximab was safe and generally well tolerated in this healthy volunteer population. The most common adverse reactions reported were headache, pruritus, upper respiratory tract infection, cough, vessel puncture site bruise, infusion site swelling, nasal congestion, infusion site pain, urticaria, and pain in extremity. Most of the events were mild to moderate in severity, and the local tolerability was acceptable. Three percent of subjects discontinued obiltoxaximab infusion or did not receive a second infusion because of hypersensitivity reactions. Hypersensitivity is a common AE associated with drugs, especially protein therapeutics. Hypersensitivity reactions manifest as a spectrum of clinical signs and symptoms and may occur during or immediately after drug exposure because of release of histamine and other vasoactive mediators. Most of the hypersensitivity reactions associated with obiltoxaximab, primarily manifesting as pruritus, rash, and urticaria, were mild to moderate in intensity, and these individuals were usually treated only with antihistamines. Only 1 subject participating in the primary safety profile studies was reported as having experienced severe hypersensitivity reactions: severe pruritus and urticaria in a subject who had not been premedicated with diphenhydramine. No hypersensitivity reactions were considered by study investigators to represent serious AEs.

Anaphylaxis is a severe, acute hypersensitivity reaction often accompanied by cardiorespiratory

symptoms.[22] Clinicians are typically well aware of anaphylaxis, although there is no universal agreement on its definition. Specific diagnostic criteria have been proposed[21] but are open to interpretation and not necessarily widely implemented clinically. During the obiltoxaximab clinical trials, anaphylaxis was reported by 1 investigator in a subject who required treatment with epinephrine. This subject experienced a diffuse, pruritic urticarial rash, shortness of breath, and coughing. Two other subjects experienced acute hypersensitivity reactions associated with cardiorespiratory symptoms but were not reported as having anaphylaxis by the investigators. Manifestations included rash, dyspnea, and cyanosis in one and urticaria, postural dizziness, chest discomfort, jaw pain, and dysarthria in the other. Neither required epinephrine for management of symptoms. None of these 3 subjects was reported as having hypotension, bronchospasm, or oropharyngeal angioedema. However, all 3 are included in the safety results of this report and in product labeling as having experienced anaphylaxis. In addition to these 3 individuals, 7 subjects stopped taking the study drug because of mild to moderate hypersensitivity reactions. None demonstrated evidence of cardiorespiratory compromise or angioedema. It is probable that the threshold for discontinuation in these healthy volunteers was lower than that for patients receiving monoclonal antibodies for active medical conditions because there was no potential benefit to continuing obiltoxaximab administration.

The approval of obiltoxaximab in the United States for the treatment of inhalational anthrax due to *B anthracis* in combination with appropriate antibacterial drugs in adult and pediatric patients[23] provides an additional treatment for this deadly disease. The recommended dosage of obiltoxaximab in adult patients is a single IV dose of 16 mg/kg during 90 minutes. Obiltoxaximab is also approved for prophylaxis when alternative therapies are not available or not appropriate but should only be used for prophylaxis when its benefit for prevention of inhalational anthrax outweighs the risk of hypersensitivity and anaphylaxis. Because human studies evaluating the effectiveness of obiltoxaximab for infections due to *B anthracis* are not ethical and field trials have not been feasible, obiltoxaximab was developed under the Animal Rule, under which a drug can be approved based on animal efficacy studies.[24] The PK profile of obiltoxaximab has been well described in these 5 healthy volunteer studies, which was necessary for the selection of an effective dose in humans. The PK studies in animals have generally produced similar results, specifically, dose-proportional increases in exposure and serum concentrations that decreased in a biexponential fashion after intravenous administration. However, the $t_{1/2}$ in animals was shorter than that in humans.[18]

The PK profile of obiltoxaximab has been examined and considered to have been adequately characterized after a 16-mg/kg IV infusion, meeting 1 of the criteria of the Animal Rule. In addition, obiltoxaximab is generally well tolerated. Obiltotoxaximab was associated with acute hypersensitivity reactions, including anaphylaxis, but the overall safety experience was consistent with other monoclonal antibodies. *B anthracis* has been identified as a top priority biowarfare target because of the potential for mass casualties in the event of spore dispersion as part of a bioterrorist attack. These data, combined with previously reported efficacy data from animal model experiments,[17] support the addition of obiltoxaximab to the therapeutic armamentarium in the treatment of inhalational anthrax.

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CONFLICTS OF INTEREST

All authors are employees of or were consultants for Elusys Therapeutics, Inc, during the conduct of this study.

SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.clinthera.2016.07.170.

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Address correspondence to: Christa Nagy, PhD, Department of Clinical Operations, Elusys Therapeutics, Inc, 25 Riverside Dr., Unit 1, Pine Brook, NJ 07058. E-mail: cnagy@elusys.com

SUPPLEMENTARY MATERIAL

Study 4 – Ciprofloxacin Concentration Determination Methods

Blood samples (3 mL) for analysis of intravenous (IV) ciprofloxacin pharmacokinetics (PK) were obtained predose (prior to IV infusion of obiltoxaximab) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 hours following the start of the ciprofloxacin 2infusion. Blood samples (3 mL) for analysis of oral ciprofloxacin PK were obtained at predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 hours after the last dose. Blood samples were collected into tubes containing K_2 EDTA, processed to plasma, and analyzed for ciprofloxacin concentrations using a validated method at KCAS Bioanalytical Services, Shawnee, KS, with an assay range of 10 to 2000 ng/mL.

Supplemental Tables I and II.

Supplemental Table 1. Study Descriptions

Study Number	Study Design	Specific Enrollment Criteria Details	Obiltoxaximab Dose, Regimen, Duration, and Number of Subjects	Obiltoxaximab PK Sampling Times	ATA Sampling Times
1	R, DB, PC, single dose, dose escalation	Inclusion ● Age 18–50 y *Exclusion* ● SBP >139 mmHg; DBP >89 mmHg ● Smoking was prohibited within 6 months	Obiltoxaximab 120, 240, or 360 mg (n=36) or placebo (n=9)	Predose and 1, 3, 6, 12, 24, and 48 hours after the end of the infusion, and on Days 7, 14, 21, 42, 56, and 70	Predose and Day 42[a]
2	R, DB, PC, single-dose, dose escalation	*Inclusion* ● Age 18–65 y *Exclusion* ● SBP >140 or <90 mmHg; DBP >90 mmHg ● More than 3 cigarettes per day were prohibited	Obiltoxaximab 4, 8, or 16 mg/kg (n=90) or placebo (n=18)	Predose, and 4, 8, 24, 36, and 48 hours after the end of the infusion, and on Days 8, 15, 29, 43, and 71	Predose on Day 1 and on Days 8, 43, and 71
3	R, DB, PC, single dose	*Inclusion* ● ≥18 y *Exclusion* ● ≥150 mmHg or ≤90 mmHg; DBP ≥95 mmHg ● Smoking was permitted	Obiltoxaximab 16 mg/kg (n=210) or placebo (n=70)	Predose, end of infusion, and 3, 8, and 24 hours after the start of infusion, and on Days 8, 15. 29, 43, and 71	Predose on Day 1 and on Days 8, 43, and 71
4	R, OL, parallel group, drug-drug interaction (ciprofloxacin)	*Inclusion* ● Age 18–60 y *Exclusion* ● SBP ≥150 mmHg or ≤90 mmHg; DBP ≥95 mmHg	Obiltoxaximab 16 mg/kg (n=20) or obiltoxaximab 16 mg/kg + ciprofloxacin 400	Predose, end of infusion, and 2.5, 4.5, 7.5, and 24 hours after the start of infusion, and	Predose on Day 1 and on Days 9, 43, and 71

(continued)

Supplemental Table 1. (continued).

Study Number	Study Design	Specific Enrollment Criteria Details	Obiltoxaximab Dose, Regimen, Duration, and Number of Subjects	Obiltoxaximab PK Sampling Times	ATA Sampling Times
		• Smoking was prohibited within 3 months • Ciprofloxacin-specific criteria: o History of hypersensitivity to fluoroquinolones o History of conditions or therapies that increase risk of *Clostridium difficile* infection o Medical conditions that required repeat courses of antibiotics (≤10-day-course of antibiotics within 6 months prior to Day 1 permitted) o History of tendon rupture o Use of cation-containing drugs or food supplements within 2 days prior to Day 1 o Use of protheophylline, theophylline, methylxanthine, tizanidine, or other drugs metabolized via cytochrome P450 1A within 30 days prior to Day 1 o Use of medications that prolong the QT interval within 30 days prior to Day 1 or within 5 half-lives of Day 1 (whichever was longer) o High risk for QT prolongation	mg IV on Day 1 followed by oral ciprofloxacin (750 mg) every 12 hrs on Days 2–8 and morning of Day 9 (n=20)	Days 9, 16, 29, 43, and 71[b]	
5	R, DB, PC, repeat dose of	*Inclusion* • Age ≥18 y	Obiltoxaximab16 mg/kg on Days 1 and 14	Predose, end of infusion, and 3 and 8 hours after the	Predose on Days 1, 14, and 120, and on

(continued)

Supplemental Table 1. (continued).

Study Number	Study Design	Specific Enrollment Criteria Details	Obiltoxaximab Dose, Regimen, Duration, and Number of Subjects	Obiltoxaximab PK Sampling Times	ATA Sampling Times
	obiltoxaximab 14 or 120 days apart	*Exclusion* ● SBP $\geq$150 mmHg or $\leq$90 mmHg; DBP $\geq$95 mmHg ● Smoking was permitted	and placebo on Day 120 (n=35) or obiltoxaximab 16 mg/kg on Days 1 and 120 and placebo on Day 14 (n=35)	start of infusion on Days 1, 14, and 120; and on Days 2, 8, 15, 28, 43, 71 ,85, 121, 128, 134, 149, 163, and 191	Days 8, 43, 85, 128, 163, and 191

DB, double-blind; DBP, diastolic blood pressure; IV, intravenously; OL, open label; PC, placebo controlled; R, randomized; SBP, systolic blood pressure; y, years.
[a]Subjects with positive results at Day 42 were followed biweekly until Day 70 and then monthly until results were negative for at least 6 months if antibody titers were $<$1:100 and up to a year if titers $\geq$1:100.
[b]Sample times for obiltoxaximab pharmacokinetic samples. Ciprofloxacin data are not presented.

Supplemental Table II. Details of Study Conduct

Study Number	Study Location	Study Dates	Sample Size Determination	Randomization
1	Clinical pharmacology unit; Columbus, OH	23 Feb 2009–26 Sept 2009	Dose-escalation design with sequential dosing per standard design of phase I human studies of therapeutic monoclonal antibodies	Subjects were randomized to groups based on a randomization schedule generated by the SAS random number procedure (SAS Institute, Cary, NC). Randomly permuted blocks of size 5 were used to achieve an active-to-placebo ratio of 3:1. Subjects were assigned to groups as they appeared at the clinic. The study included randomization of at least 4 females into each of the 3 dose-level groups to provide data on both genders.
2	Contract Research Organization Unit; Overland Park, KS	14 Sept 2011–29 June 2012	Study was designed to detect at least a 20% difference in pharmacokinetic parameters (C_{max} and AUC) with 80% power based on the variability observed in previous clinical studies. Additionally, the sample size was sufficient to examine dose proportionality. In a two-sided tests analysis for equivalence of sample log-normal geometric means with bounds of 0.8 and 1.25 for the mean ratio and a significance level of 0.05, assuming variability observed in previous studies, a sample size of 30 per group yielded a power of greater than 80%. A sample size of 30 per group achieved a power of at least 80% to conclude dose proportionality	Subjects were randomly assigned to groups in 3 sequential cohorts. Within each cohort, subjects were randomly assigned to receive obiltoxaximab or placebo in a ratio of 5:1, using a block size of 6. A "meet in the middle" rule was used to assign randomization numbers for males and females. An enrolled female volunteer was assigned to the lowest available randomization number and a male volunteer to the highest available randomization number, until all 8 volunteers for a given cohort were included.

(continued)

Supplemental Table II. (continued).

Study Number	Study Location	Study Dates	Sample Size Determination	Randomization
			of the dose range using a power model and a 0.8 to 1.25 criterion.	
3	Contract Research Organization Units; Daytona Beach, FL; Dallas, TX; Evansville, IN; Madison, WI	9 July 2013–30 Sept 2014	Based on the randomization ratio, approximately 210 subjects in this study were expected to receive a single 16 mg/kg IV dose of obiltoxaximab. This sample size was selected to ensure that the total number of subjects exposed to a single 16 mg/kg IV dose of obiltoxaximab in the clinical development program was at least 350 subjects.	Subjects were randomized in a block design in a 3:1 ratio to either obiltoxaximab or matching placebo by an unblinded pharmacist at the study center using a randomization schedule provided by the contract research organization. Randomization was stratified by study center.
4	Contract Research Organization Unit, Overland Park, KS	29 Oct 2013–Nov 2014	The sample size (20 subjects/group) was based on the assumption of 20% between-subject arithmetic coefficient of variation for both AUC and C_{max} for obiltoxaximab and there was no difference in the true geometric means in the presence or absence of ciprofloxacin. Based on this assumption, completion of study drug by 18 subjects/group in the parallel group design would yield 80% overall power to conclude that there was no effect of ciprofloxacin on the $AUC_{0\text{-inf}}$ or C_{max} of obiltoxaximab under the standard 2 one-sided testing procedure using 80 to 125 equivalence limits. Based on data in an earlier human clinical study with obiltoxaximab, the CV% of	This was an open-label, randomized, parallel group study. Subjects were randomized in a 1:1 ratio by the pharmacist at the study center according to a randomization schedule generated by the contract research organization.

(continued)

Supplemental Table II. (continued).

Study Number	Study Location	Study Dates	Sample Size Determination	Randomization
			obiltoxaximab for $AUC_{0\text{-}inf}$ was approximately 22% and for C_{max} ranged from 19-40%.	
5	Contract Research Organization Unit, Overland Park, KS; Minneapolis, MN	23 July 2013–Jan 2015	No formal sample size calculation was performed since formal statistical comparisons were not planned. The sample size of approximately 70 subjects was considered adequate to characterize the safety and pharmacokinetic profiles of the repeat dosing of obiltoxaximab.	Subjects were randomized by an unblinded pharmacist at the study center using a randomization schedule provided by the contract research organization. The randomization schedule was generated using SAS software in a randomized block design, with a 1:1 ratio and stratified by site.

$AUC_{(0\text{–}inf)}$, area under the concentration vs. time curve from time 0 extrapolated to infinity; C_{max}, maximum concentration; CV%, coefficient of variation.