FOOD AND DRUG ADMINISTRATION

Center for Biologics Evaluation and Research

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Issue:

FDA seeks the advice of the Advisory Committee on whether or not studies performed under the 21 CFR 601 Subpart H "Animal Rule" have provided reasonable evidence that Botulism Antitoxin (Equine), Heptavalent (A, B, C, D, E, F, G) has an acceptable safety profile and is likely to be effective in the patient populations for the indications being sought.

Executive summary

Cangene Corporation submitted a Biologics License Application (BLA) for Botulism Antitoxin (Equine), Heptavalent (A, B, C, D, E, F, G); proposed proprietary name "BAT", seeking approval under the 21 CFR 601.90 "Animal Rule" regulations. Per 21 CFR 601.90, these regulations pertain to the approval of "certain biological products that have been studied for their safety and efficacy in ameliorating or preventing serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substances." Cangene is seeking an indication for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin (BoNT) serotypes A, B, C, D, E, F, or G. In support of this BLA, Cangene has submitted the results of therapeutic efficacy studies in two non-human animal species (guinea pigs and rhesus macaques) challenged with lethal doses of BoNT and administered BAT or a placebo upon the development of clinical signs of botulism; the study endpoint of survival was based on a comparison between the treatment groups in the two animal species. Cangene has also submitted model development and pharmacokinetic/pharmacodynamics (PK/PD) data to demonstrate that these animal models are reasonably likely to predict clinical benefit in humans, and has performed studies to allow for the extrapolation of a human dose from the animal models. A study examining the safety of BAT in healthy human subjects was performed and concluded that the biological product was well tolerated. Finally, data from use of BAT under an investigational new drug (IND) application managed by the Centers for Disease Control and Prevention was submitted to supplement the safety database and provide additional evidence of BAT clinical benefit and potential adverse events in humans with botulism. The Agency is seeking advice whether 1) the animal efficacy studies are reasonably likely to predict a clinical benefit in humans; 2) whether the totality of the data (including use under the CDC IND) supports an acceptable risk/benefit profile for the use of BAT; and 3) whether the animal and human studies provide a sufficient basis for dosing recommendations.

Botulism

Botulism is caused by a potent neurotoxin (botulinum neurotoxin; BoNT) produced by *Clostridium botulinum* or specific strains of *C. baratii* and *C. butyricum*. Seven distinct serotypes of toxin (A-G) have been identified. Typically any one bacterial strain will produce only a single toxin. These toxins block release of the neurotransmitter acetylcholine from synaptic vesicles at the neuromuscular junction of peripheral motor nerves and autonomic cholinergic nerves. Exposure to *C. botulinum* toxins may be due to ingestion of pre-formed toxins, often from improperly canned foods, or due to wound or intestinal colonization with the organism which then produces the toxin. Iatrogenic exposures to BoNT have occurred due to overdoses of BoNT-based therapies used to treat cervical dystonia or for cosmetic purposes. Animal studies and an accidental laboratory exposure of humans have shown that BoNT inhalation can also result in botulism, although this route is inefficient. There are no available rapid tests to diagnose botulism, so presumptive clinical diagnoses are made for the purpose of early treatment. Initial signs and symptoms due to cranial nerve paralysis include diplopia, ptosis, dysarthria, and dysphagia. Autonomic symptoms such as dry mouth and blurred vision may also occur. As toxicity progresses, a symmetric descending paralysis, including diaphragmatic paralysis occurs, requiring ventilatory support. Symptoms in infants include lethargy, poor suck reflex, constipation, weak cry, and diminished muscle tone, which progresses to paralysis (Centers for Disease Control and Prevention, n.d.-a). Patients with severe toxicity experience respiratory arrest and often require weeks of mechanical ventilation.

Botulinum neurotoxin as a biological threat agent

BoNT has long been considered a potential biological warfare agent. Fox, in his 1933 treatise on the use of biologics in warfare, cited BoNT as a "single terror-inspiring example" of a toxin with capacity for use in offensive biological warfare (Fox, 1942). BoNT was one of a handful of agents considered for retaliatory use by the Allies in World War II, and research continued under the U.S. and U.S.S.R. offensive programs after the war (Dembek, Smith, & Rusnak, 2007). In the late 1980's and early 1990's, Iraq pursued a biological warfare program that included the production of large quantities of BoNT (Davis, 1999). More recently, BoNT has been considered or even exploited as a potential weapon by non-state entities such as Al Qaeda (Salama & Hansell, 2005) and the Aum Shinrikiyo cult (Stern, 1999). The most likely exposure to BoNT as an intentional release would be related to the inhalation or ingestion of the toxin. While generation of a small particle aerosol of BoNT would be technically challenging, it could lead to considerable casualties (Arnon et al., 2001). A similar amount of toxin used to contaminate food in a manner analogous to that used by the Rajneeshee cult in 1984 could result in hundreds of fatal exposures (Dembek et al., 2007).

Botulism toxin and Antitoxin Treatment

The pathophysiology of BoNT is reasonably well understood. Botulism toxins are zinc metalloproteases consisting of a light chain (Lc) and heavy chain (Hc). The 50 kDa Lc contains the metalloprotease activity, while the Hc binds neuronal gangliosides or phosphatidyl-ethanolamine and mediates translocation into the cell. The fundamental mechanism of action is BoNT Lc-mediated cleavage of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins in neurons. Loss of

SNARE proteins prevents acetylcholine-containing vesicles from fusing with the plasma membrane at neuromuscular junctions, preventing neurotransmitter release and triggering paralysis. Humans are susceptible to all seven serotypes, although most naturally occurring cases of botulism are due to serotypes A, B, and E. BoNT-mediated SNARE cleavage can persist from days to months in an intoxicated neuron (Keller, Neale, Oyler, & Adler, 1999).

The only available specific treatment for botulism is infusion of antitoxin antibodies to remove circulating toxin, preventing further neuronal compromise. BAT is a polyclonal equine $F(ab')_2$ antibody preparation, manufactured from sera of horses immunized with botulinum toxoids and toxins. All other care is supportive, including mechanical ventilation and standard intensive care in the setting of complete paralysis. Due to the persistence of BoNT effects on the acetylcholine release from nerves, early treatment with antitoxin is critical in order to halt further entry of the toxin into nerve cells. (Tacket, Shandera, Mann, Hargrett, & Blake, 1984). The mechanism of action of antitoxin antibodies is well understood, and is due to specific binding of toxin by the antigen binding portion of the antibody, preventing toxin entry into neurons and facilitating clearance via non Fc-mediated mechanisms.

Unmet Medical Need

There has been no licensed treatment available for adult botulism since Botulism Antitoxin Bivalent (Equine) Types A and B produced by Sanofi Pasteur Limited was withdrawn from distribution after expiration of the last remaining lot and the license subsequently revoked. There are no licensed vaccines for prevention of botulism. Botulism Immune Globulin Intravenous (Human) (BabyBIG) is licensed and available for treatment of infant botulism due to intestinal colonization with *C. botulinum* secreting A or B toxins. While suitable for this indication, the potency of BabyBIG is 1-4% of the nominal potency of BAT, and the product supply is limited. BabyBIG does not contain significant quantities of antibodies against toxin serotypes other than A and B.

Regulatory context

The original biologics license application for BAT, Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-(Equine) was submitted on September 20, 2012. A 6-month priority review timeline was granted because of unmet medical need. The product previously received orphan drug designation on June 29, 2011. The pathway for licensure of BAT is 21CFR 601 Subpart H, Approval of Biologic Products when Human Efficacy Studies are Not Ethical or Feasible. This is commonly referred to as the Animal Efficacy Rule or the Animal Rule, and is codified in 21 CFR 601.90.

Product attributes

BAT is a polyclonal hyperimmune product manufactured from the plasma of horses immunized with BoNT or BoNT toxoids. The equine IgG has been digested with pepsin to yield $F(ab')_2$ fragments in order to decrease immunogenicity arising from the equine Fc portion of the molecule. The product contains

$F(ab')_2$ fragments specific for each of the seven BoNT serotypes. The labeled potency (per vial) is at least 4,500 units for serotype A, 3,300 units for serotype B, 3,000 units for serotype C, 600 units for serotype D, 5,100 units for serotype E, 3,000 units for serotype F, and 600 units for serotype G. Each unit is defined as the amount of antitoxin that can neutralize 10,000 mouse intraperitoneal lethal dose 50% ($MIPLD_{50}$) of serotypes A, B, C, D, F, and G, and 1,000 $MIPLD_{50}$ of serotype E.

Equine botulism antitoxins have been previously licensed in the United States, and an equine $F(ab')_2$ antitoxin for use in scorpion envenomation was licensed in 2011.

Use of the Animal Efficacy Rule to support licensure

In 2002 the FDA amended the regulations applicable to the approval of drugs and biologics to allow the use of studies performed in animals to provide key evidence of the effectiveness of a new drug or biological product (21 CFR 601 Subpart H). These changes were made in order to address concerns over licensure pathways for products used to reduce or prevent toxicity from chemical, biological, radiological, or nuclear (CBRN) substances when human efficacy studies are not ethical or feasible. This regulation identifies four essential elements that must be met in order for the FDA to consider licensure based on efficacy studies in animals.

1. There is a reasonably well-understood pathophysiological mechanism for the toxicity of the chemical, biological, radiological, or nuclear substance and its amelioration or prevention by the product;

2. The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model (meaning the model has been adequately evaluated for its responsiveness) for predicting the response in humans;

3. The animal study endpoint is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity; and

4. The data or information on the pharmacokinetics and pharmacodynamics of the product or other relevant data or information in animals and humans is sufficiently well understood to allow selection of an effective dose in humans, and it is therefore reasonable to expect the effectiveness of the product in animals to be a reliable indicator of its effectiveness in humans.

Human safety trials are still required, and the applicant must conduct postmarketing studies to verify and describe the product's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical.

To address these essential elements, Cangene has performed studies in two animal species (guinea pigs and rhesus macaques), has performed a safety study in human volunteers, and has committed to a postmarketing study. BAT has also been used to treat botulism in humans under an IND held by CDC.

However, the data from this program is not sufficient to support an efficacy determination since it was not collected from a well-controlled study.

Animal Models

The mechanism of BoNT induced pathophysiology has been examined in a number of animal species, including guinea pigs and rhesus macaques. Both species have been used for botulism challenge studies in the past, and are sensitive to multiple BoNT serotypes delivered via several different exposure routes (intravenous, intramuscular, orally, or via inhalation). The animal models chosen by Cangene utilize either intramuscular (guinea pig model) or intravenous (rhesus macaque) injection routes for the BoNT used for challenge. The Agency agreed with Cangene's choice of parenteral toxin challenge routes since variability in the animal models could be reduced through more accurate challenge dosing and development of botulism is dependent on the establishment of circulating BoNT in the host. As human exposures to BoNT are varied and unpredictable, these animal models also represent a 'worst-case' scenario in terms of disease onset and severity. Cangene quantified the lethality of an intravenous BoNT serotype A challenge in rhesus macaques in dose ranging studies, which were also used to document the clinical course of botulism in this species. Likewise, dose ranging studies were undertaken in guinea pigs for each of the seven BoNT serotypes (A, B, C, D, E, F, and G) delivered via intramuscular injection and the clinical course determined. In both species, the dose of BoNT required to achieve 50% mortality in challenged animals was determined through a regression analysis of the dose response data. The clinical course in both species also demonstrated a dose response, with higher toxin levels leading to a more rapid onset of clinical signs. Based on the results of the model development studies, Cangene determined (and the Agency concurred) that observation of clinical signs of botulism would be an appropriate trigger for therapy in the efficacy studies, and that a survival endpoint was acceptable. The animal efficacy studies were designed to test the null hypothesis that there would be no improvement in survival at 21 days post-BoNT challenge between animals administered BAT and animals administered placebo.

For the pivotal animal efficacy studies, Cangene utilized a scaled human dose of BAT based on weight. The target potency of the BAT product was based on previously licensed and investigational botulism antitoxin/immune globulin products. Since it was planned that a nominal 70 kg human would receive 1 vial, the dose used in the animal studies was adjusted proportionally such that a 1kg animal would receive the equivalent of $1/70^{th}$ of a vial. In order to demonstrate that the proposed human dose of 1 vial of BAT is supported by the animal efficacy studies, Cangene examined the PK of BAT in unchallenged guinea pigs and rhesus macaques as well as in healthy human volunteers. Post-exposure prophylaxis studies in the animal models, and a pre-exposure prophylaxis study in a human extensor digitorum brevis muscle model provided PD data. The resulting PK and PD data from the human and animal studies were used to perform population based modeling and simulation.

In conclusion, the Agency believes that the model development data provided by Cangene adequately addressed the essential elements as required by the "Animal Rule" and provided adequate justification for the use of the guinea pig and rhesus macaque BoNT challenge models.

Efficacy in guinea pigs

In order to demonstrate the efficacy of BAT treatment against each BoNT serotype in a small animal model, Cangene performed a randomized, blinded, placebo-controlled study in guinea pigs. The study was performed in seven stages, with one serotype evaluated at each stage. For each study stage, guinea pigs with implanted jugular catheters were randomized into two groups containing 34 animals (17 per sex) each. The guinea pigs were injected with 1.5x guinea pig intramuscular lethal dose 50% ($GPIMLD_{50}$) of the indicated BoNT in the right hind limb, and monitored every 30 minutes to 1 hour (depending on serotype) for the onset of clinical signs. After the fourth consecutive observation of a moderate or severe sign of BoNT intoxication, guinea pigs were treated with a single infusion of BAT (0.16 mL/kg; 1x scaled human dose based on weight) or placebo delivered into the jugular catheter. Animals were then observed for 21 days and were euthanized if they exhibited 25% or greater loss of body weight in conjunction with any concurrent severe sign of intoxication, two consecutive observations of complete paralysis, or if a determination was made by the study director or a veterinarian that the animal was moribund.

Clinical signs developed at a comparable incidence and timing between both BAT and placebo groups and were consistent with BoNT intoxication. Mortality in placebo treated animals was variable between serotypes and (with the exception of serotype G) was greater than 85%, while a statistically significant increase in survival rate was observed in BAT treated guinea pigs. The survival comparisons are provided in Table 1, below. The guinea pigs did continue to show disease progression after treatment with BAT as indicated by the development of labored breathing sounds or patterns; however, the incidence of forced abdominal respirations and total paralysis (severe clinical signs) was decreased when compared to the placebo group.

In summary, a 1x human dose BAT treatment in guinea pigs exhibiting clinical signs consistent with botulism statistically increased survival and decreased severe clinical sign incidence for all seven BoNT serotypes.

Table 1. Summary of survival and statistical significance in pivotal guinea pig efficacy study 1180.

Serotype	Single-dose Treatment	Survival (%)	Fisher's Exact Test (p-value)
A	BAT	34/34 (100%)	< 0.0001
	Placebo control	0/34 (0%)	
B	BAT	34/34 (100%)	< 0.0001
	Placebo control	1/34 (3%)	
C	BAT	33/34 (97%)	< 0.0001
	Placebo control	4/34 (12%)	
D	BAT	33/34 (97%)	< 0.0001
	Placebo control	5/34 (15%)	
E	BAT	34/34 (100%)	< 0.0001
	Placebo control	0/34 (0%)	
F	BAT	34/34 (100%)	< 0.0001
	Placebo control	4/34 (12%)	
G	BAT	34/34 (100%)	< 0.0001
	Placebo control	17/34 (50%)	

Efficacy in rhesus macaques

Sixty rhesus macaques (29 males and 31 females) were used in this study. Animals were first randomly allocated to two treatment groups; Group 1 animals (n = 30, 14 males and 16 females, one male was removed from the original 15 immediately before the start of the study because of health reasons and only a female was available to replace him) for BAT treatment, and Group 2 animals (n= 30, 15/sex) for treatment with placebo. Animals from each treatment group were then randomly allocated to cohorts. On Day 0, all animals received a dose equivalent to 1.7x nonhuman primate intravenous lethal dose 50% ($NHPLD_{50}$)/kg (~44 mouse intraperitoneal median lethal dose [MIPLD50]/kg) of BoNT/A as a single intravenous injection via a catheter placed in a saphenous vein. Starting 23 hours after intoxication, all animals were monitored hourly (± 10 minutes) for clinical signs of intoxication and assessment of food consumption. Immediately after the onset of clinical signs indicative of BoNT intoxication in each animal (ptosis, muscular weakness, and/or respiratory distress), BAT at a 1x scaled human dose (0.26 mL/kg) or

placebo (0.31 mL/kg, based on equivalent protein dose) was administered intravenously via the central venous catheter. Minimal supportive care (nutrition) was initiated in all animals (Groups 1 and 2) within 23 minutes of antitoxin or placebo administration and was either administered parenterally through the central venous catheter via constant rate infusion by use of an ambulatory pump, or via oral gavage of liquid rhesus monkey diet. Animals were observed for 21 days.

Clinical signs in all animals in both groups were consistent with botulism. Initial clinical signs included ptosis (n = 13), muscular weakness (n = 22), respiratory distress (n = 14), ptosis and muscular weakness (n = 1), ptosis and respiratory distress (n = 4), and muscular weakness and respiratory distress (n = 6).

All (30/30) placebo-treated Group 2 animals were euthanized prior to study scheduled termination due to signs of botulism and poor physical condition, with a median time to death of 74.5 hours. However, fourteen of thirty (14/30) Group 1 animals recovered (i.e., without clinical signs excluding food consumption) after the onset of clinical signs, and survived to the end of the study (Day 21). The remaining Group 1 animals (16/30) died or were euthanized due to poor physical condition, with a median time to death of 189.5 hr post-intoxication.

Treatment with BAT delayed the progression of BoNT/A intoxication resulting in euthanasia when compared with placebo. Analysis of these results confirmed a statistically significant improvement in survival of BAT-treated animals (See Table 2). The median time interval between the onset of clinical signs and recovery (resolution of clinical signs) for Group 1 animals was 137 hours. None of the nonhuman primates challenged with BoNT/A and subsequently treated with placebo survived to the end of the study, whereas 14 of 30 (46.7%) nonhuman primates treated with BAT survived to the end of the study. This difference in survival rate between the two groups was statistically significant.

Table 2. Summary of Survival and Median Time-to-Death for Rhesus Macaques in Study FY10-066.

Treatment	**Survival (%)**	**Fisher's Exact Test (p-value)***	**Median Time to Death in Hours (95% Confidence Interval)**	**Log- Rank Test (p-value)***
1xBAT	14/30 (47%)	p<0.0001	189.5 (102, –)[a]	p<0.0001
Placebo control	0/30 (0%)		74.5 (63, 81)	

[a] The upper bound of the confidence interval could not be estimated due to the limited number of events (i.e., 14 animals survived until study termination). *Comparison significant at the 0.05 level of significance.

In conclusion, a 1x human dose BAT treatment of rhesus macaques challenged with BoNT and exhibiting clinical signs consistent with botulism statistically increased survival and median time-to-death.

Human dose justification

In order for a meaningful dose extrapolation to be made to humans from the animal models, the PK properties of BAT were determined in guinea pigs, rhesus macaques, and humans.

- PK studies were undertaken in guinea pigs receiving a single 0.2x or 1.0x scaled human dose of intravenously administered BAT. Neutralizing activity against each of the seven BoNT serotypes was measured in serum collected at up to 12 days post administration.
- Likewise, rhesus macaques were administered a 1x or 5x scaled human intravenous dose of BAT and the BoNT serotype A neutralizing activity measured in serum samples. A single study was performed to establish the PKs of BAT in rhesus macaques exposed to BoNT serotype A.
- Finally, human volunteers received either one or two vials of BAT (representing a single or double dose) via intravenous administration and serum samples collected for up to day 28 post administration.

A non-compartmental analysis was used to derive PK parameters in all three species, and demonstrated that clearance of BoNT serotype A neutralizing activity is less rapid in humans when compared to guinea pigs or rhesus macaques. Human clearance of neutralizing activity against the other serotypes (B, C, D, E, F, and G) was also substantially (2.7-6.2 fold) lower in humans than in guinea pigs.

Two post-exposure prophylaxis studies were performed, one in guinea pigs with all seven BoNT serotypes and one in rhesus macaques with BoNT serotype A. In these studies, animals were intoxicated with BoNT and treated prior to the onset of clinical signs. Groups of guinea pigs received a 4x $GPIMLD_{50}$ challenge with the appropriate BoNT serotype followed by a single intravenous infusion of a 1x, 0.2x, 0.04x, or 0.008x scaled human dose of BAT either 12 hours (serotypes A, B, C, D, F, and G) or 6 hours (serotype E) later. In the nonhuman primate post-exposure prophylaxis study, rhesus macaques were challenged with 4x $NHPLD_{50}$ BoNT serotype A by intravenous injection and received a single intravenous infusion of either a 0.1x or 1x scaled human dose of BAT 4 hours later. Administration of BAT prior to the onset of symptoms was highly effective at preventing morbidity and mortality in both animal species.

A population based PK/PD modeling and simulation study was performed to integrate the PK data obtained from all three species. The model used PD data from the two pre-exposure prophylaxis studies, and the PK data from humans, guinea pigs, and rhesus macaques. A compartmental PK model was constructed and a traditional allometric power model used to scale the PK parameters in humans. An exposure response model was also constructed that used output from the PK model to simulate BAT levels and predicted a response based on the results from the animal post-exposure prophylaxis studies. The conclusion of this study was that the predicted probability of survival in humans for all BoNT serotypes was greater than 95%, and that a margin of efficacy (defined as $AUC_{human\ dose}/AUC_{minimum\ effective\ exposure}$, based on the PK results of the clinical lot of BAT, and the minimum effective dose calculated from the animal studies) ranged from a ratio of 6.6 to a ratio of 257.5, depending on serotype.

A comparison was also performed in terms of the amount of neutralizing capacity provided by a single vial of BAT based on the PK result in humans (see Table 3). While the clinical lot used in the human and animal studies was more potent than the minimum potency specification, the PK data indicates that an adequate potency buffer exists considering the BoNT serum levels in human botulism ranges from undetectable levels to 160 $MIPLD_{50}$/mL.

Further background information including additional data on relevant animal models and pharmacology of BAT are provided in the Appendix.

Table 3. BAT proposed specification, clinical lot 2060401X potency, observed C_{max}, and neutralizing capacity against BoNT

BoNT Serotype	**Proposed specification [U/vial]**	**Clinical Lot 2060401X Potency (actual) [U/vial]**	**BAT C_{max} (human)** Mean data, N = 20 [U/mL]	**Clinical Lot 2060401X Neutralizing Capacity against BoNT** [$MIPLD_{50}$/mL]
A	>4,500	10,399	2.69	26,900
B	>3,300	7,071	1.90	19,000
C	>3,000	7,138	2.26	22,600
D	>600	1,452	0.81	8,100
E	>5,100	10,690	0.94	940
F	>3,000	6,546	2.37	23,700
G	>600	1,229	0.59	5,900

Human safety

Three clinical trials in healthy human volunteers provided safety information for BAT.

- Study BT-001: a pharmacokinetic and safety study of single or double-dose administration in 40 normal volunteers
- Trial BT-002 stages A and B studied Botulism Antitoxin effects on paralysis induced by Type A and Type B Botulinum Neurotoxins in the Extensor Digitorum Brevis Muscle

- Stage A: single center double-blind, randomized, placebo-controlled, parallel armed, safety and pharmacodynamic trial that studied [previously available] licensed botulism antitoxin types A and B ("comparator").
- Stage B: single center double-blind, randomized, placebo-controlled, parallel armed, safety and pharmacodynamic trial that studied BAT (16 subjects)

- Under an IND held by CDC, 148 symptomatic subjects with documented or suspected botulism poisoning received BAT.

In the two clinical trials, BT-001, BT-002B, and throughout the CDC IND program, a total of 204 subjects were given at least one dose of BAT. Two doses were given to 25 subjects.

Common adverse reactions

The most common adverse reactions reported in the clinical trials were headache, somnolence, fever, rash, lymphadenopathy, tonsillar hypertrophy, nausea, extremity pain, and upper respiratory tract infections. The distribution of adverse reactions is provided in Table 4 and Table 5.

Table 4. Distribution of adverse reactions between active treatment and placebo arms in BT-002B based on severity

		eBAT BAT 1 vial	**Placebo**
		Reactions (%)	Reactions (%)
Number of reactions		50	31
Severity	Mild	35 (70%)	31 (100%)
	Moderate	8 (16%)	0 (%)
	Severe	7 (14%)	0 (%)
Not serious		50 (100%)	31 (100%)

Mild: Awareness of a sign or symptom but subject can tolerate
Moderate: Discomfort enough to cause interference with normal daily activity
Severe: Resulting in an inability to do work or do usual daily activity

Table 5. Type and distribution of severe and moderate adverse reactions between active treatment and placebo arms in BT-002B.

		eBAT BAT 1 vial	**Placebo**
		Reactions (%)	Reactions (%)
Types of reactions in severe and moderate categories of severity			
Severe adverse reactions	Pain	1 (2%)	0 (0%)
	Viral infection	1 (2%)	0 (0%)
	Skin lacerations	3 (6%)	0 (0%)
	Tonsillar disorder	1 (2%)	0 (0%)
	Upper respiratory tract infection	1 (2%)	0 (0%)
Moderate adverse reactions	Conjunctivitis	1 (2%)	0 (0%)
	Pyrexia	1 (2%)	0 (0%)
	Thermal burn	1 (2%)	0 (0%)
	Somnolence	1 (2%)	0 (0%)
	Panic attack	1 (2%)	0 (0%)
	Dysmenorrhea	1 (2%)	0 (0%)
	Rash	1 (2%)	0 (0%)
	Urticaria	1 (2%)	0 (0%)

Adverse reactions of special medical interest

Allergic

Two moderate acute allergic reactions were reported, one each in the single-dose treatment arm of BT-001 and the active treatment arm of BT-002B.

Anaphylaxis
No cases of anaphylaxis occurred in any trial.

Serum sickness
Serum sickness was reported in two of the 204 exposed subjects (1%).

- A 64 year old male developed serum sickness, including hematuria, after receiving BAT under CDC'S IND and died 52 days after treatment.
- A 26 year old female had a moderate acute allergic reaction followed after ten days by limited lymphadenopathy, considered by Cangene as consistent with mild serum sickness. No other positive laboratory findings were reported that confirmed the diagnosis of serum sickness.

Lymphadenopathy
Lymphadenopathy occurred more often with BAT (3/16, 19%) than comparator (0/5, 0%), but approximately equally between BAT (3/19, 19%) and placebo (2/10, 20%). Lymphadenopathy was considered unrelated to treatment except in the subject who was diagnosed with serum sickness.

Tonsillar hypertrophy
Discussed in the same context as lymphadenopathy and usually considered by Cangene as unrelated to treatment or part of a viral syndrome, the frequency of tonsillar hypertrophy (4/16, 25%) was increased both over placebo (1/10, 10%) in BT-002B and over comparator in BT-002A (0%).

Bleeding
Bleeding was reported in 5 subjects in the treatment arms. There were two cases of dysmenorrhea not otherwise specified, one case of menorrhagia, one case of venipuncture site bruising, and one case of epistaxis. All of these were considered as unrelated. No cases of thrombosis were detected in any arm of any clinical trial.

Serious Adverse Reactions (SAR)
No SARs were reported in BT-001 or BT-002A/B. Two SARs were reported under CDC's IND.

A 10-year old male experienced asystole during initial BAT infusion and bradycardia upon rechallenge. Further treatment was discontinued. Although the investigator considered this reaction only possibly related, based on recurrent cardiac instability upon rechallenge, FDA considers the reaction to be treatment-related.

A 27 year old male subject died 27 hours after receiving BAT. The immediate cause of death was mucous plugging during that occurred during tracheostomy manipulation. FDA does not consider the death to be due to BAT.

Deaths

No deaths occurred in studies BT-001 or BT-002. Six deaths were reported under CDC's IND. All were deemed by Cangene to be unrelated to BAT. FDA has requested additional information for three of these six cases.

- A 64 year old male died 52 days after BAT infusion. This patient had myalgia, arthralgia, and hematuria consistent with serum sickness. Additional information has been requested to determine whether serum sickness may have contributed to the patient's death.
- An 82 year old female died of respiratory failure and pneumonia three days after infusion. The death was considered to be unrelated to BAT.
- A 77 year old male died of unknown causes ninety four days after BAT administration. No cause of death or details of clinical course were available to the reviewer, and additional information has been requested. However the death is likely unrelated to BAT based on the long time elapsed between infusion and death.
- An 88 year old female died of Miller Fisher variant of Guillain-Barré syndrome 7 days after BAT administration. Because of the temporal relationship, the death was considered by the clinical reviewer to be potentially related to BAT and additional information has been requested.
- A 64 year old male died of metastatic cancer and respiratory failure 49 days after BAT infusion. The death was considered to be unrelated to BAT.
- A 27 year old male suffered respiratory and cardiac arrest during a tracheostomy manipulation, considered secondary to mucous plugging. The death was considered to be unrelated to BAT.

Dropouts and discontinuations

There were no study dropouts in the controlled clinical trials, although two subjects discontinued infusions due to allergic reactions. One patient experienced pruritis, headache, urticaria, fever, pharyngeal pain and somnolence, and was treated with epinephrine, diphenhydramine, and ibuprofen. Another subject experienced urticaria, fever, and chest discomfort, and was treated with diphenhydramine and methylprednisolone.

Under CDC's IND, a 10 year old male discontinued BAT infusion as previously described, due to cardiac instability (asystole and bradycardia).

Special populations

Pediatrics

No pediatric subjects were studied in BT-001 or BT-002. Seven pediatric subjects were under CDC's IND. Two of the 7 pediatric subjects suffered adverse reactions including one related SAR as described above. The second subject was a 3-year old male who developed fever and was treated with acetaminophen. One infant less than one year old was treated for serotype F infant botulism. This infant had no reported

adverse reactions and responded rapidly to treatment. Although safety data in pediatric populations are limited, it is expected that the safety profile will be similar to that of adults.

Geriatrics

No geriatric subjects were studied in BT-001 or BT-002. Twenty geriatric subjects were enrolled in the CDC's IND. Five of the six deaths in CDC's IND occurred in the geriatric population (age ≥ 64 years), and to date are considered unrelated to treatment.

Immunogenicity

Compared with the previously available licensed botulism antitoxin for types A and B, a lower percentage of subjects treated with BAT developed anti-equine antibodies by day 28. Treatment-emergent anti-equine antibodies against BAT developed in 7/26 (27%) of subjects in BT-001 and 2/23 (17%) subjects in BT-002B, compared with 3/9 (33%) from subjects receiving the previously available comparator product in BT-002A. Anti-equine antibodies were not observed in any placebo infused subjects. There was no relationship between immunogenicity and adverse reactions, and most subjects who suffered allergic adverse reactions were skin test and serology negative.

Benefit:Risk Assessment

Botulism is a life-threatening condition often requiring mechanical ventilation and intensive supportive care. Data from well controlled, pivotal efficacy trials in symptomatic BoNT-challenged guinea pigs and rhesus macaques demonstrate that BAT substantially reduces the risk of death. It is anticipated that treatment of symptomatic BoNT-exposed humans with BAT should prevent the need for mechanical ventilation or reduce the time of ventilator dependence and hospitalization. The risk of severe allergic reactions and serum sickness after BAT is relatively low, and these conditions are responsive to treatment. Overall, based upon its assessment, FDA considers that the benefits exceed the risks associated with the use of BAT to treat symptomatic botulism.

Questions for the committee

1. Do the results from the efficacy studies of botulism antitoxin heptavalent (A, B, C, D, E, F, G)-(Equine) in guinea pigs and nonhuman primates provide sufficient evidence that the product is

reasonably likely to provide clinical benefit for the treatment of humans with symptomatic botulism?

2. Do the results from safety studies in healthy human volunteers, efficacy studies in animal models, and clinical data from CDC's use of BAT under IND support an acceptable risk benefit profile for use of BAT?
3. Do the animal and human studies provide a sufficient basis for dosing?
4. Since the Animal Rule requires postmarketing studies to monitor safety and efficacy of products approved under the Rule, the Agency suggests that Cangene utilize a registry to capture data on safety and efficacy of the use of BAT for sporadic cases of botulism for a 3 year period after licensure, and that this registry be reactivated and utilized to capture data on the use of BAT in any mass casualty scenario. Please comment whether such a registry would be adequate:
    a. To add to the safety data for use of BAT in patients with botulism, and/or

To monitor the effectiveness of the recommended human dose in cases of botulism

APPENDIX

Additional Background on Botulism

Prevalence

The most recent annual survey of botulism in the United States available is the National Enteric Disease Surveillance Botulism Annual Summary for 2010. There were 112 laboratory confirmed cases reported: 8% were foodborne, 76% were infant botulism, 15% were due to wound botulism, and 1% were of unknown or other etiology (Centers for Disease Control and Prevention, n.d.-b).

Botulism Routes of exposure

Food borne botulism

Foodstuffs can be contaminated with spores of *Clostridum* spp. and if stored under anaerobic conditions these spores germinate into vegetative bacteria that can produce botulinum neurotoxins (BoNT). This process usually involves inadequately cooked home-prepared canned foodstuffs, improperly stored food, or, in Alaska, fermentation of seal meat in tightly closed plastic containers. In 2010 only 8% of U.S. botulism cases were attributable to food-borne routes of exposure.

Wound botulism

Wound botulism is caused by colonization of deep wounds with spores of *C. botulinum*. The anaerobic conditions allow germination of the spores and production of BoNT by vegetative bacteria resulting in a clinical presentation similar to that of foodborne botulism (Sobel, 2005). Historically, wound botulism was observed rarely after traumatic injuries. Currently, the majority of wound botulism cases are related to subcutaneous or intradermal injection of heroin contaminated with *Clostridium* spores (Werner, Passaro, McGee, Schechter, & Vugia, 2000). Approximately 15% of botulism cases in the U.S. occurring in 2010 were classified as wound botulism.

Intestinal botulism

In infants*, C. botulinum spores*, colonize the intestine and produce toxin, which then enters the circulation. The source of the spores in infants can be related to the ingestion of corn syrup or honey, often added to infant formula. It is suspected that in these cases the normal intestinal flora has not developed to the degree necessary for resistance to *C. botulinum* overgrowth. Adult intestinal colonization has also been described, but occurs at a much lower incidence and is typically associated with gastrointestinal tract abnormalities or antibiotic use that has disrupted the normal adult intestinal flora. The majority of botulism cases in the U.S. are attributable to infant botulism (76% in 2010).

Inhalational botulism

Inhalational botulism in humans has only been documented in one laboratory-associated exposure.

Three laboratory workers exhibited symptoms consistent with botulism three days following the necropsy of rabbits and guinea pigs that had been exposed during a whole body BoNT/A aerosol experiment; presumably residual toxin on the fur was aerosolized during the skinning process (Holzer, 1962). These individuals were treated with a horse-derived antiserum, but no information on the potency of the serum or the levels of circulating toxin were recorded. Lyophilized, aerosolized BoNT was explored as an offensive biological warfare agent in World War II and animal model experiments in various species have demonstrated that a lethal dose may be delivered in this manner, albeit less efficiently compared to parenteral routes (Cardella, Jemski, Tonik, & Fiock, 1963; Herrero, Ecklung, Streett, Ford, & King, 1967; Park & Simpson, 2003; Sanford et al., 2010).

Iatrogenic botulism

Iatrogenic exposures to BoNT have occurred due to overdoses of toxins as therapeutic agents. BoNT serotypes A and B (BoNT/A and BoNT/B) are licensed in the U.S. for cosmetic use and for treating cervical dystonia, respectively. One recent case involved use of a non-licensed BoNT/A preparation and resulted in several lengthy hospitalizations (Chertow et al., 2006). Circulating toxin was measured in three of the four patients and ranged from 12 to 24 mouse intraperitoneal lethal dose 50% ($MIPLD_{50}$) units/mL of serum, estimated to represent a total circulating BoNT dose of 21-43 times the estimated human lethal dose of 40 $MIPLD_{50}$ /kg. Toxin was not detectable after the patients were administered a bivalent botulism antitoxin product with a labeled potency against BoNT/A of 7500 IU/vial (one IU of antitoxin neutralizes 10,000 $MIPLD_{50}$), however the amount of antitoxin administered to each patient was not published.

Botulinum neurotoxin as a biological threat agent: a scenario

One mass casualty scenario that has been publicly discussed involves the intentional contamination of the milk supply with BoNT. In 2005, such an attack was modeled and provided an estimate of the number of intoxicated individuals with variable toxin quantities (0.1 g-1 kg) and detection scenarios (Wein & Liu, 2005). This scenario was later generalized to account for non-uniform distribution of the attack material, as would be expected with solid food contamination (Liu & Wein, 2008). These scenarios used a dose response relationship based on an assumption of an effective dose 50% level of 1 µg for adults and 0.43 µg for children. These models did not take into account treatment timing or antitoxin potency when making casualty determinations. However, to put these toxin quantities into perspective the specific activity of the BoNT/A used in the animal studies for BLA 125462 was 6902 $MIPLD_{50}$/µg protein, and the neutralization capacity of the antitoxin for serotype A is at least 4500 units/vial, where 1 unit of antitoxin is capable of neutralizing 10,000 $MIPLD_{50}$ units of toxin.[1]

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[1] Note that toxin units are not synonymous with antitoxin units. Moreover, while 1 antitoxin unit will neutralize 10,000 units of serotypes A, B, C, D, F, and G, it by definition neutralizes 1,000 units of serotype E.

Mechanism of botulism toxicity

Botulism toxins are zinc metalloproteases that can be categorized based on seven antigenically different serotypes (and are designated accordingly with letters A-G). They are produced as a single 150 kDa polypeptide, which undergoes proteolytic cleavage into a disulfide linked light chain (Lc) and heavy chain (Hc). The 50 kDa light chain contains the metalloprotease activity, while the 100 kDa heavy chain incorporates receptor binding and translocation domains. The neurotoxins are released from the bacteria as part of a multimeric protein complex containing hemagglutinin and other components. The accessory proteins do not contribute directly to BoNT toxicity, although they may stabilize the active toxin in the presence of low pH environments or proteases found in the stomach.

The pathophysiology of BoNT is reasonably well understood. The BoNT heavy chain binds gangliosides or phosphatidylethanolamine on neurons and then interfaces with a protein receptor that triggers endocytosis. The heavy chain then undergoes a conformational shift due to the low endosomal pH and forms a transmembrane channel that allows the light chain to enter the cytosol. The fundamental mechanism of action is BoNT light chain mediated cleavage of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins in neurons. Loss of SNARE proteins prevents acetylcholine-containing vesicles from fusing with the plasma membrane at neuromuscular junctions, thereby preventing neurotransmitter release and triggering a flaccid paralysis. While all BoNT serotypes are exceedingly potent neurotoxins, toxicity can vary widely between the serotypes. Humans are susceptible to all seven serotypes, although most naturally occurring cases of botulism are due to serotypes A, B, and E. Toxins of different serotypes exhibit specificity for different SNARE substrates and also demonstrate different kinetics in terms of recovery, with serotype. BoNT-mediated SNARE cleavage can persist from days to months in an intoxicated neuron (Keller et al., 1999).

Treatment of botulism

The currently recommended treatment of botulism involves passive immunization with antitoxin to remove circulating toxin, and (in severe cases) supportive care including enteral or parenteral nutrition, fluid management, and mechanical ventilation. Due to the persistence of BoNT Lc mediated cleavage of SNARE proteins and the resulting long term paralysis, early treatment with antitoxin is critical in order to stop progression of the disease (Tacket et al., 1984).

Previously licensed or investigational use therapeutics

In the early 1940's the Lederle Laboratories Division of American Cyanamid was granted U.S. license number 17 for botulism antitoxin; a bivalent equine product against BoNT serotypes A and B. The license was revoked by an FDA efficacy panel in August 1979 since Lederle had ceased manufacturing or supporting the product. A trivalent botulism antitoxin with activity against serotypes A, B, and E was licensed in 1972 and marketed in the United States by Connaught Laboratories Limited (who became Aventis Pasteur, now Sanofi Pasteur); the product was also reviewed by an FDA efficacy panel in 1979, classified as Category I, and the license continued. Botulism Antitoxin Bivalent (Equine) Types A and B was a $F(ab')_2$ product produced from the plasma of horses immunized with BoNT/A, /B, or /E toxoids. Circa 1999, this product lost potency against serotype E, therefore the serotype E specificity was

removed from the label claim (although low titers of anti-E activity were still present) and the product continued on the market as a bivalent antitoxin against serotypes A and B. A separate Type E antitoxin, also manufactured by Sanofi, was made available under an IND sponsored by CDC. Sanofi ceased manufacture of their botulism antitoxin products in 2003, and on 12 March 2010 the last lot of the licensed product reached its expiry date. The CDC subsequently ceased distribution of the Sanofi antitoxin and announced the decision to replace it with the Cangene BAT in a Morbidity and Mortality Weekly Report (Centers for Disease Control and Prevention, 2010). The Sanofi license was voluntarily revoked in June 2011 leaving Cangene's BAT as the only botulism antitoxin available for the treatment of botulism.

Vaccines

There are no U.S. licensed vaccines against botulism at this time. A toxoid based vaccine (Pentavalent [ABCDE] Botulinum Toxoid) had been available since 1965 under an IND held by CDC, however due to declining immunogenicity and increasing reactogenicity the use of this product was discontinued in November 2011 (Centers for Disease Control and Prevention, 2011). A recombinant vaccine for serotypes A and B (rBV A/B) is under development by the Department of Defense and has progressed to human trials (Smith, 2009).

Currently licensed products

Botulinum Immune Globulin Intravenous (Human); BabyBIG

Licensed for treatment of infant botulism in patients below one year of age in 2003, BabyBIG (BIG-IV) is a human hyperimmune product manufactured from the plasma of individuals who were immunized with pentavalent botulism toxoid and selected for their high titers of neutralizing antibody against botulism neurotoxins A and B. (BabyBIG package insert) The labeled potency is at least 15 IU/mL for BoNT/A and at least 2.7 IU/mL for BoNT/B, which while sufficient for treating infant botulism, is approximately 1-4% of the nominal potency of the equine antitoxins. BIG-IV is made available through the Infant Botulism Treatment and Prevention Program and limited quantities (typically a single lot) are in distribution.

Regulatory context

The original biologics license application for BAT, Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-(Equine) was submitted on September 20, 2012. A 6-month priority review timeline was granted because of unmet medical need. The product received orphan drug designation on June 29, 2011. The pathway for licensure of BAT is defined in 21CFR Subpart H, Approval of Biologic Products when Human Efficacy Studies are Not Ethical or Feasible, commonly referred to as the Animal Efficacy Rule or the Animal Rule. This is codified in 21 CFR 601.90 for biologics.

Animal Efficacy Rule

Rationale

In 1999, FDA determined that the requirement for human studies to demonstrate efficacy has the effect of preventing development and availability of approved drug and biological products to reduce or prevent serious or life-threatening toxicity resulting from exposure to lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substances."[2]. FDA published a Final Rule in 2002 entitled "New Drug and Biological Products: Evidence Needed to Demonstrate Effectiveness of New Drugs when Human Safety Studies are not Ethical or Feasible." As noted above, this rule is commonly referred to as the Animal Efficacy Rule or Animal Rule; because it defines a pathway to licensure of treatments for toxic substances based upon carefully controlled and executed efficacy studies in animals.

Specifically, the Animal Rule may be used when human efficacy studies are not ethical or feasible.

To date, six drugs or biological therapies have been approved by CDER under the Animal Efficacy Rule. These included: Cyanokit for cyanide exposure, pyridostigmine bromide for Soman pre-exposure prophylaxis, doxycycline for plague, levofloxacin for plague, ciprofloxacin for anthrax, and raxibacumab for anthrax. Of the five approvals, raxibacumab (approved December 2012) was the first novel molecular entity approved under the Animal Rule; the other compounds had been previously approved for other indications.

Criteria for Submission

As outlined in FDA's 2009 Animal Rule Guidance,[3] characteristics of the disease agent, host susceptibility, disease pathogenesis and natural history, proposed treatment mode of action, comparability of human and animal models should be addressed to assure that the biological product is reasonably likely to produce clinical benefits in humans. In assessing the sufficiency of animal data, FDA may also consider any other data available, such as human data, in support of licensure.

Criteria for Approval

FDA can rely on studies from animals to provide substantial evidence of effectiveness only when:

- There is a reasonably well understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;

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[2] 64 FR 53960.

[3] http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078923.pdf

- The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represent a sufficiently well-characterized animal model for predicting the response in humans;
- The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and
- The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective does in humans.

Sufficient data to address each of these points must be contained in the submission.

Following Approval

Approval under the Animal Rule is subject to three requirements:[4]

- Postmarketing studies – the applicant must conduct postmarketing studies, such as field studies, to verify and describe the biologic product's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical (for example, during a bioterrorism event)
- Approval with restrictions to ensure safe use – if FDA concludes that a biologic product shown to be effective under this regulation can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to ensure safe use of the biological product;
- Information to be provided to patient recipients – For biologic products or specific indication approved, applicants must prepare, as part of their proposed labeling, labeling to be provided to patient recipients that includes: an explanation that the drug's approval was based solely on animal efficacy studies, the indication, directions for use, contraindications, adverse reactions, foreseeable risks and benefits, drug interactions, and any other relevant information required by FDA at the time of approval.

[4] 21 CFR 601.90

Essential Data Elements of an Animal Model[5]

Characteristics of the Chemical, Biological, Radiological, or Nuclear Agent that Influences the Disease or Condition

Botulism Challenge Agent

The partially purified BoNT complexes used for animal studies submitted in support of this application were originally purchased from ----------(b)(4)----------. The serotype A, B, E, F, and G challenge materials may be considered reasonably similar to the etiologic agents responsible for human botulism and were purified from the appropriate *C. botulinum* cultures. Certificates of analysis were provided for each toxin serotype. Serotypes C and D have not been associated with human disease but are responsible for botulism in animals (birds, cattle, and horses). ----------(b)(4)----------. The potency of toxin dilutions used in the pivotal animal studies was verified via mouse bioassay.

Pathogenic Determinants

The pathogenic determinants for botulism are identical in the guinea pig, non-human primate, and human. Regardless of the exposure scenario, pathophysiology depends on the transport of toxin into the general circulation, distribution to and internalization of the toxin into peripheral neurons and the resulting light chain mediated cleavage of SNARE proteins. The inability of the neuron to release acetylcholine results in flaccid paralysis and the clinical signs/symptoms associated with clinical botulism, with fatal outcomes attributed to respiratory paralysis (or complications thereof).

Route of exposure

Humans can be exposed to BoNT through a variety of different exposure routes, with inhalation and ingestion the most likely avenues for a bioterrorism event. Parenteral, inhalation, and oral routes of BoNT exposure have been studied in guinea pigs and nonhuman primates. In general, the parenteral routes appear to be the most efficient means of intoxication, followed by inhalation and oral exposure. ----------(b)(4)----------

[5] As outlined in the 2009 "Draft Guidance for Industry: Animal Models-Essential Elements to Address Efficacy Under the Animal Rule"

-------(b)(4)---------- ; however like the guinea pig model, parenteral routes of exposure appear to be similar while the oral and inhalational routes require higher toxin levels to achieve similar mortalities (Herrero et al., 1967; Sanford et al., 2010; Scott & Suzuki, 1988).

An important factor to consider in terms of animal model development is the reproducibility of dosage. Aerosol and oral deliveries are associated with high variability, and for oral exposure the matrix in which the toxin is suspended can affect bioavailability (Cheng & Henderson, 2011). The toxin form also influences oral toxicity, with accessory proteins playing an important role in protecting the protein from proteolysis during the transition through the gastrointestinal tract (Chen, Kuziemko, & Stevens, 1998; Cheng et al., 2008; Ohishi, Sugii, & Sakaguchi, 1977).

At the suggestion of the Agency, Cangene sponsored a study to examine the utility of an oral intoxication (study 975-G005630) and established a guinea pig oral lethal dose 50% level of 388 $MIPLD_{50}$, although the time to onset of clinical signs was more variable in comparison to the IM model. Robustness of the animal model depends on reproducibility of challenge agent quantities, and since parenteral routes of intoxication provide similar results in terms of clinical progression and mortality, an IV or IM route was preferred for the efficacy model. These issues were discussed with Cangene at the IND stage and the Agency agreed that parenteral intoxication would be acceptable in order to decrease experimental variability, especially given the uncertainty around the various human exposure scenarios.

Limited data are available on the pharmacokinetics of BoNT. Bioavailability and metabolism have been examined for BoNT/A in the mouse and rat (Ravichandran et al., 2006) and BoNT/B in the rat (Al-Saleem et al., 2008). These studies suggest that the toxin is not subject to significant biotransformation and is held in the plasma compartment in an unbound state. One consideration in terms of using the parenteral routes of exposure chosen for the BAT development program is that the disease course is shortened due to the bypass of absorption mechanisms.

Quantification of exposure

While in vitro assays for quantifying BoNT are in development and have shown some promise in terms of sensitivity and turn-around time, the most widely accepted assay is still the mouse potency bioassay. Serially diluted samples are injected into mice and a probit analysis is performed to calculate the $MIPLD_{50}$ value. This assay provides a direct readout of the biological potency of the toxin; unfortunately the assay requires large numbers of mice and 4-6 days to complete.

The mouse bioassay was used to monitor potency of the BoNT serotype batches used in the BAT development program, and was also used to verify the potency of dilutions used in the various animal studies. Circulating BoNT levels in intoxicated animals was not monitored. To establish the quantity of each BoNT serotype required for lethality in the guinea pig following intramuscular injection, study 621-G005630 was performed at Battelle. Dilutions of BoNT serotypes A-E were prepared and injected into the muscles of the right hind leg of guinea pigs. The animals were monitored twice a day for 14 days for clinical signs and mortality. Probit dose response models applied to the mortality data were used to determine the guinea pig intramuscular lethal dose 50% ($GPIMLD_{50}$) value for each toxin serotype. The

data was also compared to historical data from a previous Battelle study in guinea pigs using BoNT serotypes A-E as the challenge agent. The comparisons are provided in Table 6. The newly established $GPIMLD_{50}$ values and 95% confidence intervals for BoNT/F and BoNT/G were 25 (20.8-30.0) and 53.2 (48.7-58.0) $MIPLD_{50}$, respectively.

Table 6. Summary of LD_{50} data for BoNT serotypes A-E from study 621 compared to historical data (task 97-51)

Sero-type	$GPIMLD_{50}$ Estimates from Common Slopes Probit Models		LD_{50} Ratio Comparing Study 621-G005630 / Task 97-51			P-value from Likelihood Ratio Test to Compare Probit Slopes
	Study 621-G005630	Task 97-51	LD_{50} Ratio	95% Confidence Interval	P-value for Comparing Ratio to 1.0	
A	4.5	4.3	1.04	(0.76, 1.43)	0.7894	0.5493
B	8.6	7.1	1.20	(0.92, 1.56)	0.1886	0.0433
C	1.9	3.2	0.61*	(0.43, 0.87)	0.0072	0.6769
D	5.7	8.8	0.65*	(0.49, 0.87)	0.0034	0.1931
E	72.9	101.4	0.72*	(0.64, 0.81)	<0.0001	0.3747

* Ratio was significantly different from unity at the 0.05 significance level.

Cangene originally chose a 4x $GPIMLD_{50}$ challenge dose for their efficacy model. However, study 843-G005630 demonstrated that initiation of BAT treatment at the onset of clinical signs did not provide a survival benefit, although the median time to death was increased for serotypes A, C, and D (Table 7). Since time to death was 16-38 hours after the onset of symptoms, it was speculated that disease progression was too rapid to allow for an accurate test of BAT efficacy.

Table 7. Summary results of guinea pig study 843. The BoNT challenge dose was 4x GPIMLD$_{50}$ for each serotype.

BoNT Serotype	Treatment Dose Level	Survival (%)	Two-sided Fisher's Exact Test Comparison (P-value)	Kaplan-Meier Median Time to Death in Hours (95% Confidence Interval)	Log-Rank Test Time to Death Comparison (p-value)
A	1x eBAT NP-018	0/33 (0%)	p=1.0000	63 (59, 64)	p<0.0001*
	Placebo control	0/33 (0%)		56 (47, 59)	
C	1x eBAT NP-018	5/35 (14%)	p=0.0536	107 (86, 131)	p<0.0001*
	Placebo control	0/34 (0%)		66 (66, 71)	
D	1x eBAT NP-018	0/31 (0%)	p=1.0000	74 (70, 95)	p<0.0001*
	Placebo control	0/32 (0%)		59 (55, 62)	
F	1x eBAT NP-018	2/31 (6%)	p=0.2381	41 (37, 47)	p=0.8827
	Placebo control	0/32 (0%)		41 (37, 47)	
* Comparison significant at the 0.05 level of significance					

In order to address these concerns, Cangene sponsored studies 964-G005630, 993-G005630, and 1005-G005630 in order to establish the clinical course of a lower (1.5 x GPIMLD$_{50}$) toxin challenge dose and test efficacy in the revised model. Study 964-G005630 did demonstrate that lowering the toxin dose could extend the clinical course of disease for serotypes A and E (Table 8).

Table 8. Summary of results of guinea pig study 964.

Serotype	Toxin Dose in GPIMLD$_{50}$ (MIPLD$_{50}$)	Survival (%)	Kaplan-Meier Median (95% Confidence Interval) (Hours)		
			Time to Onset of Moderate Clinical Signs	Duration of Clinical Signs	Time to Death
A	1.5x (6.75)	0/10 (0)	37 (32, 42)	121 (72, 129)	165 (102, 165)
	2.0x (9.0)	0/10 (0)	27 (27, 31)	63 (52, 71)	92 (79, 99)
	4.0x (18.0)	0/10 (0)	24 (21, 25)	26 (25, 34)	51 (47, 57)
E	1.5x (109.8)	0/10 (0)	14 (10, 17)	21 (12, 24)	32 (30, 39)
	2.0x (146.4)	0/10 (0)	16 (12, 18)	14 (11, 19)	30 (28, 32)
	4.0x (292.8)	0/10 (0)	13 (12, 13)	8 (7, 9)	20 (20, 22)

However, in studies 993 and 1005, although BAT was effective at increasing survival, an unexpectedly large proportion of control animals survived. It was determined that the precision of the original

$GPIMLD_{50}$ calculations was insufficient due to a small sample size. The toxin doses were reexamined by combining control animal data from studies 621, 964, 993, 1005 (and for serotype F, study 1124); it was determined that the original $GPIMLD_{50}$ values for serotypes B, C, D, E, F, and G had been overestimated (Table 9). The toxin dose was increased accordingly for the pivotal study 1180.

Table 9. Summary of toxin dose recalculation

Serotype	Number of $MIPLD_{50}$ per $GPIMLD_{50}$		Difference (%)	Recalculated $1.5xGPIMLD_{50}$ ($MIPLD_{50}$)
	Original BBRC 621-G005630 $GPIMLD_{50}$ Values	Recalculated $GPIMLD_{50}$ Values		
A	4.5	4.5	0	6.8
B	8.5	11.7	38	17.6
C	2.0	2.6	30	3.9
D	5.7	6.2	9	9.3
E	73.2	81.2	10	121.8
F	25.0	43.3*	73	65.0
G	53.2	56.4	6	84.6

A similar approach was utilized to verify the previously established LD_{50} dose for BoNT/A delivered via IV in the rhesus macaque ($NHPLD_{50}$). Study FY07-027 was performed at Lovelace Biomedical and Environmental Research Institute (Lovelace), and the results compared to the data published by Herrero et al. (Herrero et al., 1967). The newly established values were significantly different, and Cangene attributed this difference to the small samples size used in FY07-027 (4 dose groups, n=4 per group) compared to the Herrero study (8 dose groups, n=6 per dose group). The results of study FY07-027 and the comparison to the historical study are provided in Table 10. A dose response was observed in both mortality and clinical disease progression (Table 11). Based on these results, an initial challenge dose of 4x $NHPIV_{50}$ was chosen for the nonhuman primate efficacy studies.

Table 10. Rhesus macaque intravenous LD_{50} for BoNT/A

Serotype	LBERI FY07-027 Study		Herrero et al. (145) Study	
	$MIPLD_{50}$/kg	Confidence Interval ($MIPLD_{50}$)	$MIPLD_{50}$/kg	Confidence Interval ($MIPLD_{50}$)
A	26	24, 28	40	31, 53

Table 11. Median time to onset of clinical signs and/or death, rhesus study FY07-027.

Clinical Sign	Study LBERI FY07-027				Study LBERI FY08-061
	25 $MIPLD_{50}$/kg (1.0x $NHPLD_{50}$)	40 $MIPLD_{50}$/kg (1.5x $NHPLD_{50}$)	60 $MIPLD_{50}$/kg (2.3x $NHPLD_{50}$)	160 $MIPLD_{50}$/kg (6.0x $NHPLD_{50}$)	104 $MIPLD_{50}$/kg (4.0x $NHPLD_{50}$)
Ptosis	42 (40, 61)	30 (19, 41)	21.5 (17, 32)	17.5 (17, 19)	27.5 (23, 29)
Muscular Weakness	51 (40, 62)	39.5 (19, 41)	30.5 (26, 36)	18.5 (16, 20)	28.5 (23, 29)
Respiratory distress	64.5 (62, 89)	39.5 (36, 43)	35 (35, 36)	19 (18, 23)	30 (29, 31)
Oral Discharge	- (102, -)	47 (34, -)	36 (35, 41)	- (20, -)	37 (27, 38)
Death	- (-, -)	47 (40, 105)	36.5 (35, 43)	23 (20, 24)	36.5 (28, 39)
Mortality	1/4	4/4	4/4	4/4	10/10

Study FY08-061 subsequently challenged a group of 10 animals to 4x $NHPLD_{50}$/kg of BoNT/A. The results were unexpected in that the median time to onset of signs was much shorter and the progression of clinical signs more rapid than had been estimated from study FY07-027. The time difference between the first clinical sign and respiratory distress was only 3 hours. Due to these results, Cangene lowered the challenge dose to 1.7x $NHPLD_{50}$/kg for the next study (FY09-016). Uniform lethality was achieved in the control group, and the median onset of clinical signs was delayed to 39 hours compared to the majority of animals in study FY08-061 that demonstrated clinical signs at 11 hours as shown in Table 12. Based on the results of study FY09-016, Cangene chose 1.7x $NHPLD_{50}$/kg as the challenge dose for BoNT/A in the pivotal nonhuman primate study FY10-066.

Table 12. Median time to onset of clinical signs, rhesus study FY09-016.

Group	Median Time to Onset (95% Confidence Interval) of Clinical Signs in Hours	Median Duration of clinical signs (hours)	Survival (%)
1x eBAT NP-018	38 (38, 40)	126*	6/6 (100%)
Placebo Control	39 (35, 43)	34**	0/4 (0%)
* Median time from onset of signs to resolution of signs. ** Median time from onset of signs to death.			

Host Susceptibility and Response to Etiologic Agent

Naturally occurring botulism has been documented in numerous animal species including wild ducks, chickens, cattle, and horses (Aiello, 2010). Toxin serotypes C and D are usually associated with animal botulism, although serotype B is responsible for shaker foal syndrome (analogous to infant botulism in humans) in the eastern United States. In general, most species are susceptible at some level of toxin exposure although rats are relatively resistant to BoNT/B intoxication due to sequence variation in the BoNT Lc cleavage target in VAMP proteins (Patarnello, Bargelloni, Rossetto, Schiavo, & Montecucco, 1993), and carrion eaters are also relatively resistant (Gupta, 2012). Experimental botulism has been demonstrated in mice, rats, guinea pigs, rabbits, dogs, and nonhuman primates. Cangene utilized two of these species for its animal model development program, the guinea pig and the nonhuman primate.

Guinea pig

The guinea pig has been utilized as a botulism model for many years, primarily for vaccine research in the biodefense field (Cardella et al., 1963). Cangene justified the use of the guinea pig based on its sensitivity to all seven BoNT serotypes, similarities in the clinical course of disease compared to other species, a similar pathophysiology compared to human botulism, and the reproducibility of the model. The guinea pig also provides a small animal model that allows for studies to be adequately powered through the use of sufficient animals; an important consideration when all seven BoNT serotypes are being tested in the model system. Limitations of the guinea pig system include a truncated clinical progression; the time between intoxication and the development of clinical signs was typically ~ 1 day (Table 13), in contrast to humans that may develop symptoms from 12 hours to 8 days after ingesting BoNT contaminated food (Arnon et al., 2001) or 72 hours after inhaling aerosolized toxin (Holzer, 1962). The ability to deliver supportive care in guinea pigs is also quite limited.

Table 13. Median time (hours) to onset of clinical signs and/or death in guinea pigs, study 1124

Clinical sign	Challenge BoNT at 1.5x $GPIMLD_{50}$						
	Serotype A	Serotype B	Serotype C	Serotype D	Serotype E	Serotype F	Serotype G
Any mild clinical sign		162		183	21		
Any moderate clinical sign	20	25	19	28	8	20	26
Any severe clinical sign	102	117	75	111	29	127	-
Death	115	121	87	120	30	168	-

Nonhuman primate

The rhesus macaque has also been a historically acceptable animal model for botulinum intoxication (Herrero et al., 1967; Oberst, Cresthull, Crook, & House, 1965a, 1965b). Like the guinea pig, the clinical course and pathophysiology of disease are comparable to the human condition and nonhuman primates are sensitive to intoxication with all seven BoNT serotypes. The rhesus does offer advantages in that the clinical signs (ptosis, muscular weakness, descending paralysis) are more directly comparable to human botulism and are easily observable in the model. Moreover, minimum supportive care (nutritional and fluid support) can be provided as needed in order to model a worst case scenario for when the standard level of care (e.g. respiratory support) is not available. Like the guinea pig model however, the nonhuman primate model also demonstrates a truncated clinical course compared to human botulism, with a short interval between the onset of clinical signs and death (Table 14).

Table 14. Median time (hours) to clinical signs and/or death in rhesus macaques challenged intravenously to the indicated dose of BoNT/A, study FY07-027.

Clinical sign	Toxin Dose			
	25 $MIPLD_{50}$/kg	40 $MIPLD_{50}$/kg	60 $MIPLD_{50}$/kg	160 $MIPLD_{50}$/kg
Ptosis	42 (40, 61)	30 (19, 41)	21.5 (17, 32)	17.5 (17, 19)
Muscular Weakness	51 (40, 62)	39.5 (19, 41)	30.5 (26, 36)	18.5 (16, 20)
Respiratory Distress	64.5 (62, 89)	39.5 (36, 43)	34.5 (35, 36)	19 (18, 23)
Oral Discharge	-- (102, --)	47 (34, --)	36 (35, 41)	-- (20, --)
Nasal Discharge	--	--	-- (33,--)	-- (20,--)
Death	-- (--, --)	47 (40, 105)	36.5 (35, 43)	23 (20, 24)

-- Estimate could not be calculated due to limited number of events.

Natural History of Disease

Time to Onset of Disease/Condition

The time to onset in human botulism following exposure to BoNT is highly variable and depends on serotype, dose, and route of exposure. Botulism caused by intestinal or wound colonization typically exhibits a time to onset greater than disease triggered by ingestion or inhalation. Interestingly, in the 2004 iatrogenic exposure case, patients who received intramuscular injections of up to $8x10^6$ $MIPLD_{50}$ of BoNT/A in the facial region did not present with clinical botulism until 48-72 hours after the injections (Chertow et al., 2006).

As is the case with human botulism, the time to onset of clinical signs for botulism in guinea pigs experimentally intoxicated with BoNT is also dependent on serotype, dose, and route of exposure. Study 621-G005630 established a dose response relationship between BoNT serotypes A-G and onset of clinical signs with the exception of high doses where animals died rapidly and animal observation frequency was not high enough to capture the disease progression. The data from this study was used to perform a regression analysis and predict mean time to onset of clinical signs for multiples of the $GPIMLD_{50}$ as shown in Figure 1 below. Time to onset of clinical signs was also monitored during the model development studies and the pivotal efficacy study. Considerable deviation from the predicted values was observed at the 4x $GPIMLD_{50}$ dose for serotypes A, B, C, and E. For studies where the 1.5x $GPIMLD_{50}$ challenge dose was utilized, the onset of signs was considerably more rapid than predicted, especially for the pivotal study 1180 (see Table 15). The variability may be due to the inherent variability in the mouse potency assay used to quantify the challenge dose of each serotype or due to dosing errors; the sponsor should provide some rationale for the rapid onset of signs in the pivotal trial.

Figure 1. Mean times to onset of clinical signs at multiples of the $GPIMLD_{50}$ for each serotype as predicted by the regression model, guinea pig study 621.

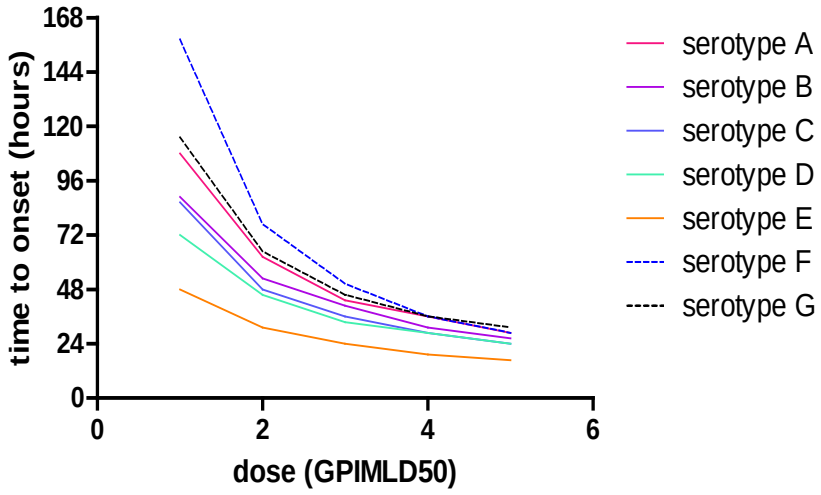


Table 15. Summary of median/mean time (hours) to onset of the first clinical sign across control groups for guinea pig IM challenge studies performed for STN 125462. *Times for study 621 are estimates based on the regression model from that study.

Study	Challenge dose ($GPIMLD_{50}$)	Serotype						
		A	B	C	D	E	F	G
621	4x*	34	31	28	28	19	35	36
670	4x	28	33	30	27	12	26	36
731	4x	46	51	44	32	13	33	38
843	4x	35	-	34	28	30	-	-
964	4x	24	-	-	-	13	-	-
621	1.5x*	81	68	64	57	38	109	85
993	1.5x	33	-	-	-	17	-	-
964	1.5x	41	-	-	-	15	-	-
1005	1.5x	-	36	30	26	-	24	23
1124	1.5x	20	25	19	28	8	20	26
1180	1.5x	14	21	19	21	7	14	17

Similarly, a regression analysis based on observed clinical signs in study FY07-027 (Figure 2) was used to predict time-to-onset in the nonhuman primate model. While time to onset was less variable between studies using the same challenge dose in the rhesus macaque than in the guinea pig, the results did deviate considerably from those calculated in the regression analysis. Moreover, the time to onset in the pivotal efficacy study FY10-066 was significantly delayed compared to that observed in studies FY09-016 and FY09-114 (see Figure 3).

Figure 2. Mean times to onset of clinical signs at multiples of the NHPIVLD$_{50}$ for rhesus macaques challenged with BoNT/A, study FY07-027.

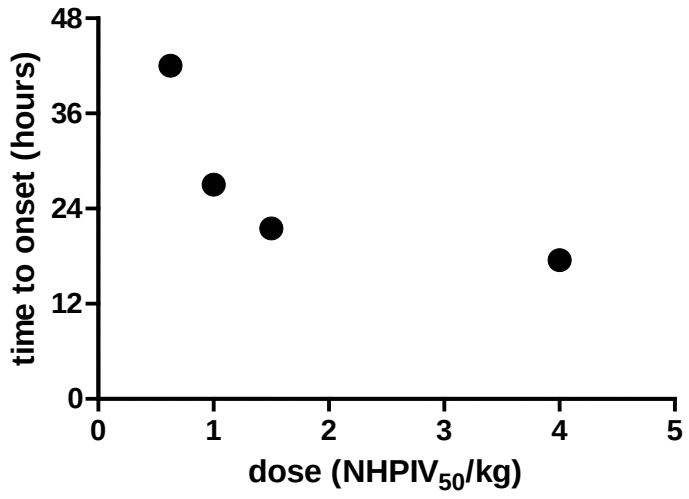
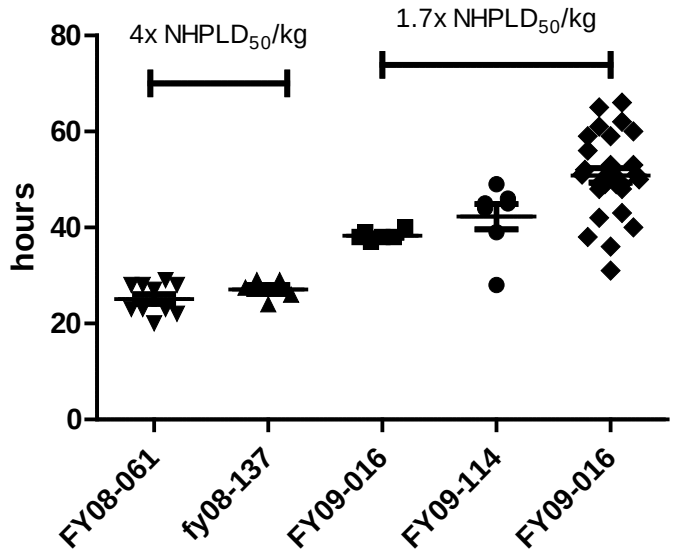


Figure 3. Summary of median time to onset of the first clinical sign across control groups for nonhuman primate intravenous challenge studies performed for STN 125462. The predicted median time to onset for a 1.7x and 4x NHPLD50 BoNT/A challenge is 18.1 and 25.5 hours, respectively, based on study FY07-027.



Time Course of Progression of Disease/Condition and Manifestations (Signs and Symptoms)

Humans with botulism may present with mild signs or symptoms such as ptosis, speech difficulties, or diplopia or rapid progress to severe symptoms including full respiratory paralysis. The disease progression involves cranial nerve dysfunction, followed by a descending flaccid paralysis along with autonomic instability. Patients usually present with vision or swallowing difficulties and in the absence of treatment progress to a loss of control of facial and neck muscles, generalized weakness, and finally to dysphagia, loss of pharyngeal reflex, and respiratory paralysis (Arnon et al., 2001). Depending on the dose of toxin and the route of exposure, disease progression may be rapid; a patient who was intoxicated with BoNT/F progressed to respiratory failure within 24 hours of the onset of symptoms with an estimated circulating toxin level of ~ 1 $MIPLD_{50}$/mL plasma (Sobel et al., 2009).

Like the human, disease progression in the guinea pig is a function of BoNT serotype, dose, and route of exposure and follows a characteristic, reproducible pattern (within a specific serotype). Animals usually display mild signs such as lethargy or ruffled fur, then progress to moderate signs including limb weakness, changes in breathing rate and/or pattern, salivation, or lacrimation. Severe signs of intoxication present last and include total paralysis or forced abdominal respirations. The most consistent clinical signs across all BoNT serotypes were right hind limb weakness and changes in breathing pattern or sounds. Guinea pigs challenged with BoNT/E were interesting in that lacrimation was observed at a higher frequency than with the other serotypes.

Disease progression in nonhuman primates challenged with BoNT/A was different compared to guinea pigs, but did follow the general pattern of a descending flaccid paralysis. The first clinical sign observed was typically ptosis, sometimes in combination with muscle weakness and/or respiratory distress. A comparison of the disease manifestations between humans, guinea pigs, and rhesus macaques is presented in Table 16.

Table 16. Interspecies comparison of the frequency of signs and symptoms following exposure to BoNT/A

Human (Hughes et al. (198)		Guinea Pig (BBRC 670-G005630)		Rhesus Macaque (LBERI FY08-061)	
Symptom/Sign	Frequency (%)	Symptom/Sign	Frequency (%)	Symptom/Sign	Frequency (%)
Fatigue	77	Lethargy	99	Lethargy	ND
Dysphagia	96	Salivation	51	Oral discharge	70
Ptosis	73	Ptosis	ND	Ptosis	100
Lacrimation	ND	Lacrimation	30	Lacrimation	ND
Dyspnea	60	NCBP	90	Respiratory distress	90
Arm weakness	75	Weak limbs	99	Muscular weakness	100
Leg weakness	69				

ND = Not Determined; NCBP = Noticeable Change in Breathing Pattern.

Trigger for intervention

Diagnosis of botulism in humans can be difficult due to the nonspecific initial symptoms (see Table 16, above). In the animal models this is further complicated by the difficulties in recognizing clinical signs such as dry mouth, dysphagia, diplopia, and dilated/fixed pupils. Since Cangene is seeking a treatment indication, it was necessary to identify clinical signs in intoxicated animals that could be reproducibly recognized and serve as a consistent trigger for intervention. Bioassays would not suffice for this purpose due to the rapid disease course and low levels of circulating toxin in the animals.

Based on the model development studies (621-G005630, 670-G005630), pilot post-exposure prophylaxis study 731-G005630, and pilot efficacy studies (993-G005630, 1005-G005630, and 1124-G005630) it was determined that observation of moderate or severe signs of intoxication would be an appropriate treatment trigger in the pivotal efficacy study. In order to ensure reproducibility and minimize the risk that animals might be dosed prematurely, the fourth consecutive observation of any moderate or severe sign of intoxication (salivation, lacrimation, right hind limb weakness, weak limbs, change in breathing sounds or patterns, forced abdominal respirations, or total paralysis) was set as the treatment trigger for the pivotal study 1180-G005630.

Likewise, for rhesus macaques the clinical observations made in model development study FY07-027, pilot post-exposure prophylaxis study FY08-061, and pilot efficacy studies (FY08-137, FY09-016, and FY09-114) were used to define the treatment trigger for the pivotal study FY10-066.

Personnel involved in clinical observation of intoxicated guinea pigs were trained and qualified in this procedure. One performance qualification plan (QD-294) was executed to train and qualify the study personnel at Battelle and involved a written exam, hands-on-training, and a series of clinical observation training sessions on intoxicated animals. Pivotal observations were made by pairs of qualified personnel and verified by the lead technician or Study Director.

Personnel involved in clinical observation of intoxicated nonhuman primates at Lovelace received pre-study training. At least one person during each observation period had participated in at least two previous studies, and a veterinarian was present during each observation shift during study days 1-14.

Characterization of Medical Intervention

Product Class

BAT is a polyclonal hyperimmune product manufactured from the plasma of horses immunized with BoNT or BoNT toxoids. The equine IgG has been digested with pepsin to yield $F(ab')_2$ fragments in order to decrease immunogenicity arising from the equine Fc portion of the molecule. The product contains Fab and $F(ab')_2$ fragments specific for each of the seven BoNT serotypes. The labeled potency (per vial) is at least 4,500 units for serotype A, 3,300 units for serotype B, 3,000 units for serotype C, 600 units for serotype D, 5,100 units for serotype E, 3,000 units for serotype F, and 600 units for serotype G. Each unit is defined as the amount of antitoxin that can neutralize 10,000 $MIPLD_{50}$ of serotypes A, B, C, D, F, and G, and 1,000 $MIPLD_{50}$ of serotype E.

Equine botulism antitoxins have been previously licensed in the United States and an equine $F(ab')_2$ antitoxin for use in scorpion envenomation was licensed in 2011.

Mechanism of Action

Cangene has not studied the *in vitro* activity of BAT. The product potency is measured, however, using a bioassay whereby dilutions of product are mixed with each serotype of BoNT prior to injection into mice and neutralization capacity determined by probit analysis of the resulting survival curves. Neutralization in this assay is a result of the sequestration of toxin by the polyclonal antibody fragments, thus precluding BoNT binding to neurons and resulting toxicity. In vivo, the protective effect of antibodies directed against BoNT involves both steric hindrance between the antibody-bound toxin and its receptors as well as immune-mediated clearance of the antibody-toxin complexes(Cheng, Stanker, Henderson, Lou, & Marks, 2009). These immune complexes are likely removed via hepatic clearance mechanisms (Al-Saleem et al., 2008; Ravichandran et al., 2006). Since the mechanism of action depends on clearance of circulating toxin prior to entry into the neuron, BAT will not reverse clinical symptoms or signs once they develop and should be administered as soon as possible after the onset of neurological symptoms

Pharmacokinetics

Pharmacokinetics of BAT in guinea pigs, rhesus macaques, and humans are described in the **Pharmacokinetic studies** section, below.

Synergy or antagonism of medical products likely to be used in combination

Antihistamines and/or corticosteroids are frequently dosed concomitantly with non-human plasma derivative products in order to reduce the risk of allergic reactions, and NSAIDS are often used to manage minor adverse reactions. Non-aminoglycoside antibiotics may be used to manage secondary infections acquired during the lengthy hospitalization stays associated with severe botulism.

No combination treatments were studied in the BAT animal model development program, and pretreatments with antihistamines/corticosteroids were not utilized in the human safety trials. No information was available on the use of pretreatments in CDC's IND.

Design considerations for Animal Efficacy Studies

Endpoints

The primary endpoint for both guinea pig and rhesus macaque pivotal animal efficacy studies was survival at 21 days post-intoxication. Predetermined euthanasia criteria were utilized to provide a humane and consistent survival endpoint.

Timing of intervention

For the pivotal therapeutic studies, animals were dosed with placebo or BAT upon clinical observations defined as the treatment trigger. Each animal was evaluated individually and treated separately upon exhibiting the treatment trigger.

Route of Administration

The route of administration of BAT in the animal studies is identical to that used in humans, specifically via intravenous infusion.

Dosing Regimen

Both guinea pigs and rhesus macaques received a 1x scaled human dose (based on weight) delivered as a single intravenous infusion. For a description of the dose scaling, please refer to the Population PK/PD modeling and simulations to support section below.

Animal Efficacy studies

Guinea pigs

Initial Efficacy Study 1124-G005630

Study 1124-G005630 was a non- Good Laboratory Practice compliant study performed at Battelle Biomedical Research Center in West Jefferson, OH. The goal of the study was to demonstrate efficacy of BAT when administered to guinea pigs challenged with a lethal dose of any one of BoNT serotypes A, B, C, D, E, F, or G. Efficacy was tested by comparing survival in the BAT groups to a placebo control. Guinea pigs were randomized to fourteen groups consisting of 12 animals per sex. Each paired group was challenged with 1.5x $GPIMLD_{50}$ of a single BoNT serotype delivered as an intramuscular injection to the right hind limb, and upon the fourth consecutive observation of any moderate or severe sign of botulism individual animals were treated with a 1x scaled human dose of BAT or a placebo. Animals were monitored for clinical signs, disease progression, and survival for 21 days.

Results from the study are summarized in Table 17. The majority of guinea pigs were treated upon the fourth observation of weakness in the right hind limb. In addition to an increase in survival, animals treated with BAT tended to demonstrate a shortened duration of clinical signs

Table 17. Summary of survival, median time to death, and log-rank comparisons between BAT and placebo treated groups of guinea pigs challenged with 1.5x GPIMLD$_{50}$ of BoNT serotypes A, B, C, D, E, F, or G.

BoNT Serotype	Group	Treatment Dose Level	Survival (percent)	Kaplan-Meier Median Time to Death (95% Confidence Interval) in Hours [4]	Log-Rank Test Time-to-Death Comparison (p-value)
A	1	1.0x NP-018 [1]	24/24 (100%)	-- (--)	<0.0001 [3]
	2	Placebo Control [2]	0/24 (0%)	115 (103, 118)	
B	3	1.0x NP-018 [1]	24/24 (100%)	-- (--)	<0.0001 [3]
	4	Placebo Control [2]	1/24 (4%)	121 (100, 124)	
C	5	1.0x NP-018 [1]	24/24 (100%)	-- (--)	<0.0001 [3]
	6	Placebo Control [2]	0/24 (0%)	87 (66, 99)	
D	7	1.0x NP-018 [1]	22/23 (96%)	-- (--)	<0.0001 [3]
	8	Placebo Control [2]	2/19 (11%)	120 (99, 123)	
E	9	1.0x NP-018 [1]	23/23 (100%)	-- (--)	<0.0001 [3]
	10	Placebo Control [2]	1/24 (4%)	30 (28, 33)	
F	11	1.0x NP-018 [1]	24/24 (100%)	-- (--)	<0.0001 [3]
	12	Placebo Control [2]	11/23 (48%)	168 (92, --) [4]	
G	13	1.0x NP-018 [1]	24/24 (100%)	-- (--)	0.0021 [3]
	14	Placebo Control [2]	16/24 (67%)	-- (--)	

[1] = Compared to proposed human clinical NP-018 dose (mL/kg basis)
[2] = Normal Equine Immune Globulin
-- = Either the animal death was not observed or the Kaplan-Meier estimates could not be calculated due to censoring.
[3] = Comparison significant at the 0.05 level of significance.
[4] = The upper bound of the 95 percent confidence interval could not be estimated due to the high incidence (48%) of censoring in the data.

Pivotal Efficacy Study 1180-G005630

Study 1180-G005630 was the pivotal efficacy study performed in guinea pigs at Battelle Biomedical Research Center in West Jefferson, OH. The study was performed under Good Laboratory Practices per 21 CFR Part 58. The study was conducted in phases, with a single serotype challenge conducted in each phase. Guinea pigs equipped with jugular catheters were randomized into fourteen groups with each group containing seventeen animals per sex, and paired groups were challenged with 1.5x GPIMLD$_{50}$ of the appropriate BoNT serotype delivered via intramuscular injection into the right hind limb. An additional six animals (three per sex) were intoxicated with each serotype to serve as replacements in the event that a group-assigned animal could not be treated for any reason; the number of animals actually used in the study and the target GPIMLD$_{50}$ compared to the MIPLD$_{50}$ potency is provided in Table 20. The guinea pigs were monitored frequently (every 30min-1 hour, depending on serotype) for

clinical signs, and upon the fourth consecutive moderate or severe sign of botulism were treated with a single infusion of 0.16 mL/kg of BAT lot 2060401Y or placebo delivered into the jugular catheter. The null hypothesis that there would be no improvement in survival at 21 days post-challenge between BAT treated and placebo treated groups for each BoNT serotype. Secondary endpoints included median time to death, incidence of clinical signs, time to onset of clinical signs, and time to resolution of clinical signs. Predefined euthanasia criteria were specified in the study protocol and used during the study; animals were euthanized if they exhibited 25% or greater loss of body weight in conjunction with any concurrent severe sign of intoxication, two consecutive observations of complete paralysis, or if a determination was made by the study director or a veterinarian that the animal was moribund.

Table 18. Group assignments and target BoNT doses used in study 1180.

BoNT Serotype	Group ID	BoNT Dose		Intoxication Route	Treatment	Number of Animals	
		$GPIMLD_{50}$	$MIPLD_{50}$			F	M
A	A1	x1.5	6.8	IM	NP-018	17	17
	A2				Placebo Control	17	17
B	B1	x1.5	17.6	IM	NP-018	17	17
	B2				Placebo Control	17	17
C	C1	x1.5	3.9	IM	NP-018	16	18
	C2				Placebo Control	14	20
D	D1	x1.5	9.3	IM	NP-018	18	16
	D2				Placebo Control	17	17
E	E1	x1.5	121.8	IM	NP-018	16	18
	E2				Placebo Control	17	17
F	F1	x1.5	65.0	IM	NP-018	18	16
	F2				Placebo Control	17	17
G	G1	x1.5	84.6	IM	NP-018	17	17
	G2				Placebo Control	17	17

A single lot of each BoNT serotype was used in this study, and each had been reasonably well characterized in the model development studies. The potency of each BoNT serotype dilution used in the study was confirmed via mouse bioassay and each was within ± 20% of the target dose. Likewise, the BAT dilutions used for treatment were sampled pre and post infusion and tested for potency using the mouse bioassay. The dilutions were within ± 20% of the target doses. Treatments were initiated within 45 minutes of the treatment trigger observation, with the exception of several animals in the serotype E challenge group that received treatment between 1 and 10 minutes late. The majority of animals across all serotypes were treated based on four consecutive observations of weakness in the right hind limb. Overall, the majority of animals on this study were euthanized based on two consecutive observations of paralysis, although the proportion varied based on toxin serotype. Challenge groups for serotypes D, F, and G were more variable in terms of euthanasia trigger and

serotypes F and G in particular tended to show fewer animals euthanized due to paralysis and more animals found dead or moribund. Serotypes D and G were remarkable due to the increased incidence of weight loss in the control animals. For an overview of the disposition of animals in study 1180, please refer to Table 19.

Table 19. Disposition of control (placebo-treated) animals, study 1180.

BoNT serotype	# of animals dead	# of animals euthanized	# of animals euthanized due to paralysis	# of animals euthanized due to weight loss	# of animals euthanized when found moribund	# of animals found dead
A	34	34	32	0	2	0
B	33	33	29	1	3	0
C	30	29	21	0	8	1
D	29	28	16	10	2	1
E	34	34	34	0	0	0
F	30	24	9	3	12	6
G	17	13	4	6	0	4

Survival results and a statistical comparison of the treatment and placebo groups from study 1180 are presented in Table 20. The intent to treat analysis set included all animals that were intoxicated with BoNT and survived to receive the test or placebo articles. High mortality was observed in all placebo groups, with the exception of serotype G where 50% of challenged animals survived. The median time-to-death could not be calculated for the treatment groups due to low mortality, and the upper limit for the serotype G control group could not be estimated due to the level of censoring in the data. The median time to death in serotype A, B, C, D, and E control groups was consistent with preliminary study 1124. The median time to death was markedly shorter in study 1180 for serotypes G and F; however the animals were underdosed with these serotypes in study 1124 based on analysis of the toxin challenge material with the mouse potency assay.

Prior to treatment, clinical signs developed at a comparable incidence and timing between the treatment and control groups. Clinical signs in animals treated with BAT continued to progress for a time after administration of BAT; however, the median time-to-onset of signs was decreased and the duration of signs was shorter; see Figure 4. In control animals, clinical signs typically began with right hind limb weakness and progressed through a change in breathing and generalized weakness before the animals exhibited severe signs such as total paralysis or forced respiratory respirations. Clinical signs in treated animals would arrest at right hind limb weakness and/or breathing changes, and would slowly improve over the study. Notably, no treated animals exhibited a regression of clinical signs that would be expected if an insufficient amount of BoNT had been neutralized.

Finally, there was a statistically significant difference in survival across all BoNT challenge groups treated with BAT, indicating that the product was effective in providing a clinically relevant benefit after a lethal exposure to BoNT (see Table 20).

Figure 4. Mean clinical severity scores for each BoNT serotype challenge, study 1180. Time of treatment is indicated by a dotted vertical line.

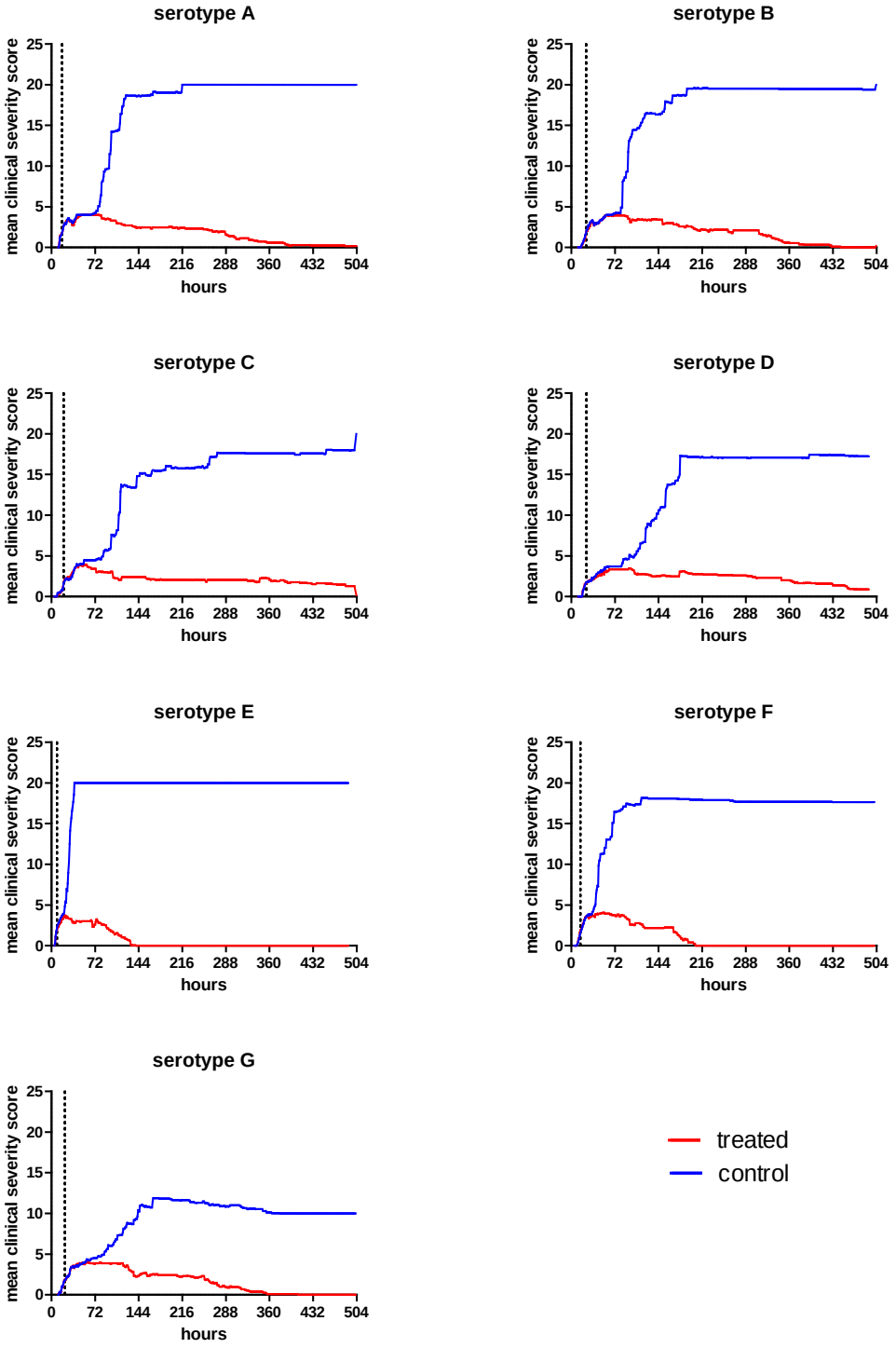


Table 20. Intent to treat analysis set survival comparisons, study 1180.

Serotype	Group	Number Survived/Total Animals (Percent Survived) (95 Percent Confidence Interval)	Two-Sided Fisher's Exact Test Comparison (P-Value)
A	A1 (Treated)	34/34 (100%) (90%, 100%)	<0.0001*
	A2 (Control)	0/34 (0%) (0%, 10%)	
B	B1 (Treated)	34/34 (100%) (90%, 100%)	<0.0001*
	B2 (Control)	1/34 (3%) (0%, 15%)	
C	C1 (Treated)	33/34 (97%) (85%, 100%)	<0.0001*
	C2 (Control)	4/34 (12%) (3%, 27%)	
D	D1 (Treated)	33/34 (97%) (85%, 100%)	<0.0001*
	D2 (Control)	5/34 (15%) (5%, 31%)	
E	E1 (Treated)	34/34 (100%) (90%, 100%)	<0.0001*
	E2 (Control)	0/34 (0%) (0%, 10%)	
F	F1 (Treated)	34/34 (100%) (90%, 100%)	<0.0001*
	F2 (Control)	4/34 (12%) (3%, 27%)	
G	G1 (Treated)	34/34 (100%) (90%, 100%)	<0.0001*
	G2 (Control)	17/34 (50%) (32%, 68%)	

Nonhuman primates

Two nonhuman primate (NHP) studies were conducted by Cangene in order to support the efficacy of BAT for the treatment of intoxication by BoNT/A under the Animal Rule. These NHP studies were performed at Lovelace Biomedical and Environmental Research Institute in Albuquerque, New Mexico under Good Laboratory Practices (GLP) using rhesus macaques (*Macaca mulatta*). Both studies utilized a 1.7x/kg $NHPLD_{50}$/kg challenge of BoNT/A administered intravenously (IV) through a surgically implanted indwelling central venous catheter. Twenty-three hours following the toxin challenge, the animals were monitored hourly for clinical signs of intoxication (ptosis, muscular weakness, and/or respiratory distress), and an assessment of food consumption. Immediately after the onset of clinical signs in each animal, BAT at 1x scaled human dose (0.26 mL/kg) or Botulinum Antitoxin Placebo (0.31 mL/kg, based on equivalent protein dose) was administered intravenously via the central venous catheter.

Initial Efficacy Study FY09-114

Eighteen (18) rhesus macaques (9 per sex) were randomly allocated into two treatment groups; Group 1 animals (n = 10, 5/sex) for BAT treatment, and Group 2 animals (n = 7, 4 males and 3 females) for placebo control. One Group 2 female animal was removed from the study because of catheter damage. On Day 0, all animals received a dose equivalent to ~44 $MIPLD_{50}$/kg (~1.7x $NHPLD_{50}$/kg) of BoNT/A Complex as a single intravenous injection via a catheter placed in a saphenous vein. Starting about 23 hours after toxin administration, all animals were monitored approximately hourly for clinical signs of intoxication and assessment of food consumption. Immediately after the onset of clinical signs in each animal either BAT at 1x scaled human dose (0.16 mL/kg) or Botulinum Antitoxin Placebo (0.31 mL/kg, based on equivalent protein dose) was administered intravenously via the central venous catheter. Within 26 minutes of antitoxin or placebo administration, minimal support was initiated in all animals (Groups 1 and 2) as parenteral nutrition, administered through the central venous catheter via constant

rate infusion by use of an ambulatory infusion pump. Dehydration was detected in four animals on Day 5, treated by administration of lactated Ringer's solution via the central venous catheter. Clinical signs in all animals in both groups were consistent with BoNT intoxication. The Kaplan-Meier median time-to-onset of initial clinical signs was 42.5 hours and 47 hours for Groups 1 and 2, respectively. Initial clinical signs included ptosis (n = 7), muscular weakness (n = 6), ptosis and muscular weakness (n = 3), and ptosis and respiratory distress (n = 1) (See Figure 5)

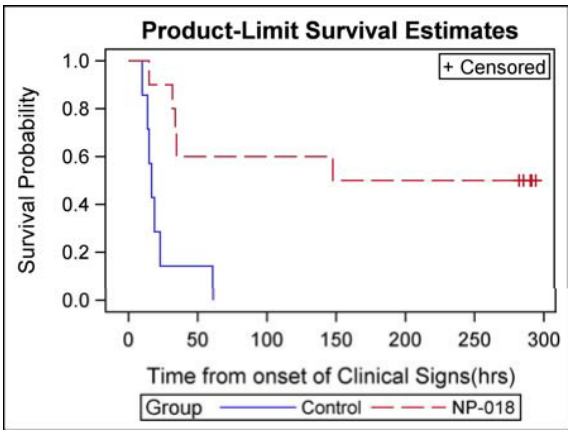
All (7/7) placebo-treated Group 2 animals were euthanized prior to study scheduled termination due to poor physical condition, with a median time to euthanasia of 65 hours. Five out of ten (5/10) Group 1 animals recovered (i.e., without clinical signs excluding food consumption), and survived to the end of the study (Day 14). The remaining Group 1 animals (5/10) were euthanized due to poor physical condition, with a median time to death of 79 hours post-intoxication.

Analysis of these results confirmed a statistically significant improvement in survival of BAT treated animals (see Table 21). The median time interval between the onset of clinical signs and euthanasia for Group 1 and Group 2 animals was 35 hours and 17 hours, respectively.

Table 21. Comparison of survival between rhesus macaques challenged with BoNT/A and administered BAT or placebo.

Treatment Group	Treatment description	Survival rate (%)	Two-sided Fisher's exact test
Group 1	121 U/kg (0.26 mL/kg) BAT (1x scaled human dose)	5/10 (50%)	P=0.044
Group 2	0.31 mL/kg botulism antitoxin placebo	0/7 (0%)	

Figure 5. Kaplan-Meier Curves Associated with Duration of Clinical Signs Defined as the Interval Between Onset of Clinical Signs (sum > 0) to Death (h).



Pivotal Efficacy Study FY10-066

Sixty rhesus macaques (29 males and 31 females) were used in this study. Animals were first randomly allocated to two treatment groups; Group 1 animals (n = 30, 14 males and 16 females, one male was removed from the original 15 immediately before the start of the study because of health reasons and only a female was available to replace him) for BAT treatment, and Group 2 animals (n= 30, 15/sex) for Placebo Control. Animals from each treatment group were then randomly allocated to cohorts. On Day 0, all animals received a dose equivalent to 1.7x $NHPLD_{50}$/kg (~44 $MIPLD_{50}$/kg) of BoNT/A Complex as a single intravenous injection via a catheter placed in a saphenous vein. Starting 23 hr after intoxication, all animals were monitored hourly (± 10 minutes) for clinical signs of intoxication and assessment of food consumption. Immediately after the onset of clinical signs indicative of BoNT intoxication in each animal (ptosis, muscular weakness, and/or respiratory distress), BAT at a 1x scaled human dose (0.26 L/kg) or Botulinum Anitoxin Placebo (0.31 mL/kg, based on equivalent protein dose) was administered intravenously via the central venous catheter. Animals were observed for 21 days.

Minimal supportive care (nutrition) was initiated in all animals (Groups 1 and 2) within 23 min of antitoxin or placebo administration and was either administered parenterally through the central venous catheter via constant rate infusion by use of an ambulatory pump, or via oral gavage of liquid rhesus monkey diet. None of the nonhuman primates challenged with BoNT/A complex and treated with placebo survived to the end of the study, whereas, 14 of 30 (46.7%) nonhuman primates treated with BAT survived to the end of the study. This difference in survival rate between the two groups was statistically significant.

Clinical signs in all animals in both groups were consistent with BoNT intoxication. Initial clinical signs included ptosis (n = 13), muscular weakness (n = 22), respiratory distress (n = 14), ptosis and muscular weakness (n = 1), ptosis and respiratory distress (n = 4), and muscular weakness and respiratory distress (n = 6).

All (30/30) placebo-treated Group 2 animals were euthanized prior to study scheduled termination due to signs of botulism and poor physical condition, with a median time to death of 74.5 hours. However, fourteen of thirty (14/30) Group 1 animals recovered (i.e., without clinical signs excluding food consumption) after the onset of clinical signs, and survived to the end of the study (Day 21). The remaining Group 1 animals (16/30) died or were euthanized due to poor physical condition, with a median time to death of 189.5 hr post-intoxication.

Treatment with BAT delayed the progression of BoNT intoxication resulting in euthanasia, when compared with placebo. Analysis of these results confirmed a statistically significant improvement in survival of BAT-treated animals (see Table 22). The median time interval between the onset of clinical signs and recovery (resolution of clinical signs) for Group 1 animals was 137 hr.

Table 22. Summary of survival and median time to death for rhesus macaques in study FY10-066

Treatment	Survival (%)	Fisher's Exact Test (p-value)*	Median Time to Death in Hours (95% Confidence Interval)	Log- Rank Test (p-value)*
1x eBAT NP-	14/30 (47%)	p<0.0001	189.5 (102, –)[a]	p<0.0001
Placebo control	0/30 (0%)		74.5 (63, 81)	

[a] The upper bound of the confidence interval could not be estimated due to the limited number of events (i.e., 14 animals survived until study termination). *Comparison significant at the 0.05 level of significance.

Additional NHP Studies

Prior to conducting the 2 GLP efficacy studies in NHP, Cangene performed the following natural history, PK, PEP, and pilot NHP studies:

- FY07- 027 GLP Lethal Dose and Clinical Course - Determine the lethal dose (NHPLD50) of botulinum serotype A in the Rhesus macaque - Animals administered a toxin dose equivalent to 0.625x, 1.0x, 1.5x, and 4.0x/kg of the expected $NHPLD_{50}$ as an IV injection and observed to determine survival and clinical progression. 16 animals, 8/sex, 4/toxin dose group
- FY07- 056 GLP PK Study – Single IV Dose BAT to determine PK in 12 healthy non-intoxicated animals.

- FY08-061 GLP Post-Exposure Prophylaxis (PEP) efficacy Study - Single IV Dose (0.1X or 1X scaled dose) BAT administered 4 hours after 4X $NHPLD_{50}$/kg challenge of botulinum serotype A neurotoxin. All animals survived in both treatment groups and there was 100% mortality in the placebo control group. 30 animals, 10 per group (1x, 0.1x, placebo)

- FY08-137 non-GLP Exploratory Therapeutic Efficacy - Single IV Dose (1X scaled dose) BAT administered after development of clinical signs of intoxication following a 4X $NHPLD_{50}$/kg challenge of botulinum serotype A neurotoxin with some animals receiving full respiratory support. 4 animals were challenged with BoNT/A and treated with 1x scaled human dose of BAT at the first sign of intoxication, then 2 animals were placed on supportive respiratory care including anesthesia and full mechanical ventilation as well as parenteral nutrition and fluid management.

- FY09-016 non-GLP Pilot Therapeutic Efficacy - Single IV Dose (1X scaled dose) BAT or placebo administered after development of clinical signs of intoxication following a 1.7X $NHPLD_{50}$/kg challenge of botulinum serotype A neurotoxin. 14 animals, 10 in treatment group 5/sex, and 4 in placebo group 2/sex.

Clinical Pharmacology

Pharmacokinetic studies

Pharmacokinetic (PK) studies of BAT were conducted in guinea pig, rhesus monkey, and humans. The following is the summary of PK studies in these species.

Guinea Pigs:

Two hundred and sixty-four male guinea pigs (mean body weight 0.5 kg) were dosed intravenously at two doses (x0.2 (low) and x1.0 (high) human dose of one vial per 70 kg person). The study was designed to analyze all 7 serotypes. Blood samples were collected from 12 animals per group up to 12 days post-dose. Serum samples were analyzed for each serotype (U/mL) by a mouse neutralization assay. Concentration-time profiles for the Serotype D and E low dose groups were incomplete due to insufficient number of measurable concentrations which prevented adequate PK analysis. PK parameters were estimated by non-compartmental analysis.

Non-compartmental analysis showed that the high dose was 5-fold higher than the low dose for all serotypes and the AUC was dose proportional for serotypes B, C, and G. For serotype A, the AUC was about 7.4-fold higher than the low dose indicating serotype A was not dose proportional. The half-life ranged from 2 to 7 hours at the low dose level and from 3 to 15 hours at the high dose level.

Rhesus Macaque:

Twelve non-human primates were randomly assigned to two treatment groups. On Day 0, all animals received a single BAT IV dose. Group 1 animals were dosed at x5 scaled human dose, and Group 2 animals were dosed at x1 scaled human.

Blood samples were collected from all animals prior to dose administration and up to 20 post-dose. BAT concentrations (only serotype A) were measured in serum using a mouse neutralization assay. The PK parameters were estimated by non-compartmental analysis.

Results showed that the pharmacokinetics of BAT (serotype A) is non-linear (AUC did not increase proportionally with dose). The half-life of BAT is about 2 hours longer at the high dose as compared to low dose. There was no gender difference in the PK of BAT.

Humans:

This was a phase 1, single-center, randomized, double-blind, parallel arm study. BAT was intravenously administered to healthy, male and female volunteers between the ages of 19 and 52 years. Forty subjects were randomized to receive either one or two vials of BAT, representing a single or double dose of botulinum antitoxin. Each dose was administered by slow intravenous infusion over 2.5 hours. The infusion rate was incremental, starting slowly and increasing if no safety related events were evident.

Blood samples for pharmacokinetic study were collected after BAT administration for botulinum toxin up to Day 28. Pharmacokinetic parameters were calculated by non-compartmental analysis using the concentration-time data generated from the mouse neutralization assay (see Table 23).

Table 23. Pharmacokinetic parameters (Mean and CV%) for antitoxin serotypes A-G in humans following intravenous administration of either one or two vials of BAT

Antitoxin Serotype	Treatment Group	AUC_{0-t} U*hr/mL	$AUC_{0-\infty}$ U*hr/mL	C_{max} U/mL	T_{max} hr	$T_{1/2}$ hr	CL mL/hr	Vd mL
A	1 Vial	21.40 (20.1)	26.00 (13.1)	2.69 (28.2)	0.70 (115.0)	8.64 (15.5)	293 (13.5)	3637 (17.1)
	2 Vials	51.92 (27.4)	56.09 (24.7)	6.23 (22.0)	0.53 (4.1)	10.20 (34.7)	285 (27.1)	3993 (27.7)
B	1 Vial	27.16 (23.0)	29.30 (19.2)	1.90 (42.7)	0.89 (125.0)	34.20 (40.4)	196 (24.0)	9607 (48.1)
	2 Vials	58.53 (16.6)	62.55 (16.4)	4.28 (33.8)	0.54 (8.9)	57.10 (69.4)	181 (19.3)	14865 (71.5)
C	1 Vial	36.63 (25.2)	37.34 (27.3)	2.26 (38.1)	1.90 (150.0)	29.60 (44.9)	144 (28.1)	6066 (52.8)
	2 Vials	78.75 (29.0)	86.25 (28.8)	4.89 (41.7)	0.53 (4.1)	45.60 (34.0)	127 (33.9)	8486 (52.4)
D	1 Vial	5.58 (48.5)	7.62 (23.3)	0.81 (55.0)	0.89 (124.0)	7.51 (24.4)	137 (19.9)	1465 (25.8)
	2 Vials	13.27 (33.7)	14.83 (39.4)	1.60 (38.8)	0.70 (111.0)	7.77 (21.6)	151 (30.5)	1653 (30.5)
E	1 Vial	6.65 (32.4)	7.16 (23.9)	0.94 (41.6)	0.52 (3.9)	7.75 (19.1)	1250 (25.1)	14172 (32.1)
	2 Vials	13.47 (20.2)	15.66 (15.6)	1.75 (32.9)	0.53 (9.4)	7.32 (23.9)	1110 (16.4)	11596 (22.4)
F	1 Vial	29.12 (29.1)	31.40 (25.4)	2.37 (25.8)	1.07 (122.0)	14.10 (16.7)	169 (25.5)	3413 (29.3)
	2 Vials	59.63 (26.5)	63.19 (25.0)	4.29 (31.0)	1.63 (149.0)	18.20 (42.9)	168 (26.4)	4334 (50.9)
G	1 Vial	6.33 (26.2)	7.05 (23.3)	0.59 (27.7)	1.28 (154.0)	11.70 (38.1)	149 (22.8)	2372 (29.5)
	2 Vials	13.40 (27.3)	14.66 (23.6)	1.19 (43.8)	0.70 (111.0)	14.70 (16.5)	144 (23.8)	3063 (29.4)

AUC = Area Under the Concentration Curve; CL = Systemic Clearance; C_{max} = Maximum Serum Concentration; CV = Coefficient of Variation; eBAT NP-018 = Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) – (Equine); $T_{1/2}$ = Half-life; T_{max} = Time to Maximum Serum Concentration; U = Units; Vd = Volume of Distribution.

The estimated pharmacokinetic parameters AUC, clearance, and half-life varied based upon the antitoxin serotype measured (Table 1). Both $AUC_{(0-\infty)}$ and C_{max} values increased in a dose proportional manner as BAT doses increased from one to two vials. The half-lives of the different antitoxin serotypes varied with the serotype. Antitoxin serotypes D and E had the shortest mean half-lives, ranging from 7.5 to 7.8 hours whereas, antitoxin serotypes B had the longest mean half-lives, ranging from 34.2 (one vial) to 57.1 (2 vials) hours. Comparison of the pharmacokinetic parameters between male and female subjects for antitoxin serotypes A through G showed that there were no gender related differences following a single intravenous administration of either one or two vials of BAT.

Comparison of Clearance among Species:
The clearance of BAT appears to be much slower in humans than guinea pig and monkey for serotype A. Based on per kg basis; human clearance for serotype A is approximately 3.5-fold lower than guinea pig and monkey. The human clearance of serotypes B to G is almost 2.7 to 6.2 fold lower than guinea pigs. This comparison is helpful in justifying the proposed human dose (discussed later).

Population PK/PD modeling and simulations to support human dosing regimen of BAT

The objective of this study was to present pharmacokinetic (PK) and pharmacodynamic (PD) modeling results using data related to guinea pigs, non-human primates (NHP) and humans in order to support the human dosing regimen of BAT.

PK and PD information of BAT collected in a total of 5 studies (3 PK, 2 post-exposure prophylaxis PD) performed by Cangene Corporation that were included in the analysis. The results from treatment studies performed in guinea pigs and non-human primates were not included in this analysis.

Population PK analysis of BAT for all serotypes were best fitted using a 3-compartment model, with the exception of Serotype E which was fitted using a 2-compartment model. Logistic regression was used to explore the relationship between BAT exposure (AUC) predicted by the population PK model and the probability of survival. The human projected probabilities of survival for serotypes A to G following a human dosing of 1x BAT ranged from 96% to 99.9%.

Clinical pharmacology based human dose justification

BAT is indicated for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin (BoNT) serotypes A, B, C, D, E, F or G. The proposed dose of BAT in adults is 1 vial, with a final fill volume (range ---(b)(4)--) depending on actual drug potencies. There are no human efficacy data for BAT. As a result the human efficacious dose was derived from animal pharmacokinetics and efficacy studies and a human pharmacokinetic study.

In guinea pigs, efficacy (post-exposure prophylaxis) of BAT was assessed following a single intravenous (IV) dose of BAT at ×1, ×0.2, ×0.04, or ×0.008 scaled human doses. Not more than 1 death, out of a total of 20 animals per serotype, was noted in the BAT group for all 7 serotype at a scaled human dose of ×1. A similar observation was noted in a separate pivotal efficacy (treatment) study.

In non-human primates (NHP), efficacy (post-exposure prophylaxis) of BAT was assessed following a single intravenous dose of BAT at ×1, and ×0.1 scaled human dose. In both dose groups, all animals survived. In a separate treatment study, animals received BAT at x1 scaled human dose. Only 47% of animals survived. All efficacy studies in NHP only assessed serotype A.

Pharmacokinetic studies in guinea pig, NHP, and humans indicated that the clearance of BAT (x1 scaled human dose) in humans is 3 to 5 times slower than guinea pigs and NHP, indicating higher exposure of BAT in humans than animals. This indicates that the humans will have higher protective drug levels against botulinum toxin compared to animals.

Pharmacokinetic and pharmacodynamic modeling and simulation supported the efficacy of an adult dose of 1 vial BAT (11.2 mL) in humans. Based on the results of an exposure-response analysis, the predicted probabilities of human survival for the serotypes A to G following a dosing of 1x BAT ranged from 95.9%, to 99.9%.

In humans, a pharmacokinetic study of BAT was analyzed following a single intravenous dose of BAT at ×1, and ×2 scaled human doses. The sponsor's goal stated that BAT serum concentrations after IV administration of 1 vial (×1) should be able to counter BoNT serum concentrations of 400 to 20,000 MIPLD50/mL.

Table 24 displays the relationships between the 7 BoNT subtypes, measured (observed) maximal serum drug concentrations (at 30 min after start of the IV infusion) using actual potencies, and the calculated neutralizing capacity (NC) against the different BoNT subtypes. The neutralizing concentrations (NC) were calculated as follows: 1 U of BAT neutralizes 10,000 $MIPLD_{50}$/mL BoNT for all serotypes except for serotype E, which equates 1 U of BAT to 1,000 $MIPLD_{50}$/mL. The range of the NC based on observed drug concentration is between 940 and 26,900 $MIPLD_{50}$/mL. Even using the lower limit specifications for potencies the NC against BoNT is still between 400 and 20,000 $MIPLD_{50}$/mL. Compared to an actual measurement of BoNT in a patient's serum (≈ 160 $MIPLD_{50}$/mL, foodborne, serotype E) the predicted NC of 1 vial BAT, based on PK results of a clinical study (study no. BT-001), seems to be more than sufficient to counter expected BoNT concentrations in humans.

Table 24. BAT proposed specification, clinical lot 2060401X potency, observed C_{max}, and neutralizing capacity against BoNT

BoNT Serotype	Proposed specification [U/vial]	Clinical Lot 2060401X Potency (actual) [U/vial]	BAT C_{max} (human) Mean data, N = 20 [U/mL]	Clinical Lot 2060401X Neutralizing Capacity against BoNT [$MIPLD_{50}$/mL]
A	>4,500	10,399	2.69	26,900
B	>3,300	7,071	1.90	19,000
C	>3,000	7,138	2.26	22,600
D	>600	1,452	0.81	8,100
E	>5,100	10,690	0.94	940
F	>3,000	6,546	2.37	23,700
G	>600	1,229	0.59	5,900

In short, the study design and conclusions of the pharmacokinetic and pharmacodynamic studies are acceptable, and, based on these studies it appears that an adult human dose of one vial of BAT will be efficacious in adult humans intoxicated with BoNT.

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