Safety and Pharmacokinetics of a Four Monoclonal Antibody Combination Against Botulinum C and D Neurotoxins

Doris M. Snow¹, Kathryn Riling¹, Angie Kimbler², Yero Espinoza³, David Wong³, Khanh Pham³, Zachary Martinez³, Carl N. Kraus⁴, Fraser Conrad⁵, Consuelo Garcia-Rodriguez⁵, Ronald R. Cobb², James D. Marks⁵ and Milan T. Tomic³*  

¹Ology Bioservices, Inc., 8490 Progress Drive, Suite 150, Frederick MD  
²Ology Bioservices, Inc., 13200 NW Nano Ct, Alachua, FL  
³Ology Bioservices, Inc., 626 Bancroft Way Suite D, Berkeley, CA  
⁴Arrevus, 2443 Lynn Road, Suite 210, Raleigh, NC  
⁵Department of Anesthesia and Perioperative Care, University of California, San Francisco, CA  

Keywords: Botulinum neurotoxin, safety, pharmacokinetics, monoclonal antibody, antibody combinations, Phase 1 clinical trial, antitoxin  

Running Title: Safety and pharmacokinetics of NTM-1634 in Phase 1  

Corresponding Author  
*To whom correspondence should be addressed  
Dr. Milan Tomic  
Research and Development, Antibody Development  
Ology Bioservices, Inc.  
626 Bancroft Way, Suite D  
Berkeley, CA 94710  
milan.tomic@ologybio.com
ABSTRACT

Botulism is caused by botulinum neurotoxin (BoNT), the most poisonous substance known. BoNTs are also classified as Tier 1 bioterror agents due to their high potency and lethality. The existence of seven BoNT serotypes (A-G), which differ between 35% to 68% in amino acid sequence, necessitates the development of serotype specific countermeasures. We present results of a Phase 1 clinical study of an anti-toxin to BoNT serotypes C and D, NTM-1634, which consists of an equimolar mixture of four fully human IgG1 monoclonal antibodies (mAbs), each binding to non-overlapping epitopes on BoNT serotypes C and D resulting in potent toxin neutralization in rodents. This first-in-human study evaluated the safety and pharmacokinetics of escalating doses of NTM-1634 administered intravenously to healthy adults (NCT03046550). Three cohorts of eight healthy subjects received a single intravenous dose of NTM-1634 or placebo at 0.33 mg/kg, 0.66 mg/kg or 1 mg/kg. Follow-up examinations and pharmacokinetic evaluations were continued up to 121 days post-infusion. Subjects were monitored using physical examinations, hematology and chemistry blood tests, and electrocardiograms. Pharmacokinetic parameters were estimated using noncompartmental methods. The results demonstrated that the materials were safe and well-tolerated with the expected half-lives for human mAbs and with minimal anti-drug antibodies detected over the dose ranges and duration of the study.
INTRODUCTION

Botulism, an acute life threatening flaccid paralysis affecting both humans and animals, is caused by botulinum neurotoxins (BoNTs) produced by the bacterium *Clostridium botulinum* and additional *Clostridia* species (1, 2). Naturally occurring botulism is an orphan disease, with approximately 120 cases/year in the United States. BoNTs are also classified as Tier 1 biothreat agents, the highest level of classification, due to their high potency and lethality (3). As such, the US government has funded development of botulinum antitoxins, including those reported here.

Of the seven immunologically distinct types of BoNTs (A-G) (4-6) serotypes A, B, E and F cause most of the naturally occurring human disease including foodborne, wound and intestinal botulism. Sequence analysis of BoNT/C and BoNT/D strains reveals the existence of mosaic toxins that contain portions of both BoNT/C and BoNT/D as well as sequence unique to the mosaics (7, 8). BoNT C/D has the sequence of BoNT/C for the amino-terminal 2/3 of the toxin but is 95% identical to the sequence of BoNT/D for the carboxy-terminal 1/3. BoNT D/C has high identity with the BoNT/D amino terminus but shares a lower identity with the BoNT/C and BoNT/D carboxy-terminus (8). BoNT/C and BoNT/D most frequently intoxicate non-humans with BoNT/C and C/D causing botulism in avian species (9, 10) as well as felines and canine species BoNT/D and D/C most frequently causing botulism in cattle (11, 12). BoNT/C and BoNT/D, however, can also cause botulism in humans (13). Two cases of foodborne botulism and one case of infant botulism has been attributed to BoNT/C (14). BoNT/D organisms have also been found in tainted ham that caused botulism in several individuals (15). BoNT/C blocks neuromuscular transmission in human neuromuscular junction preparations and causes prolonged inhibition of exocytosis in cerebellar granular neurons. Both BoNT/C and BoNT D/C cause lethal botulism in non-human primates exposed via the aerosol route. Finally, BoNT C/D is therapeutically active in treating dystonia in humans (16). These studies indicate that BoNT/C and BoNT/D and their mosaic toxins pose a similar biothreat as other BoNT serotypes. Thus, the development of countermeasures for all seven serotypes is a high priority of the National Institute for Allergy and Infectious Diseases (NIAID) and the Department of Health and Human Services (17).

The only treatment for botulism is antitoxin. As a result, the Public Health Emergency Medical Countermeasure Enterprise (PHEMCE) has a requirement for polyclonal BoNT antitoxin for the National Stockpile for intentional botulism (17). The current treatment for adult
botulism is heptavalent (serotypes A-G) equine botulism antitoxin (BAT) (18). BAT is immunogenic, and hypersensitivity reactions have been reported, including serum sickness and asystole (18). BAT is a F(ab’); product with short serum half-lives (7.5 - 34.2 hours), which eliminates its use for prevention of botulism and limits its effectiveness as a treatment. Relapses of human botulism after treatment have been noted presumably due to the short half-life of BAT and poorer potency against some BoNT subtypes (19). BAT requires slow IV infusion after dilution into a total volume of 200 mL. This combined with hypersensitivity reactions makes it a challenge to administer in mass casualty scenarios. As an alternative, we have been developing serotype-specific monoclonal antibody combinations (three mAbs/serotype) that potently and neutralize BoNT by eliciting first pass clearance through the liver. Combining the three mAbs increases the potency of BoNT/C neutralization by at least three orders of magnitude over individual antibodies (Garcia-Rodriguez, in preparation). In addition, we have reported generation of three-mAb combinations to BoNT/A (20-22), BoNT/B (23), BoNT/E (24), BoNT/F (25) and BoNT/H (26) that are effective in mouse models of botulism and where the three mAb combinations are more potent than single mAbs by two to three orders of magnitude.

The products for botulism due to serotype A (NTM-1631, formerly known as XOMA 3AB) having completed a Phase 1 clinical trial with no serious adverse effects (27). Phase 1 clinical trials for three antibody combinations to treat botulism due to BoNT/B (NCT02779140) and /E (NCT03603665) are ongoing.

For the treatment and prevention of BoNT/C and BoNT/D botulism we have developed a four mAb combination, NTM-1634, that consists of an equimolar co-formulated mixture of four fully human IgG1 monoclonal antibodies (mAbs) all with the same constant regions, referred to as XCD-a, XCD-b, XCD-c, and XCD-d. These mAbs bind non-overlapping epitopes on BoNT C and D and their mosaic toxins with high affinity and which neutralizes each of these BoNTs in the mouse neutralization assay. Here we report the results of a Phase 1, single center, placebo-controlled, double blind, randomized, dose-escalation study whose purpose was to evaluate the safety and pharmacokinetic properties of NTM-1634.
MATERIALS AND METHODS

This was a Phase 1, single-center, placebo-controlled, double-blind dose escalation study to evaluate the safety, pharmacokinetic characteristics, and immunogenicity of NTM-1634 in healthy adults. This first-in-human study consisted of three cohorts (A: 0.33 mg/kg; B: 0.66 mg/kg; C: 1 mg/kg) of eight subjects each. Each subject received a single IV infusion of NTM-1634 or placebo administered over one hour. The placebo was normal saline.

NTM-1634

NTM-1634 consists of four human single chain Fv (scFv) mAbs isolated from yeast display libraries constructed from the antibody variable region genes of humans immunized with pentavalent botulinum toxoid (Garcia Rodriquez et al, in preparation). The heavy and light chain variable region genes of the scFv were inserted into vectors containing the human kappa and IgG1 constant region genes to create human IgG1/kappa mAbs and four separate stable CHO-K1 cell lines established. The IgG1 isotype was selected based on studies showing the importance of Fc receptor engagement for the synergy in potency seen when the mAbs were combined (JDM, unpublished data). Each mAb (XCD-a, XCD-b, XCD-c, and XCD-d) was expressed and purified individually using Protein A and ion-exchange chromatography and then combined in an equimolar amount to create NTM-1634.

Subjects

The study enrolled a total of 24 healthy adult volunteers. Subjects in all cohorts participated in the study for approximately 21 weeks, including a 4-week screening period, a 3-day inpatient stay and approximately 17-week follow-up study after drug administration. Eligible research participants were healthy male or healthy, non-pregnant, non-lactating females between the ages of 18 and 45 years old. Subjects had a body mass index between 18.5 and 30 kg/m², a negative illicit drug screen, and adequate venous access for the infusion. Subjects were considered study ineligible if they had a history of a chronic medical condition that would interfere with the accurate assessment of the objectives of the study, a history of severe allergic reaction to any type of medications, bee stings, food or environmental factor or reaction to immunoglobulins, positive serology for HIV, HBsAg or HCV antibodies, were pregnant or breastfeeding, were previously exposed to BoNT and active drug or have alcohol dependence. Research subjects who had received any monoclonal antibody in the past or any antibody or blood products, treatment with another investigational drug within 28 days of dosing, donated blood within 56
days of enrollment, or use of H1 antihistamines or beta-blockers within 5 days of dosing were also excluded. Additional exclusion criteria included a marked baseline prolongation of QT/QTc interval, clinically significant electrocardiogram, systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg, resting heart rate <50 or >100 beats per minute or oral temperature ≥38°C.

**Study Design**

Subjects were randomized into three cohorts of eight subjects and admitted to the study unit on Day -1. Infusion occurred on Day 1 and subjects remained at the clinic until discharge on Day 2 (25 hrs after the infusion had ended). Follow-up visits occurred on Days 3, 4, 8, 15, 29, 43, 57, 91, and 121. Safety parameters included physical examination, 12-lead ECG, vital signs, and clinical laboratory values. Pharmacokinetic samples were collected pre-dose, at the end of infusion, 1, 3, 7, 23, 47, and 71 hours post end of infusion as well as on Days 8, 15, 29, 43, 57, 91 and 121 for all cohorts. Dose escalation did not occur until safety data from at least 7 subjects per cohort through Day 8 were reviewed by the Safety Review Committee comprised of the principal investigator (D.M.S.), Ology Bioservices Medical Representative (C.N.K.), and the NIAID Division of Microbiology and Infectious Diseases Medical Monitor.

For each dosing cohort, the first two subjects were randomized 1:1 to active and placebo so that one of the first two subjects receives active treatment and the other control. The treatment assignment of the remaining six subjects in each cohort was 3:1, active:placebo. The randomization list was generated by the unblinded study biostatistician and transferred to the unblinded study pharmacist prior to the start of the study. The study staff participating in the administration of the study product and assessment of subjects were not aware of the contents of the IV bag. Drug and placebo appeared identical.

The study drug was manufactured by XOMA Corporation (Berkeley, CA) and filled by Althea Technologies (San Diego, CA). Assays developed for NTM-1643 characterization and release were qualified using biological testing including a mAb-specific ELISA binding assay which used recombinant BoNT/C protein domains (ref. (22, 28) and Manzanarez et al., in preparation), developed and qualified by Ology Bioservices, and an in vivo quadruple antibody protection assay in mice, developed and qualified in collaboration with SRI International (Menlo Park, CA). The investigational product was formulated as a 5 mg/mL, clear colorless, sterile aqueous solution in a pH 6.0 buffered vehicle without any preservatives. The drug product was
supplied in 2 mL, pyrogen-free, Type 1 glass vials. Placebo was a sterile, nonpyrogenic, isotonic solution of 0.9% sodium chloride injection, USP grade and water for injection. The placebo did not contain preservatives, bacteriostatic, antimicrobial agents or added buffer. Normal saline was used to dilute the NTM-1634 for IV infusion and were supplied in single-dose plastic containers.

**Safety Analyses**

The safety endpoints were the occurrence of serious adverse events (SAEs) following administration of NTM-1634 to the final follow-up visit, the occurrence of adverse events (AEs) from administration of NTM-1634 to Day 57, the occurrence of changes from baseline in physical examination, vital signs, and clinical safety laboratory values following administration of NTM-1634 to the final follow-up visit, and the occurrence of changes from baseline in ECG parameters post administration of NTM-1634 on Day 1 (day of infusion). Immunogenicity was assessed by determining the presence of human anti-human antibodies.

**Pharmacokinetic Analyses**

Four immunoassays were developed to measure the concentrations of XCD-a, XCD-b, XCD-c, and XCD-d in serum samples. A bridging electrochemiluminescence (ECL) assay was used (22, 27, 28). Biotinylated (b-) and ruthenylated (ru-) BoNT/C domains were used in the assay as the capturing and detecting reagents, respectively. The assay uses the bivalent binding capability of the antibodies to form a bridging complex with biotinylated-domain and ruthenylated-domain to generate ECL signals for the measurement of the target antibody concentration in serum. Six assays were developed using the same format for each antibody assay. ECL signals generated from captured immune complexes formed when one arm of the mAb bound to a biotinylated domain and the other arm to the ruthenylated domain were detected by a Meso-Scale Discovery (MSD) SECTOR Imager 6000 and reported in ECL units. Calibration standards and quality control samples were prepared by spiking known amounts of each mAb into human serum. With the minimum required dilution of 1:10, the lower limit of quantitation (LLOQ) was 10 ng/mL with a quantifiable range of 10 – 2000 ng/mL.

Immunogenicity assays were developed and validated to detect anti-XCD-a, XCD-b, XCD-c, and XCD-d antibodies in human serum. Bridging ECL assays used labeled mAbs for the capture and detection of anti-drug antibodies (ADA) in serum. A mixture of biotinylated- and ru- XCD-a, XCD-b, XCD-c, and XCD-d mAbs were incubated with human serum and ECL signals were...
Samples that generated a signal above the screening cut point (SCP) were considered positive and confirmed with the use of a competitive assay in which the serum samples were incubated with and without nonlabelled mAbs. Samples with ECL signals above the confirmation cut point were considered to be positive and further analyzed for titers.

**Statistical Analyses**

Pharmacokinetic parameters for each of the four monoclonal antibodies of NTM-1634 (XCD-a, XCD-b, XCD-c, and XCD-d) were calculated from the serum concentration-time data using non-compartmental techniques (Phoenix-WinNonlin Version 6.3.0, Pharsight Corporation, St. Louis, MO) and actual sampling times based on the start of the infusion. The following PK parameters were reported: area under the curve concentration-time curve (AUC) to the last concentration above the lower limit of quantitation (AUC$_{0-t}$), maximum observed concentration (C$_{\text{max}}$), time that C$_{\text{max}}$ was observed (T$_{\text{max}}$), terminal elimination rate constant ($\lambda_z$), AUC from time 0 extrapolated to infinity (AUC$_{0-\infty}$), terminal elimination half-life (t$_{1/2}$), total clearance (CL), and volume of distribution (V$_z$). Summary of T$_{\text{max}}$ included number of non-missing observations (n), median, minimum, and maximum only. For all other PK parameters and concentrations, n, mean, standard deviation (SD), median, minimum, and maximum values were presented. In addition, geometric mean (GeoMean) and geometric coefficient of variation (GeoCV%) were presented for serum concentrations, C$_{\text{max}}$, AUC$_{0-t}$, AUC$_{0-\infty}$, t$_{1/2}$, CL, and V$_z$.

The overall incidence of treatment-emergent AEs (TEAEs) (number and percentage of subjects) were summarized by dose cohort, and overall for categories of degree of severity, SAEs, causally related TEAEs and SAEs by causality. The TEAEs were summarized and tabulated at both the subject (number [%] of subjects) and event (number of events) level for each dose cohort and overall. Treatment-emergent laboratory abnormalities by toxicity grades are summarized for each cohort and overall. Observed values and change from baseline of continuous ECG measurements (heart rate, QRS duration (ms), PR interval (ms), QT interval (ms), QTcF interval (ms)) are summarized by cohort and overall. The overall ECG interpretation is summarized by “Normal”, “Abnormal, not clinically significant,” and “Abnormal, clinically significant.”

**RESULTS**

**Antibody Characteristics**
NTM-1634 consists of four human mAbs isolated from yeast display libraries constructed from the antibody variable region genes of humans immunized with pentavalent botulinum toxoid (Garcia Rodriquez et al, in preparation). Each mAb bound a non-overlapping BoNT epitope with high affinity (Table 1). Three of the mAbs bind all four BoNTs with a $K_D < 10^{-9}$ M while mAb XCD-b only binds BoNT/C and BoNT D/C. A combination of the four parental mAbs from which mAbs XCD-a, XCD-b, XCD-c, and XCD-d were derived had an effective dose that protected 50% of mice challenged with 40,000 LD50s (ED$_{50}$) of BoNT/C of 5.0 $\mu$g/mouse, of BoNT C/D of 7.5 $\mu$g/mouse and of BoNT D/C of 7.5 $\mu$g/mouse (Garcia Rodriquez et al, in preparation).

Subject Demographic Characteristics

Twenty-four research participants were planned for these studies (Figure 1). Twenty-five were enrolled, randomized and included in the study. Randomized participants were administered either the NTM-1634 or placebo intravenously. No deaths or AEs leading to study discontinuation were reported. A summary of subject disposition is presented in Table 2. Two subjects from Cohort B (0.66 mg/kg) were lost to follow-up. One subject who received placebo was lost to follow-up after Day 2 and the other was lost to follow-up after day 57. The subject that was lost to follow-up after day 2 was replaced in Cohort B at the time of Cohort C (Figure 1). A total of eighteen research participants, six per cohort, received NTM-1634 (0.33 mg/kg; 0.66 mg/kg or 1 mg/kg) and two research participants per cohort, six in total, received placebo. The doses were chosen to: 1) deliver a dose of BoNT antitoxin that exceeded the current dose of BAT; 2) achieve a serum concentration greater than the mAb $K_D$ for BoNT and 3) allow measurement of the concentration of each component mAb. For example, a 70 kg subject, these doses would provide a total neutralizing capacity of $1.85 \times 10^8$, $3.70 \times 10^8$ and $5.6 \times 10^8$ mouse LD50s of BoNT/C based on the preclinical data described above. This compares to a single dose BoNT/C neutralizing capacity for BAT of $3.0 \times 10^7$ mouse LD50s.

The study participants ranged in age from 19-44 years of age. The majority of study subjects were white (17, 68%), of Hispanic or Latino background (13, 52%), and female (19, 74%). The demographic characteristics were similar across the treatment groups, except Cohort C where all
the participants who received active compound were female. The demographic characteristics are summarized in Table 3.

**Safety Profile**

No deaths or AEs leading to study discontinuation were reported. Overall, two non-drug related SAEs were reported. One subject in Cohort B (0.66 mg/kg) had an SAE of exacerbation of schizophrenia and one subject in Cohort C (1 mg/kg) had and SAE of wrist fracture. TEAEs were reported in 19 of 25 subjects (76%) with a total of 69 TEAEs reported over the course of the study (Table 4). Cohort A (0.33 mg/kg) had four of 6 (66.7%) reporting 18 TEAEs. Cohort B (0.66 mg/kg) had five of six (83.3%) reporting 21 TEAEs. Cohort C (1 mg/kg) had six of six (100%) subjects reporting 20 TEAEs. The placebo group has four of seven (57.1%) subjects reporting 10 TEAEs. The most frequently reported TEAE was blood creatine phosphokinase increase which occurred in 5 (20%) of the subjects. The next most frequently reported TEAEs (reported in two or more subjects overall) were hemoglobin decreases, hematocrit decreases, viral upper respiratory tract infection, white blood cell count decreases, blood calcium decreases, blood potassium increases, blood sodium increases, cough, hypernatremia, myalgia, neutrophil count decreased, and proteinuria. There were no clinically significant abnormal ECGs nor were there clinically meaningful trends identified comparing baseline vital and ECG values to subsequent timepoints. All TEAEs were considered by the investigator to be “not related” to study drug. ADA results were negative for all participants with the exception of one subject who had a positive ADA result with at titer of 1.2 ng/mL for mAb XCD-b pre-dose on Day 1. All post-dose ADA results were negative for this participant. The significance of this finding is unknown, but it may be due to the setting of the cut-point for ADA detection. The subject did not report any past medical history and experienced no AEs during the study.

**Pharmacokinetic Analysis**

A summary of the pharmacokinetic data is presented in Table 5. Peak concentrations for each of the antibodies regardless of dose, was generally observed one to two hours after the one-hour infusion. The peak concentrations of each of the four antibodies were also similar. After the peak, the concentrations of all four antibodies declined in a log-linear fashion with a distinct distribution and terminal elimination phase (Figure 2). Serum concentrations for XCD-a were quantifiable up to Day 29 in 5 of 6 subjects, Day 43 in 3 of 6 subjects and Day 57 in 1 of 6 subjects who received NTM-1634 0.33, 0.66 and 1 mg/kg, respectively. Serum concentrations of
XCD-b were quantifiable up to Day 91 in 2 of 6 subject, Day 121 in 2 of 6 subjects and Day 121 in 3 of 6 subjects who received NTM-1634 0.33, 0.66 and 1 mg/kg, respectively. Serum concentrations of XCD-c were quantifiable up to Day 91 in 1 of 6 subjects, Day 121 in 1 of 6 subjects and Day 121 in 1 of 6 subjects who received NTM-1634 0.33, 0.66 and 1 mg/kg, respectively. Serum concentrations of XCD-d were quantifiable up to day 57 in 2 of 6 subjects, Day 91 in 2 of 6 subjects, and Day 91 in 1 of 6 subjects who received NTM-1634 0.33, 0.66 and 1 mg/kg, respectively.

The dose-adjusted Cmax for all four antibodies were similar across all three dose cohorts. In addition, the area under the concentration-time curve from 0 to infinity (AUC(0-∞)) for XCD-b and XCD-c were not different across all three dose cohorts, indicating dose proportionality over the 3-fold dose range. The area under the concentration-time curve from 0 to infinity (AUC(0-∞)) for XCD-a and XCD-d were lower than XCD-b and XCD-c, with XCD-a having the lowest AUC(0-∞) across all three dose cohorts. Each of the four mAb had long half lives varying from 10.2 to 24 days. XCD-b and XCD-c had longer t1/2 than either XCD-a or XCD-d with XCD-a having the shortest t1/2 at 263 hr. The basis for the differences in half-lives between mAbs is unclear, since the mAbs have similar pl’s and have the same antibody constant regions. The overall pharmacokinetics of each of the four individual antibodies were roughly similar to each other with the rank order of XCD-b having the longest half-life and AUC followed by XCD-c, then XCD-d and then XCD-a.

DISCUSSION

BoNTs are classified by the Centers of Disease Control and Prevention (CDC) as one of the highest-risk threat agents for bioterrorism, due to their extreme potency and lethality, ease of production and transport, and need for prolonged intensive care (3). Thus, the development of countermeasures for all seven serotypes, including BoNT/C and BoNT/D, is a high research priority. Both Iraq and the former Soviet Union produced BoNT for use as weapons and at least three additional countries (Iran, North Korea and Syria) have developed or are believed to be developing BoNT as instruments of mass destruction (3, 29, 30). Iraq produced 19,000 L of concentrated BoNT, more than any other biothreat agent, of which 10,000 L were weaponized in missile warheads or bombs. The 19,000 L represent an amount of toxin capable of killing the...
The world’s population three times over. The Japanese cult Aum Shinrikyo attempted to use BoNT for bioterrorism by dispersing toxin aerosols at multiple sites in Tokyo (3).

Botulism causes significant morbidity and mortality and exposure of even a small number of civilians would paralyze the health care delivery system of any metropolitan area. Treatment of botulism requires prolonged hospitalization in an ICU and mechanical ventilation for up to six weeks. There are no current logistically feasible prophylactic agents available in the US as medical countermeasures. Antitoxin is the only effective treatment for botulism and has been shown to reduce duration of hospitalization, duration of mechanical ventilation and cost of hospitalization (31). The current treatment for adult botulism is heptavalent (serotypes A-G) equine botulism antitoxin (BAT®) (18). BAT is immunogenic, and hypersensitivity reactions have been reported, including serum sickness and asystole (18, 32).

As an alternative and potentially safer and more effective product, we have been developing serotype-specific human or humanized mAb combinations that stoichiometrically neutralize BoNT by eliciting first pass clearance through the liver (20, 27). In addition, these antibodies are being used in diagnostic tests to identify the specific serotype of botulinum neurotoxin that was causing the symptoms and provide guidance for treatment (33, 34). This study was the first-in-human assessment of NTM-1634, a four mAb combination which potently neutralizes BoNT/C, C/D, D/C and D in rodents. The results demonstrate that single escalating doses of NTM-1634 administered intravenously into healthy subjects were well-tolerated and safe. In addition, these doses also demonstrated acceptable immunogenicity profiles with little ADA detected over the dose ranges and duration of the study. No dose-related SAEs were observed and the AEs that were observed between the three cohorts and placebo group were similar in frequency, character, and severity. These results indicate that NTM-1634 may be a safer product than the equine polyclonal antibody BAT (18).

Previous studies with NTM-1634 have demonstrated that the effectiveness of NTM-1634 in neutralizing BoNT C/D is dependent on the presence of all four antibodies (Garcia Rodriquez et al, in preparation). This is consistent with observations for other anti-BoNT antibody combinations in development (20, 24, 25). It therefore follows that the duration of effectiveness of the combination will be determined by the monoclonal antibody that falls below the minimal effective levels most rapidly. The terminal t1/2 was similar across the range of doses for each antibody. At all doses given, however, XCD-a was cleared more rapidly than the other three

12
with XCD-d being the next most rapidly cleared. However, all antibodies were detectable for a minimum of 4 weeks with the most rapidly clearing mAb, XCD-a, having a serum half-life of 11 days. This contrasts with BAT, which is a F(\(\text{ab}')2\) product with short serum half-lives for BoNT/C and BoNT/D (30 and 7.5 hours, respectively). The long half-life of NTM-1634 may reduce the likelihood of a relapse of botulism that has been reported with BAT (19).

While we have not directly compared the potency of NTM-1634 to BAT for BoNT/C or BoNT/D, the potency of a BoNT/A mAb combination (NTM-1631) under development is 400- to 600-fold more potent than BAT in mouse protection studies with BoNT/A1 and a three mAb combination to BoNT/F more than 150-450 times more potent than BAT (25, 27) on a weight basis. The lowest dose of NTM-1634 studied (0.33 mg/kg), would deliver a total BoNT/C neutralizing capacity of \(1.85 \times 10^8\) mouse LD\(_{50}\)s of BoNT/C based on the preclinical data described above. This compares to a single dose BoNT/C neutralizing capacity for BAT of \(3.0 \times 10^7\) mouse LD50s, or more than 6-fold more. This greater potency is expected since polyclonal antisera rarely have more than 1% of the total IgG directed against the target antigen, compared to ~100% for recombinant IgG. The combined safety, potency and long half-life of NTM-1634 makes it possible for potential intramuscular or subcutaneous administration after exposure and prior to the development of symptoms which is not possible with BAT. These features would make NTM-1634 simpler to administer in a mass casualty scenario. The high potency and long half-life would also permit administration of NTM-1634 for prevention of botulism as an alternative to vaccination. This is important since there is no longer a vaccine available to prevent types C and D botulism.

In summary, this study provides an early evaluation of the pharmacokinetic and safety profile of NTM-1634. The demonstration of highly potent protection in animal models, and the safety profile and long half-life of over one month in humans demonstrates the potential utility of NTM-1634 for treatment of botulism due to serotypes C and D. Thus, further clinical development of NTM-1634 for the treatment and prevention of BoNT intoxication due to serotypes C and D is warranted.

Funding: This work was supported by the National Institute of Allergy and Infectious Diseases (NIAID), under contract number HHSN27220160009C to XOMA Corp., PI: M.T.T.
Table 1: Characteristics of the human monoclonal antibodies that comprise NTM-1634. NB = no detectable binding at a BoNT concentration of 1 μM.

| Antibody name | No. of amino acids | Molecular weight (kDa) | Kᵦ values (x 10⁻¹² M) |
|---------------|--------------------|------------------------|------------------------|
| XCD-a         | 1332               | 145,899                | 1.10 2.43 15.34 16.93  |
| XCD-b         | 1338               | 146,476                | 35.79 NB 0.80 NB       |
| XCD-c         | 1338               | 146,463                | 11.79 37.81 83.95 96.20|
| XCD-d         | 1334               | 145,788                | 130.37 37.90 22.44 11.63|
Table 2: Summary of Subject Disposition (all Subjects Randomized)

| Disposition                                      | 0.33 mg/Kg (n=6) | 0.66 mg/kg (n=6) | 1 mg/kg (n=6) | Placebo (n=7) | Total (n=25) |
|-------------------------------------------------|------------------|------------------|--------------|--------------|--------------|
| All subjects randomized                         | 6 (100%)         | 6 (100%)         | 6 (100%)     | 7 (100%)     | 25           |
| Subjects who were dosed (Safety Population), n (%) | 6 (100%)         | 6 (100%)         | 6 (100%)     | 7 (100%)     | 25           |
| Subject who completed study, n (%)              | 6 (100%)         | 5 (83%)          | 6 (100%)     | 6 (85.7%)    | 23 (92%)     |
| Subjects who discontinued early from study, n (%) | 0                | 1 (16.7%)        | 0            | 1 (14.3%)    | 2 (8%)       |
| Lost to follow-up, n (%)                        | 0                | 1 (16.7%)        | 0            | 1 (14.3%)    | 2 (8%)       |
Table 3: Summary of Subject Demographics

| Parameter             | Treatment                  | Total          |
|-----------------------|----------------------------|----------------|
|                      | 0.33 mg/kg (N = 6)         | 0.66 mg/kg (N = 6) | 1 mg/kg (N = 6) | Placebo (N = 7) | (N = 25) |
| Age (Years)           | Mean 33.7                  | 30.0           | 34.7           | 32.9           | 32.8     |
|                       | SD 7.53                    | 7.95           | 9.29           | 6.82           | 7.59     |
| Minimum               | 25                         | 19             | 21             | 23             | 19       |
| Maximum               | 42                         | 42             | 44             | 40             | 44       |
| Sex (n [%])           | Male 2 (33.3%)              | 2 (33.3%)      | 0              | 2 (28.6%)      | 6 (24.0%)|
|                       | Female 4 (66.7%)            | 4 (66.7%)      | 6 (100.0%)     | 5 (71.4%)      | 19 (76.0%)|
| Ethnicity (n [%])     | Hispanic or Latino 4 (66.7%)| 3 (50.0%)      | 3 (50.0%)      | 3 (42.9%)      | 13 (52.0%)|
|                       | Not Hispanic or Latino 2 (33.3%)| 3 (50.0%)   | 3 (50.0%)      | 4 (57.1%)      | 12 (48.0%)|
| Race (n [%])          | Black or African American 0| 1 (16.7%)      | 3 (50.0%)      | 2 (28.6%)      | 6 (24.0%)|
|                       | White 5 (83.3%)             | 5 (83.3%)      | 2 (33.3%)      | 5 (71.4%)      | 17 (68.0%)|
|                       | Other 1 (16.7%)             | 0              | 1 (16.7%)      | 0              | 2 (8.0%) |
Table 4: Summary of Treatment-Emergent Adverse Events (TEAE) Reported

| TEAE                          | 0.33 mg/kg (N = 6) | 0.66 mg/kg (N = 6) | 1 mg/kg (N = 6) | All Active (N = 18) | Placebo (N = 7) | Total (N = 25) |
|-------------------------------|-------------------|-------------------|----------------|-------------------|----------------|---------------|
| Blood creatine phosphokinase increased | 1 (16.7 %) | 1 (16.7 %) | 3 (50.0 %) | 5 (27.8 %) | 0 (0 %) | 5 (20.0 %) |
| Hemoglobin decreased          | 1 (16.7 %) | 0 (0 %) | 2 (33.3 %) | 3 (16.7 %) | 3 (14.3 %) | 4 (16.0 %) |
| Hematuria                     | 0 (0 %) | 0 (0 %) | 1 (16.7 %) | 1 (5.6 %) | 1 (8.6 %) | 3 (12.0 %) |
| Hematocrit decreased          | 1 (16.7 %) | 1 (16.7 %) | 2 (33.3 %) | 3 (16.7 %) | 3 (12.0 %) | 3 (12.0 %) |
| Viral upper respiratory tract infection | 2 (33.3 %) | 1 (16.7 %) | 0 (0 %) | 3 (16.7 %) | 0 (0 %) | 3 (12.0 %) |
| White blood cell count decreased | 1 (16.7 %) | 1 (16.7 %) | 1 (16.7 %) | 3 (16.7 %) | 0 (0 %) | 3 (12.0 %) |
| Blood calcium decreased       | 1 (16.7 %) | 0 (0 %) | 0 (0 %) | 1 (5.6 %) | 1 (14.3 %) | 2 (8.0 %) |
| Blood potassium increased     | 2 (33.3 %) | 0 (0 %) | 0 (0 %) | 2 (11.1 %) | 0 (0 %) | 2 (8.0 %) |
| Blood sodium increased        | 1 (16.7 %) | 0 (0 %) | 0 (0 %) | 1 (5.6 %) | 1 (14.3 %) | 2 (8.0 %) |
| Cough                         | 0 (0 %) | 1 (16.7 %) | 1 (16.7 %) | 2 (11.1 %) | 0 (0 %) | 2 (8.0 %) |
| Hypernatremia                 | 0 (0 %) | 2 (33.3 %) | 0 (0 %) | 2 (11.1 %) | 0 (0 %) | 2 (8.0 %) |
| Myalgia                       | 1 (16.7 %) | 0 (0 %) | 1 (16.7 %) | 2 (11.1 %) | 0 (0 %) | 2 (8.0 %) |
|                  | 0  | 0  | 1  | 1  | 1  | 2  | 2  | 0  | 0  | 2  | 2 |
|------------------|----|----|----|----|----|----|----|----|----|----|----|
| **Neutrophil**   |    |    |    |    |    |    |    |    |    |    |    |
| count decreased  |    |    | 1  | 1  |    |    |    |    |    |    |    |
|                  |    |    |    |    |    |    |    | 2  |    |    |    |
| **Proteinuria**  |    |    |    |    |    |    |    |    |    | 2  |    |
|                  |    |    |    |    |    |    |    |    |    |    | 2  |
| **Total**        | 11 | 7  | 12 |    | 8  | 38 |
| **TEAE**         |    |    |    |    |    |    |    |    |    |    |    |
| mAb | Cohort (dose) | C<sub>max</sub> (ng/mL)/GEOCV% | Tmax (hr) (min/max) | AUC<sub>0-t</sub> (µg·h/mL)/GEOCV% | AUC<sub>0-∞</sub> (µg·h/mL)/GEOCV% | t<sub>1/2</sub> (days)/GEOCV% |
|-----|---------------|---------------------------------|---------------------|----------------------------------|----------------------------------|-----------------------------|
| XCD-a | A (0.33mg/kg) | 1762/11.2 | 1.00 (1.00, 2.00) | 207/16.4 | 2745/8.8 | 10.9/11.4 |
|      | B (0.66mg/kg) | 3805/19.3 | 1.50 (1.00, 2.00) | 525/28.1 | 635/11.1 | 10.6/17.5 |
|      | C (1 mg/kg)  | 6347/14.0 | 2.00 (1.00, 4.02) | 961/14.3 | 1001/14.2 | 10.2/9.3 |
| XCD-b | A (0.33mg/kg) | 1969/10.7 | 2.00 (1.00, 2.08) | 591/28.9 | 739/17.5 | 24.3/16.6 |
|      | B (0.66mg/kg) | 4296/21.3 | 1.00 (1.00, 2.00) | 1530/21.4 | 1674/21.3 | 24/28.4 |
|      | C (1 mg/kg)  | 7226/10.2 | 2.00 (1.00, 4.00) | 2508/14.1 | 2612/14.3 | 23.4/15.0 |
| XCD-c | A (0.33mg/kg) | 1877/10.0 | 1.00 (1.00, 2.00) | 542/22.6 | 624/22.3 | 20.2/18.1 |
|      | B (0.66mg/kg) | 4149/23.6 | 1.00 (1.00, 2.00) | 1339/23.8 | 1492/20.7 | 22.3/27.5 |
|      | C (1 mg/kg)  | 7151/12.0 | 2.00 (1.00, 4.02) | 2193/15.9 | 2281/16.3 | 21.4/14.9 |
| XCD-d | A (0.33mg/kg) | 1830/14.0 | 2.00 (2.00, 4.00) | 403/15.6 | 463/17.8 | 15.9/19.1 |
|      | B (0.66mg/kg) | 4080/17.7 | 1.00 (1.00, 4.00) | 990/25.4 | 1083/25.2 | 17.8/35.0 |
|      | C (1 mg/kg)  | 6829/10.5 | 1.00 (1.00, 4.00) | 1553/15.6 | 1662/13.3 | 15.8/16.4 |
Figure 1: CONSORT diagram of clinical study design.

Assessed for eligibility (n=50)

Randomized (n=25)
- Not meeting inclusion criteria (n=25)
- Declined to participate (n=0)
- Other reasons (n=0)

Allocated to intervention (n=8)
- Received allocated intervention (n=8)
- Did not receive allocated intervention (n=0)

Lost to follow-up (n=0)

Analyzed (n=8)
- Excluded from analysis (n=0)

Allocated to intervention (n=9)
- Received allocated intervention (n=9)
- Did not receive allocated intervention (n=0)

Lost to follow-up (n=2)

Discontinued intervention (n=0)

Analyzed (n=7)
- Excluded from analysis (n=0)

Allocated to intervention (n=8)
- Received allocated intervention (n=8)
- Did not receive allocated intervention (n=0)

Lost to follow-up (n=0)

Discontinued intervention (n=0)

Analyzed (n=8)
- Excluded from analysis (n=0)
Figure 2: Serum concentration over time mean of 6 subjects (error bars indicate standard deviation) at three dosing levels 0.33mg/kg (blue), 0.66 mg/kg (red), 1 mg/kg (green). Curves are least square best-fit, for all curves, $R^2 \geq 0.9$). Curves are least squared fits and do not converge at zero antibody concentration in log scale as a result of the lower limit of quantitation of the assay being 10ng/mL, and the lack of sampling after 150 days. A. mAb XCD-a, B. XCD-b, C. XCD-c, D. XCD-d.
REFERENCES

1. Hatheway CL. 1995. Botulism: the present status of the disease. Curr Top Microbiol Immunol 195:55-75.
2. Anonymous. 1998. Centers for Disease Control and Prevention: Botulism in the United States, 1899-1996. Handbook for Epidemiologists, Clinicians, and Laboratory Workers, Atlanta, GA.
3. Arnon SS, Schechter R, Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Fine AD, Hauer J, Layton M, Lillibridge S, Osterholm MT, O'Toole T, Parker G, Perl TM, Russell PK, Swerdlow DL, Tonat K. 2001. Botulinum toxin as a biological weapon: medical and public health management. JAMA 285:1059-70.
4. Lacy DB, Stevens RC. 1999. Sequence homology and structural analysis of the clostridial neurotoxins. J Mol Biol 291:1091-104.
5. Barash JR, Arnon SS. 2014. A novel strain of Clostridium botulinum that produces type B and type H botulinum toxins. J Infect Dis 209:183-91.
6. Hill KK, Smith TJ. 2013. Genetic diversity within Clostridium botulinum serotypes, botulinum neurotoxin gene clusters and toxin subtypes, p 1-20. In Rummel A, Binz, T. (ed), Botulinum Neurotoxins, vol 364. Springer, Hanover, Germany.
7. Webb RP, Smith TJ, Wright PM, Montgomery VA, Meagher MM, Smith LA. 2007. Protection with recombinant Clostridium botulinum C1 and D binding domain subunit (Hc) vaccines against C and D neurotoxins. Vaccine 25:4273-82.
8. Moriishi K, Koura M, Fuji N, Fujinaga Y, Inoue K, Syuto B, Oguma K. 1996. Molecular cloning of the gene encoding the mosaic neurotoxin, composed of parts of botulinum neurotoxin types C1 and D, and PCR detection of this gene from Clostridium botulinum type C organisms. Applied and environmental microbiology 62:662-667.
9. Borland E, Morison C, Smith G. 1977. Avian botulism and the high prevalence of Clostridium botulinum in the Norflok Broads. The Veterinary Record 100:106-109.
10. Takeda M, Tsukamoto K, Kohda T, Matsui M, Mukamoto M, Kozaki S. 2005. Characterization of the neurotoxin produced by isolates associated with avian botulism. Avian Dis 49:376-81.
11. Abbitt B, Murphy M, Ray A, Reagor J, Eugster A, Gayle L, Whitford H, Sutherland R, Fiske R, Pusok J. 1984. Catastrophic death losses in a dairy herd attributed to type D botulism. Journal of the American Veterinary Medical Association 185:798-801.
12. Relun A, Dorso L, Douart A, Chartier C, Guat2eo R, Mazuet C, Popoff M, Assie S. 2017. A large outbreak of bovine botulism possibly linked to a massive contamination of grass silage by type D/C Clostridium botulinum spores on a farm with dairy and poultry operations. Epidemiology & Infection 145:3477-3485.
13. Middlebrook JL, Franz, D. R. 1997. Botulinum Toxins, Medical Aspects of Chemical and Biological Warfare.643-54.
14. Oguma K, Yokota K, Hayashi S, Takeshi K, Kumagai M, Itoh N, Tachi N, Chiba S. 1990. Infant botulism due to Clostridium botulinum type C toxin. Lancet 336:1449-50.
15. Demarchi J, Mourgues C, Orio J, Prevot AR. 1958. [Existence of type D botulism in man]. Bull Acad Natl Med 142:580-2.
16. Eleopra R, Tugnoli V, Quatralle R, Rossetto O, Montecucco C. 2004. Different types of botulinum toxin in humans. Mov Disord 19 Suppl 8:S53-9.
Anonymous. 2017. 2017-2018 Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) Strategy and Implementation Plan. Available online at: https://www.phe.gov/Preparedness/mcm/phemce/Documents/2017-phemce-sip.pdf.

BARDA,

Anonymous. 2013. Cangene Corp., BAT® [Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) - (Equine)] Sterile Solution for Injection. Available online at: https://www.fda.gov/downloads/.../UCM345147.pdf. Accessed on 11 October 2017.

Fagan RP, Neil KP, Sasich R, Luquez C, Asaad H, Maslanka S, Khalil W. 2011. Initial recovery and rebound of type f intestinal colonization botulism after administration of investigational heptavalent botulinum antitoxin. Clin Infect Dis 53:e125-8.

Nowakowski A, Wang C, Powers DB, Amersdorfer P, Smith TJ, Montgomery VA, Sheridan R, Blake R, Smith LA, Marks JD. 2002. Potent neutralization of botulinum neurotoxin by recombinant oligoclonal antibody. Proc Natl Acad Sci U S A 99:11346-50.

Lou J, Wen W, Conrad F, Meng Q, Dong J, Sun Z, Garcia-Rodriguez C, Farr-Jones S, Cheng LW, Henderson TD, Brown JL, Smith TJ, Smith LA, Cormier A, Marks JD. 2018. A Single Tri-Epitopic Antibody Virtually Recapitulates the Potency of a Combination of Three Monoclonal Antibodies in Neutralization of Botulinum Neurotoxin Serotype A. Toxins (Basel) 10.

Meng Q, Li M, Silberg MA, Conrad F, Bettencourt J, To R, Huang C, Ma J, Meyer K, Shimizu R, Cao L, Tomic MT, Marks JD. 2012. Domain-based assays of individual antibody concentrations in an oligoclonal combination targeting a single protein. Anal Biochem 421:351-61.

Fan Y, Dong J, Lou J, Wen W, Conrad F, Geren IN, Garcia-Rodriguez C, Smith TJ, Smith LA, Ho M. 2015. Monoclonal antibodies that inhibit the proteolytic activity of botulinum neurotoxin serotype/B. Toxins 7:3405-3423.

Garcia-Rodriguez C, Razai A, Geren IN, Lou J, Conrad F, Wen WH, Farr-Jones S, Smith TJ, Brown JL, Skerry JC, Smith LA, Marks JD. 2018. A Three Monoclonal Antibody Combination Potently Neutralizes Multiple Botulinum Neurotoxin Serotype E Subtypes. Toxins (Basel) 10.

Fan Y, Garcia-Rodriguez C, Lou J, Wen W, Conrad F, Zhai W, Smith TJ, Smith LA, Marks JD. 2017. A three monoclonal antibody combination potently neutralizes multiple botulinum neurotoxin serotype F subtypes. PLoS One 12:e0174187.

Fan Y, Barash JR, Lou J, Conrad F, Marks JD, Arnon SS. 2016. Immunological Characterization and Neutralizing Ability of Monoclonal Antibodies Directed Against Botulinum Neurotoxin Type H. J Infect Dis 213:1606-14.

Nayak SU, Griffiss JM, McKenzie R, Fuchs EJ, Jurao RA, An AT, Ahene A, Tomic M, Hendrix CW, Zenilman JM. 2014. Safety and pharmacokinetics of XOMA 3AB, a novel mixture of three monoclonal antibodies against botulinum toxin A. Antimicrob Agents Chemother 58:5047-53.

Meng Q, Garcia-Rodriguez C, Manzanarez G, Silberg MA, Conrad F, Bettencourt J, Pan X, Breece T, To R, Li M, Lee D, Thorner L, Tomic MT, Marks JD. 2012. Engineered domain-based assays to identify individual antibodies in oligoclonal combinations targeting the same protein. Anal Biochem 430:141-50.

Anonymous. 1995. United Nations Security Council. Tenth report of the executive committee of the special commision established by the secretary-general pursuant to
paragraph 9(b)(I) of security council resolution 687 (1991), and paragraph 3 of resolution 699 (1991) on the activities of the Special Commission. United Nations Security Council, New York, NY.

30. Zilinskas RA. 1997. Iraq's biological weapons: the past as future? Jama 278:418-424.
31. Arnon SS. 1993. Clinical trial of human botulism immune globulin., p 477-482. In DasGupta BR (ed), Botulinum and Tetanus Neurotoxins: Neurotransmission and Biomedical Aspects. Plenum Press, New York.
32. Richardson JS, Parrera GS, Astacio H, Sahota H, Anderson DM, Hall C, Babinchak T. 2019. Safety and Clinical Outcomes of an Equine-Derived Heptavalent Botulinum Antitoxin Treatment for Confirmed or Suspected Botulism in the United States. Clin Infect Dis doi:10.1093/cid/ciz515.
33. Wang D, Krilich J, Baudys J, Barr JR, Kalb SR. 2015. Enhanced detection of type C botulinum neurotoxin by the Endopep-MS assay through optimization of peptide substrates. Bioorganic & medicinal chemistry 23:3667-3673.
34. Kalb SR, Krilich JC, Dykes JK, Lúquez C, Maslanka SE, Barr JR. 2015. Detection of botulinum toxins A, B, E, and F in foods by Endopep-MS. Journal of agricultural and food chemistry 63:1133-1141.