Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) – (Equine)

Treatment of Symptomatic Botulism

BLA 125462

Briefing Document for the Biologic Products Advisory Committee

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List of Abbreviations and Definition of Terms

ADR	Adverse Drug Reaction
AE	Adverse Event
ATA	Anti-therapeutic Antibody
AUC	Area Under the Concentration Curve
$AUC_{0-\infty}$	Area Under the Concentration Curve from Zero to Infinity
AUC_{0-t}	Area Under the Concentration Curve from Zero to Time t
BabyBIG	Botulism Immune Globulin Intravenous (Human)
BARDA	Biomedical Advanced Research and Development Authority
BAT	Botulism Antitoxin
BAT AB	Botulism Antitoxin Bivalent (Equine) Types A and B
BBRC	Battelle Biomedical Research Center
BIG-IV	Botulism Immune Globulin Intravenous (Human)
BLA	Biologic License Application
BoNT	Botulinum Neurotoxin(s)
BVDM	Bovine Viral Diarrhea Virus
BW	Body Weight
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
9CFR	Code of Federal Regulations - Title 9: Animals and Animal Products
cGMP	current Good Manufacturing Practice
CI	Confidence Interval
CL	Systemic Clearance
CLd	Distribution Clearance
CLdt	Deep Tissue Distribution Clearance
CMAP	Compound Muscle Action Potential
C_{max}	Maximum Serum Concentration
CMV	Cytomegalovirus
CV	Coefficient of Variation
DoD	Department of Defense
EAP	Expanded Access Program,
ED_{50}	Median Effective Dose
EDB	Extensor Digitorum Brevis
Fab′	An immune globulin fragment (F) consisting of one antigen (ab′) domain made of the light-chain and heavy-chain fragment
$F(ab')_2$	An immune globulin fragment (F) consisting of two antigen $(ab')_2$ domains made of two light-chains and two heavy-chains joined by a disulfide bond
Fc	A region of an antibody which is constant and binds to receptors

FDA	Food and Drug Administration
GLP	Good Laboratory Practice
$GPIMLD_{50}$	Guinea Pig Intramuscular Lethal Dose 50%
H-BAT	Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) – (Equine)
HC	Heavy Chain
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IgG	Immune Globulin G
IM	Intramuscular
IND	Investigational New Drug Application
IV	Intravenous
LBERI	Lovelace Biomedical and Environmental Research Institute
LC	Light Chain
LD_{50}	Lethal Dose 50%
LLOQ	Lower Limit of Quantitation
MedDRA	Medical Dictionary for Regulatory Activities
MED	Minimum Effective Dose
MEE	Minimum Efficacious Exposure
$MIPLD_{50}$	Mouse Intraperitoneal Lethal Dose 50%
MNA	Mouse Neutralization Assay
MOE	Margin of Efficacy
N/A	Not Applicable
NHP	Non-human Primate
$NHPLD_{50}$	Non-human Primate Lethal Dose 50%
PD	Pharmacodynamics
PK	Pharmacokinetics
PPV	Porcine Parvovirus
SAE	Serious Adverse Event
SD	Standard Deviation
S/D	Solvent/Detergent
SNAP	Synaptosomal-associated protein 25kDa
SNARE	Soluble *N*-ethylmaleimide Sensitive Factor Attachment Receptor Protein
SNS	Strategic National Stockpile
$T_{1/2}$	Half-life
T_{max}	Time to Maximum Serum Concentration
U	Unit
UK	United Kingdom
US	United States
Vc	Central Volume of Distribution
Vd	Volume of Distribution

Vdt	Deep Tissue Volume of Distribution
Vp	Peripheral Volume of Distribution
Vss	Volume of Distribution at Steady State
WHO	World Health Organization
WNV	West Nile Virus

1 EXECUTIVE SUMMARY

Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) – (Equine), abbreviated H-BAT, is a sterile solution of purified antibody fragments produced from equine immune globulins (IgGs) modified by pepsin digestion. H-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.

A single vial of H-BAT is the proposed adult human dose with each vial containing sufficient antitoxin potency to neutralize all seven BoNT serotypes. The proposed dose is supported by statistically significant efficacy in multiple randomized controlled studies in two animal species, safety data from controlled clinical trials, clinical experience derived from the Centers for Disease Control and Prevention (CDC) sponsored expanded access program (EAP) in the target patient population and pharmacokinetic (PK) and pharmacodynamic (PD) modeling of animal and human data. The safety of H-BAT in humans has been favorable with no adverse events (AEs) that limit or preclude human use in this very serious disease with limited treatment options.

1.1 Unmet Medical Need for Botulism Treatment

Botulism is a rare, and sometimes fatal, paralytic illness that occurs when neuromuscular transmission is interrupted by BoNTs produced by *Clostridium botulinum* (*C. botulinum*) or other related Clostridial species. BoNTs are among the most potent neurotoxins known to science [Arnon et al., 2001] and exist in seven antigenically distinct serotypes, designated by the letters A through G.

Currently, no specific, licensed therapies are available to treat all seven known serotypes. Given the inability to rapidly detect and identify BoNT serotypes in emergency situations, there is a critical need for a single effective treatment against all BoNT serotypes.

Five forms of botulism have been defined by route of exposure, including foodborne, wound, infant/adult intestinal colonization, inhalation and iatrogenic.

- Foodborne botulism is caused by the ingestion of preformed toxin produced in food by *C. botulinum* or related Clostridial species. Its most frequent source is inadequately cooked home-canned foods, which allow spores to survive, germinate, reproduce and generate BoNT in the anaerobic environment of the canned food.
- Wound botulism, resulting from *C. botulinum* infection and BoNT production in the tissue, has been increasingly diagnosed in injection drug abusers due to contamination of illicit drugs or injection equipment with Clostridial spores [Palmateer et al., 2013].
- Intestinal colonization by *Clostridium* bacteria following ingestion of spores with subsequent *in vivo* BoNT production is most commonly referred to as infant botulism due to its prevalence in infants younger than one year of age [Arnon 2004]. Rare adult intestinal botulism has also been documented in patients with recent antimicrobial treatment or major gastrointestinal abnormalities [Fagan et al., 2011].

- Inhalation botulism results from exposure to aerosolized BoNT, a potential delivery method for a bioterrorist attack. Although primarily demonstrated experimentally in animals, inhalation botulism has been documented in a single clinical report in three patients [Holzer 1962].
- Iatrogenic botulism is an unintended consequence of treatment with BoNT. Botulinum neurotoxin serotypes A and B are commonly used clinically for the treatment of focal dystonia and localized muscle spasticity, cerebral palsy, overactive bladder syndrome and migraines, as well as for cosmetic purposes. Iatrogenic botulism occurs as a result of over dosage or spread of therapeutic BoNT outside of the target muscle into the circulation.

While an average of 110 botulism cases occurs annually in the United States (US), botulism can pose a greater risk to the public. The CDC has classified BoNTs as a category A biological warfare agent because of their extreme potency, ease of production, ability to be weaponized and the resulting lethality or prolonged incapacity, leading to substantial disruption in societal functions. The two most likely forms of botulism that could arise from the deliberate release of BoNT are foodborne and inhalational botulism.

In all forms of botulism, regardless of route of exposure, the BoNT has the same mechanism of action with absorption into the circulation, nerve uptake and inhibition of the acetylcholine release at the neuromuscular junction (Figure 3 and Figure 4). Classically, botulism is characterized by the acute onset of bilateral cranial neuropathies associated with symmetrical descending weakness. While the time course of clinical onset following exposure may vary by route, signs and symptoms of botulism remain the same.

Historically, equine-derived botulism antitoxins have been a major component of botulism treatment protocols, as this product class rapidly neutralizes circulating toxin preventing further uptake into the nerve endings and arresting progression of paralysis. Currently in North America, there are no available licensed products to treat botulism except for Botulism Immune Globulin Intravenous (Human) (BabyBIG® or BIG-IV), which is used exclusively to treat infant botulism caused by BoNT serotypes A and B.

Given the lack of licensed products and the potential bioterrorism threat, the US government requested a broad spectrum medical countermeasure to treat botulism. To meet this unmet public health need, Cangene Corporation (hereafter Cangene) has developed H-BAT for the treatment of symptomatic botulism following documented or suspected exposure to BoNT serotype A, B, C, D, E, F or G.

1.2 Overview of H-BAT Development

The evaluation of new treatment options for botulism in well-controlled clinical trials is not feasible due to the sporadic nature of natural exposure cases, the emergency setting requiring rapid intervention at locations that cannot be predetermined, and the inability to intentionally intoxicate to study humans. Therefore Cangene pursued development of H-BAT based upon the “Animal Rule” (Title 21 Code of Federal Regulation [CFR] 601 Subpart H). The “Animal Rule” provides the Food and Drug Administration (FDA) with a mechanism to grant marketing approval based upon adequate and well-controlled animal studies when the

results of those studies establish that the biological product is reasonably likely to produce clinical benefits in humans. The FDA can rely on the evidence from animal studies to provide substantial evidence of effectiveness only when the following four essential elements are met, as stated in 21 CFR 314.610(a)(1)-(4); 21 CFR 601.91(a)(1)-(4):

1. *There is a reasonably well-understood pathophysiological mechanism of the toxicity of the (chemical, biological, radiological, or nuclear) substance and its prevention or substantial reduction 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 for predicting the response in humans.*
3. *The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity.*
4. *The data or information on the PK/PD of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.*

In early consultation with Cangene, the FDA agreed that H-BAT was an ideal candidate for the "Animal Rule" and that efficacy in two well-characterized animal models along with clinical safety data would support licensure. After evaluation of existing reports and published data, the guinea pig (*Cavia porcellus*) was selected as the first model, and the Rhesus macaque (*Macaca mulatta*) as the second model in non-human primates.

The guinea pig is a well-characterized botulism intoxication animal model that has been used for botulism research for well over 100 years, with a large body of existing data on the efficacy of botulism vaccines and antitoxins. As shown in the literature, and reproduced in Cangene studies, the guinea pig is susceptible to all seven BoNT serotypes. Likewise, the Rhesus macaque primate model has also demonstrated sensitivity to the seven toxin serotypes, is well characterized, and has been used previously in botulism research.

In both animal models, the pathophysiology and disease progression of botulism is representative of botulism in humans. To extrapolate the therapeutic efficacy from the animals to the clinic, reasonable, reproducible treatment triggers similar to those in humans are also required. Identification of subtle neurological signs which characterize early disease in humans is not possible in either animal model. In addition, both models have a truncated clinical course when compared to humans. Across species, the disease course is characterized by progressive muscular weakness, leading to respiratory distress and total paralysis.

In the guinea pig, the general signs and symptoms of progressive muscular weakness include right hind limb weakness, change in breathing pattern, weak limbs, salivation, lacrimation, forced respirations and total paralysis. The progression from moderate to more severe symptoms resulting in paralysis is comparable to that in humans. Any of these symptoms were identified as a trigger for treatment with H-BAT, but in the pivotal study the most common treatment triggers were right hind limb weakness and change in breathing pattern.

In the Rhesus macaque, signs of botulism including ptosis, muscular weakness and respiratory distress were utilized as triggers for H-BAT treatment. Oral/nasal discharge and

moderate or severe muscular weakness are observed later in the disease progression similar to the presentation of botulism intoxication observed in humans [Arnon et al., 2001].

The studies comprising the H-BAT development program are listed in Table 5, in Section 4.3. Overall, animal studies were conducted not only to develop the animal model and lead up to the pivotal study for each species, but to provide supporting PK and PD data for translational dose modeling to bridge to humans. Cangene conducted ten guinea pig studies, including three model development studies, one PK study in un-intoxicated animals, one dose ranging study and five therapeutic efficacy studies, including the pivotal Good Laboratory Practice (GLP) study (BBRC 1180). An additional seven studies were conducted in non-human primates (NHP, Rhesus macaque) including one model development study, one PK study in un-intoxicated animals, one PK and dose ranging study in intoxicated animals and four therapeutic efficacy studies, including the pivotal GLP study (LBERI 066).

The clinical program was conducted to provide safety, PK and PD data on H-BAT in normal healthy human subjects in two Cangene clinical trials in healthy adult volunteers. Additional clinical experience was gained under a CDC-sponsored EAP (BB-IND 6750) in which patients with suspected or confirmed botulism were treated with H-BAT.

To bridge animal efficacy data to clinical use of H-BAT, data from PK and dose-ranging studies in guinea pigs, Rhesus macaques and humans were analyzed. Translational modeling was used to evaluate the mechanistic relationship between biological levels of H-BAT and survival as it relates to BoNT exposure. Findings from the modeling and simulation work were used to evaluate and confirm the proposed clinical dose of H-BAT.

1.3 Dose Scaling Approach for Guinea Pig and Rhesus Macaque

In the animal efficacy studies, the dose of H-BAT was adjusted by scaling based on body weight (BW). Since the adult human dose of H-BAT is one vial, the dose for a one kilogram animal would be 1/70 of a vial, based on the assumption that the average human BW is 70 kilograms. In the translational dose modeling and simulation, animal to human allometric scaling is incorporated to account for differences between species. For conduct of the animal model efficacy studies, the dose selected as equivalent to the human dose based on weight scaling is referred to as a “scaled human dose” throughout this document.

1.4 Efficacy Findings in Guinea Pig and Rhesus Macaque

1.4.1 Guinea Pig Model

The pivotal therapeutic efficacy study of H-BAT in guinea pigs was a randomized, blinded and placebo-controlled GLP study. H-BAT treatment was administered intravenously (IV) after the onset of botulism symptoms following exposure to one of the seven BoNT serotypes (A to G). The objective of the study was to demonstrate a statistically significant improvement in survival of H-BAT-treated animals over placebo controls for all seven BoNT serotypes.

Groups of 34 male and female guinea pigs were administered BoNT equivalent to 1.5x guinea pig intramuscular lethal dose 50% ($GPIMLD_{50}$) of the appropriate toxin serotype (serotype A, B, C, D, E, F or G) given as a single intramuscular (IM) injection to the right hind limb. Animals were observed frequently and treated IV, either with the scaled human dose of H-BAT or equivalent amount of placebo after the onset of four consecutive, moderate clinical signs (e.g., right hind limb weakness, and/or change in breathing). Animals were monitored at least hourly for 24 hours following treatment and frequently thereafter for 21 days.

For the primary endpoint, treatment with H-BAT resulted in a significant survival benefit at 21 days, irrespective of the serotype (Table 1).

Table 1 Summary of Survival and Statistical Significance in Pivotal Guinea Pig Efficacy Study BBRC 1180

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

Note: Placebo control used in the animal studies was a purified despeciated (pepsin treated) equine IgG.

Secondary efficacy endpoints included time to death, incidence of clinical signs and clinical severity scores. The Kaplan-Meier median time-to-death (censored at the end of the study for all surviving animals) could not be estimated for many of the H-BAT groups, since death among H-BAT-treated animals was not observed for serotypes A, B, E, F and G and only one death each was observed for serotypes C and D.

Treatment with H-BAT reduced the overall incidence of severe clinical signs, such as respiratory distress and total paralysis, compared to placebo control animals. When additional clinical signs of botulism were examined and all BoNT serotypes are considered:

- The overall incidence of weak limbs was 34% (80/238) in the H-BAT-treated groups compared to 100% (238/238) in the placebo groups.

- The overall incidence of lacrimation and salivation was 0.4% (1/238) and 4.2% (10/238) in the H-BAT-treated groups compared to 29% (68/238) and 66% (158/238) in the placebo groups, respectively.
- The overall incidence of forced abdominal respirations was 0.8% (2/238) in the H-BAT-treated groups, compared to 49% (117/238) in the placebo groups.
- The overall incidence of total paralysis (severe sign requiring euthanasia) was zero (0/238) in the H-BAT-treated groups, compared to 75% (178/238) in the placebo groups.

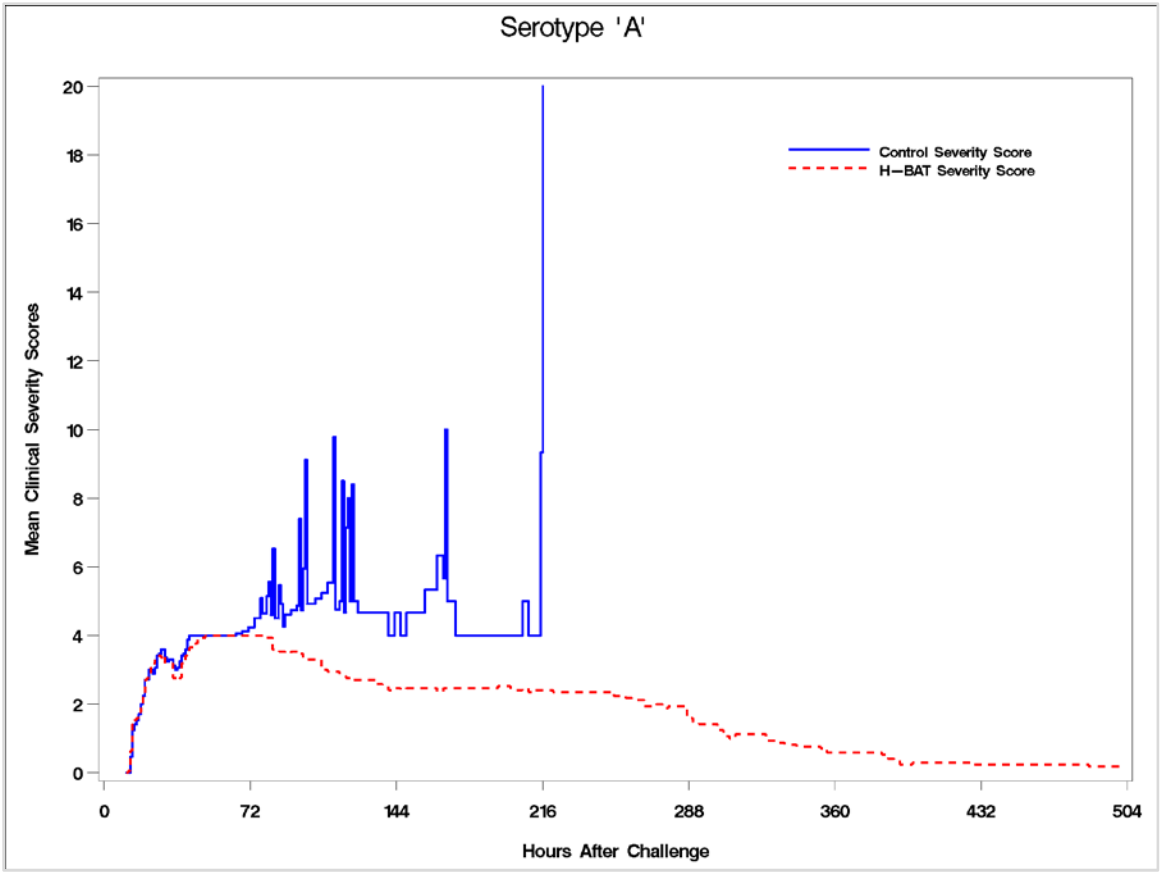
These results demonstrate a continued progression of the disease from moderate to severe clinical symptoms in placebo controls for all serotypes, while progression halted with H-BAT treatment, thus demonstrating the efficacy of a single scaled human dose of H-BAT against symptomatic botulism.

To further analyze the effect of H-BAT on the overall severity of botulism, clinical severity scores were calculated. The clinical severity score at a given time point is the sum of scores assigned to the observed clinical signs based on severity (mild = 1, moderate = 2 and severe = 3). Death is assigned a maximal value of 20. A graphical representation of the severity scores for serotype A is presented in Figure 1. All other serotypes show similar results and are presented in Section 13.5.

In general, these clinical severity scores demonstrate initial similarities in clinical progression in both the H-BAT and placebo control groups for serotype A, followed by a divergence after treatment (median 17 hours post-intoxication) within 72 hours of intoxication. In the H-BAT treatment group, the clinical severity scores decline gradually and approach zero towards the end of the study, indicating the recovery of animals to a normal clinical state. In comparison, the severity scores for the placebo group continued to worsen in the majority of the serotypes, resulting in death or euthanasia.

This response of Serotype A is considered a representative of all the toxin serotypes and the clinical severity scores for other serotypes were comparable, however, although this divergence occurred at approximately 72 hours for serotype A, it varied with each serotype, ranging from 24-72 hours post intoxication.

Figure 1: Clinical Severity Scores for H-BAT and Placebo Control Groups for Serotype A in Pivotal Guinea Pig Study Efficacy BBRC 1180



This study demonstrated that a single scaled human dose of H-BAT administered to symptomatic guinea pigs resulted in a significant survival benefit compared to placebo control animals for all seven serotypes.

1.4.2 Rhesus Macaque Model

The pivotal therapeutic efficacy study of H-BAT in Rhesus macaques was a randomized, blinded and placebo-controlled GLP study. H-BAT treatment was administered after the onset of clinical symptoms of botulism following exposure to BoNT serotype A.

Serotype A was selected as a representative BoNT serotype to study, in consultation with the FDA. Assessment of all seven toxin serotypes would require more Rhesus macaques than is ethically acceptable. Serotype A was selected as the most clinically relevant serotype as it is the most prevalent serotype found in cases of foodborne botulism in adults in the US and is the second most prevalent serotype (after serotype B) reported worldwide [Hatheway 1995]. In addition, serotype A produces a more severe clinical course in humans when compared to other well documented serotypes, B and E [Hughes et al., 1981; Woodruff et al., 1992].

Sixty rhesus macaques received a BoNT exposure equivalent to 1.7x non-human primate lethal dose 50% per kg body weight ($NHPLD_{50}$)/kg of BoNT serotype A as a single IV injection. After exposure, all animals were initially monitored hourly for clinical signs of intoxication, then every three to six hours until Day 21 or death.

Immediately after the onset of clinical signs indicative of botulinum intoxication, animals were treated according to the blinded randomization scheme with either a scaled human dose of H-BAT or placebo control *via* a central venous catheter. Initial clinical signs that triggered the administration of study treatment included ptosis, muscular weakness and respiratory distress. Both treatment groups exhibited a similar time to onset of these signs. The Kaplan-Meier median times to treatment for the H-BAT and placebo groups were 54 and 52 hours post-intoxication, respectively.

A significant improvement in the primary efficacy outcome of survival was observed in the H-BAT group compared to placebo controls (47% vs. 0%, respectively; Table 2).

Table 2: Summary of Survival and Median Time to Death for Rhesus Macaques in Study LBERI 066

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

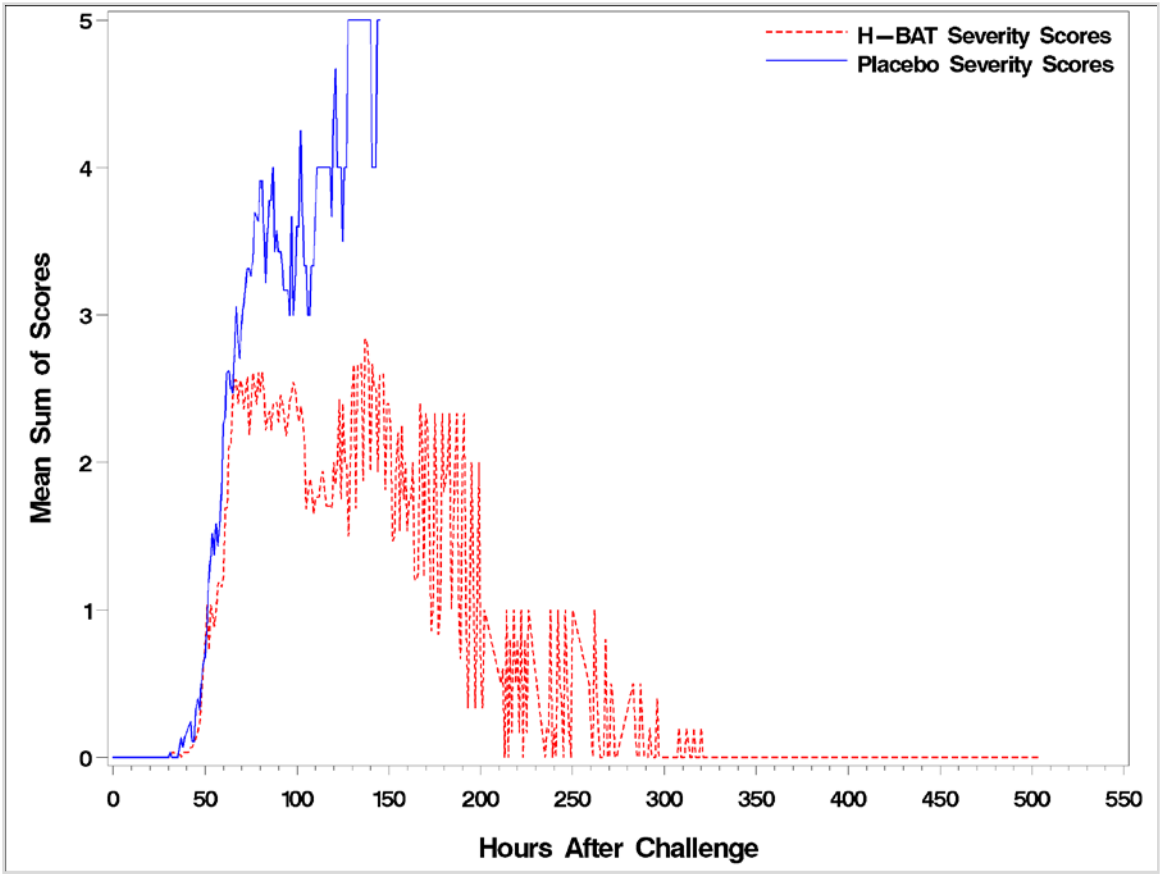
[a] The upper bound of the CI could not be estimated due to the limited number of events (i.e., 14 animals survived until study termination).

Secondary efficacy endpoints included time to death, time from onset of clinical signs to recovery and clinical severity scores. A statistically significant difference in the time to death between treatment groups was observed (Table 2).

Time from onset of clinical signs to recovery was calculated for those animals that survived to study end (Table 14). Recovery was defined as the time when the sum of scores returned to zero. The median time to recovery and 95% confidence interval (CI) was 137 hours (118, 222 hours), in H-BAT-treated animals. Animals that survived to study end without recovery were censored at the last time point that they were assessed. Since all animals in the placebo group died, the median time to recovery estimation was not possible.

To further analyze of the effect of H-BAT on clinical symptoms, clinical signs were monitored and rated according to clinical severity scores. Clinical severity scores were calculated for each individual animal at a given observation time point. The clinical severity score is calculated as the sum of scores assigned to each of the clinical signs based on severity (Table 11). The maximum sum of scores is 10, with no score assigned at the time of death. A graph of the average sum of severity scores over time by treatment group was generated to visually display the treatment effect over time (Figure 2).

Figure 2: Average Sum of Severity Scores over Time by Treatment Group for Rhesus Macaques in Efficacy Study LBERI 066



Analogous to the guinea pigs, both groups showed similar profiles in initial development of clinically severe illness (Figure 2). Subsequent to treatment, the surviving H-BAT-treated animals became stable and gradually recovered, while the clinical scores of placebo controls (and of H-BAT-treated animals that died) continued to increase until death/euthanasia. Unlike the guinea pigs, the progression of clinical signs in Rhesus macaques was more rapid, with greater than 50% of H-BAT-treated animals dying soon after treatment, likely due to the effects of toxin already taken up into the nerve endings.

This study demonstrates that a single H-BAT scaled human dose administered to symptomatic Rhesus macaques has a significant survival benefit and reduces the clinical severity score compared to placebo control animals.

Both animal models were adequately powered placebo-controlled studies with pre-specified primary and secondary endpoints that demonstrated a statistically significant survival benefit and improvement in clinical severity scores for H-BAT treated animals, addressing the requirements under the “Animal Rule”.

1.5 Experience in Humans

The focus of the clinical development program was to provide clinical safety data to support the use of H-BAT in the treatment of botulism and to provide PK data to bridge animal efficacy studies to human clinical use through modeling and simulation studies. In addition, a PD study was planned to demonstrate the ability of H-BAT to neutralize BoNT in humans. These two studies included a total of 56 normal healthy subjects administered one or two vials of H-BAT (Table 3).

Supportive clinical safety and effectiveness data was also collected by the CDC under their EAP which allowed for distribution of investigational H-BAT for treatment of symptomatic botulism as a result of naturally occurring outbreaks or isolated unintentional incidents when no other product was available. Data from 148 patients treated with H-BAT between January 15, 2008 and December 31, 2011 were included in the Biologic License Application (BLA) submission (Table 3).

Table 3: Summary of Healthy Subjects and CDC Patients Administered H-BAT

Study Identifier	Study Population	Number of Subjects	Dose	Route
BT-001	Normal Healthy Subjects	40	20 = 1 vial 20 = 2 vials	IV Infusion
BT-002B	Normal Healthy Subjects	26	16 = 1 vial 10 = placebo	IV Infusion
*CDC BB-IND 6750	Suspected Botulism Patients	148	138 = 1 vial 3 = 2 vials 5 = 1 pediatric dose 1 = 2 pediatric doses 1 = 2 infant doses	IV Infusion

* Includes patients treated with H-BAT between January 15, 2008 and December 31, 2011.

1.5.1 Safety in Humans

Equine antitoxin products have a long clinical history; equine botulism antitoxins, in particular, have been commercially available in the US since the 1960's. The primary safety concern with these heterologous IgG products is an immunologic reaction to foreign proteins. Historically, these reactions included acute anaphylactic and anaphylactoid reactions in approximately 5% of patients and delayed allergic reactions including serum sickness and possible febrile responses to immune complex formation in approximately 4% of patients [Black & Gunn 1980].

The manufacturing process for H-BAT is designed to reduce reactive heterologous proteins, utilizing cation-exchange chromatography to purify the IgG fraction, pepsin digestion to reduce the Fc portion of the IgG molecule, anion exchange chromatography to remove pepsin and residual equine protein and peptides and ultrafiltration and diafiltration to remove process-related salts, residual solvent detergent (S/D) viral inactivation reagents and digested proteins.

Additional H-BAT administration precautions in place for immunologic reactions include slow infusion at the start of dosing to allow time to detect, and respond to, severe immunologic reactions. While results of clinical trials suggest that a negative skin sensitivity test is not predictive of immunologic reactions, skin sensitivity testing is recommended in patients at risk of hypersensitivity reactions.

1.5.1.1 Safety in Healthy Subjects

The first clinical trial, BT-001, was a single center, double-blind, randomized, parallel arm study to assess the safety and PK of H-BAT following IV administration of either a single dose (one vial; n = 20) or double dose (two vials; n = 20) of H-BAT to normal healthy subjects. A second trial, BT-002 Stage B, was also a single center, double-blind, randomized, parallel arm study to assess the safety and PD of H-BAT (n = 16) or placebo (0.9% saline; n = 10) to normal healthy subjects. The healthy adults in these studies ranged from 19 to 52 years of age, with an equal number of males and females enrolled.

The most common adverse events (AEs) reported in more than 5% of all subjects in BT-001 and treatment groups from BT-002 Stage B, were headache, somnolence, contusion, tonsillar hypertrophy, nausea, pharyngolaryngeal pain, pruritus, skin laceration and urticaria. The most common related AEs were headache (9%), nausea (5%) and urticaria (5%).

During these clinical trials, there were no deaths or serious adverse events. However, two subjects (4%), one in each trial, experienced immunologic reactions characteristic of equine immune globulin products and H-BAT infusion was discontinued as a result of these AEs. The AEs in the BT-001 subject included mild and moderate headache, mild pain (body ache), mild and moderate pyrexia, and mild pharyngolaryngeal pain. The AEs in the BT-002 Stage B subject included urticaria, chest discomfort, elevated body temperature and elevated fibrinogen levels.

Overall, H-BAT was well-tolerated in healthy subjects. No difference in the number or severity of adverse events was observed between subjects who received one vial or two vials of H-BAT.

1.5.1.2 Safety in CDC Expanded Access Program Patients

The clinical exposure to H-BAT was increased through the CDC's EAP, which provided investigational H-BAT under an Investigational New Drug (IND) protocol to patients with suspected or confirmed botulism.

The CDC EAP captures case report data that includes H-BAT patient safety and effectiveness outcomes. The CDC has provided these datasets to Cangene on an ongoing basis, with data from the first 148 patients treated with H-BAT, between January 15, 2008 and December 31, 2011, available for analysis at the time of the licensure application.

In this program patients ranged in age from 10 days to 88 years (median age 47 years) with 105 (70.9%) males and 43 (29.1%) females. Seven pediatric patients 17 years of age or younger were treated in this time period, including five patients under six years. In addition 20 patients over age 65 were treated, of which nine patients were over 75 years.

Upon presentation or hospital admission, the most common symptoms and physical findings of botulism included: subjective weakness, blurred vision, dysphagia, slurred speech, ptosis, diplopia, fatigue, change in sound of voice, shortness of breath, palatal weakness, dizziness, impaired gag reflex, thick tongue, extraocular palsy and dry mouth.

Most patients (97%) received one dose of H-BAT and the majority (66%) had a definitive final diagnosis of botulism. Wound botulism was the most common (47%) route of exposure, followed by foodborne exposure (27%). Serotype A was the most commonly identified BoNT serotype isolated from patients with confirmed botulism (65%, 33 of 51 patients).

Of the 148 patients, safety information was available for 146 patients. Adverse drug reactions (ADRs) were reported for 18 patients (12%). The most common ADRs were pyrexia (5%), chills (2%) and rash (2%), nausea (1%) and edema (1%).

One patient (0.7%) experienced a delayed hypersensitivity reaction of mild serum sickness (with myalgia, arthralgia and dark urine) 12 days after H-BAT administration.

Two CDC botulism patients experienced serious adverse events (SAEs), one during and one after H-BAT administration.

- One SAE occurred during H-BAT treatment. A 10 year-old male patient experienced hemodynamic instability characterized by tachycardia, bradycardia and asystole during infusion of H-BAT. The events were rechallenge positive, with symptom improvement after initial interruptions of H-BAT infusion, and reappearance of symptoms after the infusion restarted. H-BAT infusion was stopped permanently at 70% of the planned dose. The patient recovered from both hemodynamic instability and botulism (Section 8.4).
- A second SAE was reported after treatment with H-BAT. A 27 year-old male experienced respiratory and subsequently cardiac arrest following tracheostomy change, more than 24 hours after completion of a well-tolerated H-BAT infusion. The patient was initially placed on ventilator support, which was later removed at the family's request and the patient died. The investigator assessed that the cardiac arrest was due to severe hypoxia from a mucus plug during a tracheostomy.

The CDC reported six deaths for patients treated with H-BAT (Section 8.4), including the death reported above. The causes of death in the four patients diagnosed with botulism were progressive botulism in two patients and unknown in two patients. The deaths in the two patients with a final diagnosis other than botulism intoxication were related to co-morbidities of those diseases.

Administration of H-BAT was demonstrated to be safe and well-tolerated in both healthy volunteers and patients with suspected or confirmed botulism, with most AEs being mild to moderate, and expected based on the product class.

1.5.2 Clinical Effectiveness

Efficacy of H-BAT is based on the studies in the two well-characterized guinea pig and Rhesus macaque animal models. Clinical studies to support the effectiveness of H-BAT in

humans include a PD trial (BT-002 Stage B) and a *post-hoc* analysis of clinical data from the CDC EAP. The results from these studies are consistent with the animal studies, and support the translation of animal data to clinical effectiveness.

1.5.2.1 Pharmacodynamic Clinical Trial BT-002

The effect of H-BAT on paralysis induced by BoNT serotypes A and B was evaluated in the extensor digitorum brevis (EDB) muscle model. The principle effect of exposure to botulism in humans is muscle paralysis. In this previously developed PD model, botulism toxin is injected into the encapsulated EDB muscle of the foot, and has a localized paralytic effect. H-BAT inhibition of muscle paralysis caused by BoNT serotype A (Botox®; Botox®, Prescribing Information,) or B (Myobloc®; Myobloc® Prescribing Information), was used as a surrogate endpoint to demonstrate the effectiveness of H-BAT in humans.

In BT-002 Stage B, systemic IV administration of H-BAT, 24 hours prior to intramuscular injection of BoNT was effective in neutralizing BoNT serotypes A and B and prevented subjects from experiencing a decrease in muscle function following injection of BoNT Botox® and Myobloc® (Section 8.5.1). Subjects receiving placebo control demonstrated a >50% loss of muscle function within three days of exposure to both BoNT serotype A and serotype B; while in the H-BAT treatment group muscle function was stable over time.

This study demonstrated that H-BAT is capable of neutralizing BoNT in the extravascular space, and as a result, blocking BoNT internalization into cholinergic nerve endings, supporting H-BAT's mechanism of action, which is clinically relevant for symptomatic botulism patients.

1.5.2.2 CDC Expanded Access Program with H-BAT

To support the use of H-BAT for the treatment of botulism, Cangene performed a *post-hoc* statistical analysis of patient data provided by the CDC from their EAP. The analysis was designed to determine if treating patients early after symptom onset had an impact on botulism related outcomes such as the duration of hospitalization. Early treatment was defined as ≤ two days after symptom onset and compared to later treatment (> two days).

The mean (± standard deviation, SD) duration of hospitalization for patients with botulism was 12 (± 9) days for the patients treated with H-BAT within two days of symptom onset, as compared to 26 (± 26) days for the patients treated more than two days after symptom onset. This suggests that the timing of H-BAT treatment relative to the onset of symptoms affects the duration of hospitalization. Duration of intensive care unit (ICU) stay and duration on mechanical ventilation showed similar results (Section 8.5.2), supporting the benefit of H-BAT treatment.

In summary, the clinical experience with H-BAT supports the efficacy demonstrated in the well-controlled animal models of botulism. The human EDB model showed that pre-treatment with H-BAT is capable of blocking the toxin internalization into the nerve cells, thus preventing symptom development. The CDC EAP results suggest that H-BAT may shorten the duration of hospitalization and may reduce disease progression, which are critical

measures of H-BAT benefit for both sporadic natural botulism cases and mass intentional release scenarios.

1.6 Justification for the Proposed Dose in Humans

The adult dose of H-BAT is a single vial designed to contain sufficient antitoxin potency to neutralize known clinical BoNT exposure levels for all seven BoNT serotypes. While the antitoxin potency levels were predefined at the start of the H-BAT development program based on historical information, the data collected during H-BAT development support the proposed adult dose of one vial, based on the following information:

- Historical clinical data support the effectiveness of antitoxins in the treatment of botulism.
- The neutralizing capacity of H-BAT is in excess of the maximum reported historical BoNT exposure level in humans.
- Pharmacokinetic data for H-BAT from studies in guinea pig, Rhesus macaque and humans demonstrate that the maximum serum concentration (C_{max}) is similar between species and that the AUC is the highest in humans.
- Efficacy data from guinea pig and Rhesus macaque dose-ranging studies and therapeutic efficacy studies demonstrate that the scaled human dose is effective in significantly decreasing mortality and reducing clinical severity.
- *Post-hoc* analysis of information collected from CDC EAP botulism patients treated with H-BAT support that early treatment with H-BAT, within two days of symptom onset, may reduce the duration of hospitalization compared to later treatment.
- Population PK/PD modeling and simulation work was completed to integrate data from guinea pig, Rhesus macaque and human studies to define the human dose. This translational modeling and simulation established that one vial of H-BAT is the appropriate adult dose to address most clinical situations.
- Sensitivity analysis was completed based on the H-BAT end of shelf-life potency specifications. Even at the lowest potency level, there is sufficient neutralizing capacity to protect against botulism toxin serotype A-G (Section 9.7.2).

Cangene's nonclinical and clinical studies as well as PK/PD modeling and simulation confirm that the proposed H-BAT adult dose of one vial, set early during the product development process, is justified.

1.7 Benefit/Risk Evaluation

The H-BAT development program has defined the known and potential benefits and risks, which clearly demonstrates a favorable benefit/risk profile for H-BAT treatment of suspected or documented botulism.

1.7.1 Benefits

The efficacy of H-BAT was confirmed in two well-characterized animal models of botulism (guinea pig and Rhesus macaque). Treatment with H-BAT improved animal survival rates in dose-ranging and therapeutic efficacy models in guinea pigs and rhesus macaques. Additionally, H-BAT halted clinical sign/symptom progression and reduced clinical signs over the course of BoNT intoxication in several studies. These animal efficacy studies demonstrated the potential benefits of H-BAT in a clinical setting, whether this involves a sporadic case of foodborne botulism or a mass exposure scenario following an intentional bioterrorist attack.

Further, analysis of patient data from the CDC EAP revealed that early treatment of patients with H-BAT resulted in a shorter duration of hospitalization, duration of ICU stay and duration on mechanical ventilation. Overall, this information supports the benefits of early treatment of botulism with H-BAT.

The BoNT neutralizing ability of H-BAT has also been demonstrated in the human EDB muscle model. H-BAT pretreatment prevented subjects from experiencing a decrease in muscle function following injection of BoNT serotypes A and B directly into the EDB muscle. This demonstrates that H-BAT can neutralize BoNT in the extravascular space before it enters the nerve terminal, which links to its known mechanism of action and ability to halt symptom progression in clinical botulism.

The overall demonstrated benefit of H-BAT is the significant reduction in mortality and morbidity associated with symptomatic botulism intoxication.

1.7.2 Risks

This medical risk/benefit assessment should consider whether the benefits of the drug outweigh the known and potential risks of the drug, even those indicated for life-threatening diseases.

Based on historical data for human use of equine hyperimmune products, the principal risk is immunologic reactions including anaphylactic and anaphylactoid reactions and delayed hypersensitivity reactions. Modern purified, pepsin digested immune globulin products are believed to carry an anaphylactic risk similar to or less than that of penicillin injections [Tantawichien et al., 1995].

Hypersensitivity reactions have been seen with the use of H-BAT and this information is contained within the product label. To minimize the risk of these reactions, pre-medications, including corticosteroids and antihistamines may be considered by physicians in patients at risk. Slow infusion is recommended to detect and treat potential reactions. While results of clinical trials suggest that the skin sensitivity test is not entirely predictive of immunologic reactions, skin sensitivity testing is recommended in patients at risk of hypersensitivity reactions.

The risk of serum sickness following administration of equine derived hyperimmune products is also a concern, particularly in situations where re-dosing is a possibility. One patient experienced a mild case of serum sickness after receiving a single vial of H-BAT. In

potential H-BAT re-dosing situations (i.e., wound botulism or intestinal colonization), the benefit-to-risk ratio should be assessed on a case-by-case basis.

H-BAT has been demonstrated to be well-tolerated in adults when administered at a dose of one or two vials to healthy subjects and when administered to patients with suspected or confirmed botulism. Hypersensitivity reactions occurred at a rate similar or less to that reported for other previously marketed botulism antitoxin products [Black & Gunn, 1980]. In the CDC EAP, H-BAT was well tolerated in patients with botulism and those individuals did not have botulism as a final diagnosis. This, alongside of the safety data from normal healthy subjects, is an important consideration in the event of a mass intentional release where individuals may be treated based on clinical suspicion and equivocal early clinical signs.

H-BAT is made from equine plasma and thus, there is a theoretical risk of transmitting infectious agents. As part of the manufacturing process for H-BAT, controls at the equine plasma collection stage are in place to test for the presence of specific infectious agents prior to use including testing by 9CFR (i.e., Code of Federal Regulations - Title 9: Animals and Animal Products) standards. The horses that support the H-BAT program are well-maintained herds used for repeat plasmapheresis to supply the plasma and are under the regular care of veterinary doctors.

Additional controls are incorporated into the manufacturing process for H-BAT, including two viral inactivation and removal steps. Validation of the process for viral clearance has been completed with several model viruses. The platforms used for viral inactivation and clearance are employed in the manufacture of Cangene's licensed human Hyperimmune products WinRho and HepaGamB. While unexpected, there is also the theoretical possibility that unknown infectious agents may be present in the product in the event that these are non-lipid enveloped and smaller than 20 nm in size or the plasma levels exceed the virus inactivation and removal step capabilities. No known cases of transmission of viral diseases have been associated with the use of H-BAT.

The maltose contained in H-BAT may interfere with some types of blood glucose monitoring systems [Gaines et al., 2008; Schleis 2007]. This may result in falsely elevated glucose readings and consequently in the inappropriate administration of insulin, resulting in life threatening hypoglycemia. Also, cases of true hypoglycemia may go untreated if the hypoglycemic state is masked by falsely elevated glucose readings. When administering H-BAT the measurement of blood glucose must be done with a glucose specific method.

Since early intervention has been shown to lead to improved outcomes, there is also the risk that H-BAT will be given empirically to patients with neurologic signs and symptoms similar to botulism but who are subsequently found to have other illnesses such as Guillain-Barre syndrome. This was reported in 39 of 148 patients in the CDC EAP. Release of H-BAT will remain under the control of the CDC in consultation with the requesting health care provider to limit this potential risk. Ongoing surveillance for potential adverse events not seen in the current program will occur through the proposed post-approval commitment program.

These risks must be evaluated within the context of botulinum intoxication that, if left untreated, has resulted in high mortality rates of up to 60%. The previous study by Tacket et al. [1984], established the efficacy of a trivalent equine antitoxin for foodborne serotype A

botulism with a demonstrated reduction in mortality and morbidity. The effectiveness analysis of H-BAT from the CDC EAP suggests similar results. The potential to reduce clinical severity such as mechanical ventilation time, ICU stay and shorten the duration of hospitalization with a therapy specific to the pathogenesis of the disease could have important implications for the medical infrastructure in the event of a mass exposure.

Thus, the data support the benefits of H-BAT treatment in cases of suspected or confirmed botulism and significantly outweigh the known and potential risks of the drug.

1.8 Conclusions

The H-BAT development program has established efficacy in two adequate, well-controlled, and well-characterized animal models (guinea pigs and Rhesus macaques). PK/PD studies in humans and animals support the proposed dose. Targeted safety studies in humans have affirmed safety for the proposed use. The CDC experience with H-BAT is consistent with a safe and effective product.

Based upon the regulatory path for approval that is described in the "Animal Rule", there are four Essential Elements to evaluate the scope of the evidence.

1. *There is a reasonably well-understood pathophysiological mechanism of the toxicity of the (chemical, biological, radiological, or nuclear) substance and its prevention or substantial reduction by the product.*

The pathophysiology of BoNT has been well characterized through the investigation of naturally occurring cases of botulism, the medical uses of the neurotoxins, and through *in vitro* and *in vivo* studies in animals. While there are several routes of exposure to BoNT, the pathophysiology is mediated by uptake of the toxin into the cholinergic nerve endings and inhibition of release of acetylcholine. Thus the binding of circulating toxin to prevent uptake into nerve endings is the well-established mechanism of action of H-BAT to reduce the severity and progression of symptoms from BoNT, regardless of route of toxin exposure.

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 for predicting the response in humans.*

Guinea pigs and Rhesus macaques were selected as models to demonstrate efficacy because the clinical progression of botulism in these animals is similar to that observed in humans, with each moving through stages from progressive muscle weakness to total paralysis and, ultimately, death. These animals are well-characterized models for BoNT intoxication. In both animal models, H-BAT demonstrated a strong and robust survival benefit and reduction in clinical severity. In the CDC EAP, early treatment with H-BAT suggests a reduction of the morbidity associated with botulism intoxication in patients with suspected or confirmed botulism.

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.*

Improvement in survival and reduction in morbidity are the important endpoints in humans, and they were used in the animal models. In both animal models, H-BAT demonstrated a significant and robust survival benefit compared to placebo.

4. *The data or information on the (pharmaco-) kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.*

Population PK/PD modeling and simulation work was completed to integrate data from guinea pig, Rhesus macaque and human studies that established the theoretical neutralizing capacity, PK/PD profile and efficacy in treatment and dose ranging studies and the model was used to define the human dose. The translation modeling established that one vial is the appropriate dose.

In conclusion, H-BAT is a safe and well-tolerated equine hyperimmune product with demonstrated efficacy in guinea pigs, Rhesus macaques and humans. H-BAT provides a favorable benefit-to-risk ratio, by reducing the morbidity and mortality associated with botulism intoxication in patients with suspected or confirmed botulism.

Therefore, H-BAT should be approved for the proposed indication as the overall H-BAT development program meets the regulatory requirements under the animal rule, clinical benefit/risk expectations and addresses a critical unmet medical need.

2 OVERVIEW OF BOTULISM AND UNMET MEDICAL NEED

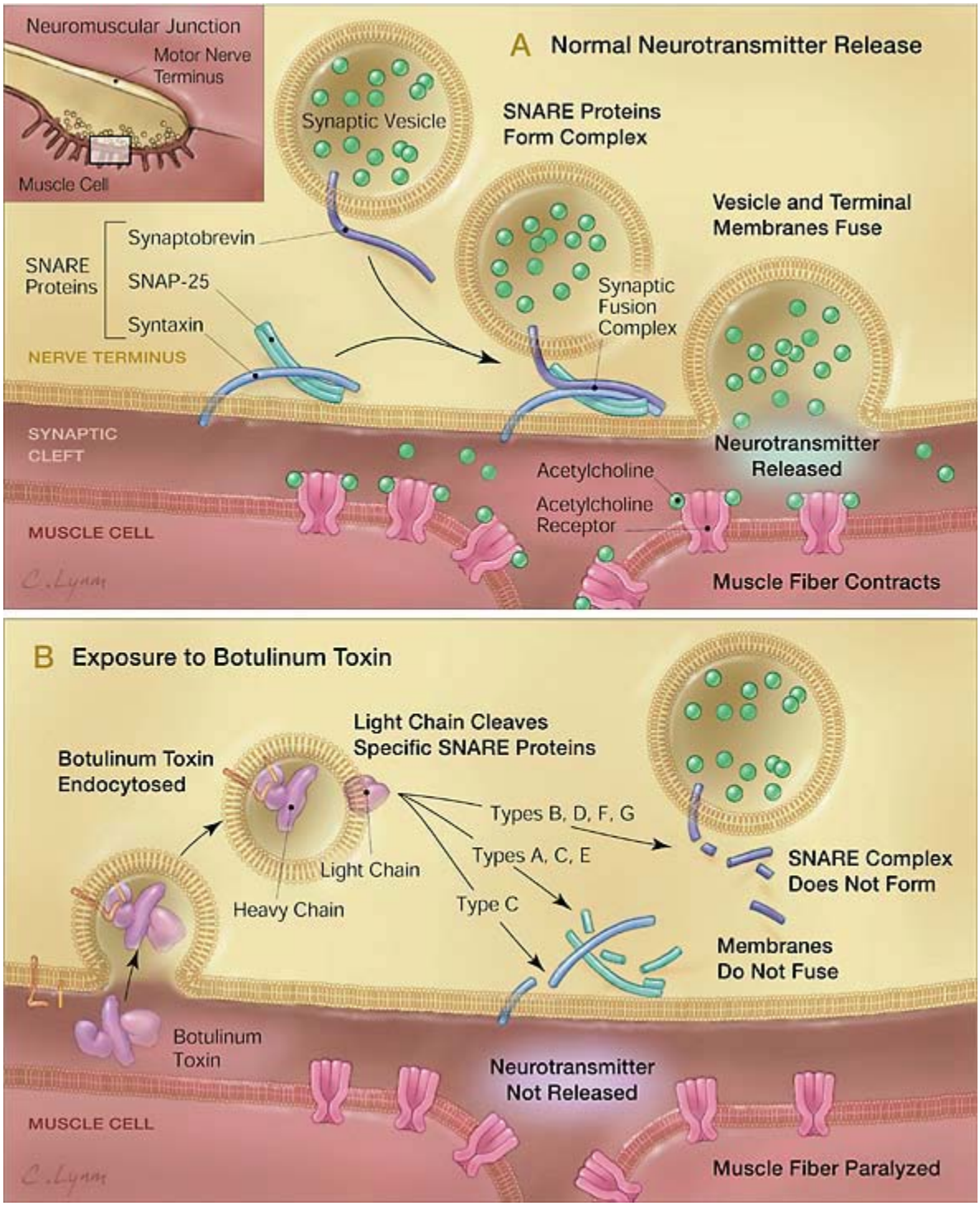
Botulism is a rare and sometimes fatal paralytic illness that affects humans, other mammals, birds and fish. Paralysis occurs when neuromuscular transmission is interrupted by botulinum neurotoxins (BoNTs) produced by *Clostridium botulinum* (*C. botulinum*) and other related clostridial species. BoNTs are some of the most potent neurotoxins known to science [Arnon et al., 2001] and exist in seven antigenically distinct serotypes, designated by the letters A through G. There are currently no specific, licensed therapies that treat all seven known serotypes.

Therapy for botulism primarily consists of supportive care, including mechanical ventilation, and passive immunization with an antitoxin, if available. Human botulism mortality rates have been reported as high as 60% [Gangarosa et al., 1971; Shapiro et al., 1998]. With improved respiratory and intensive care that includes antibiotics and antitoxins, mortality rates have decreased to less than 7% [CDC 2010]. Although mortality rates have improved, duration of hospitalization and length of stay in intensive care units (ICUs) continue to present a major burden to the healthcare system, with hospital stays up to six months to recover from botulism.

The pathophysiology of BoNT has been well characterized through the investigation of naturally occurring cases of botulism, the medical uses of the neurotoxins, and through *in vitro* and *in vivo* studies in animals. Although the routes of BoNT exposure may vary, the pathophysiology is based upon BoNT available at the neuromuscular junction; thus, the fundamental mechanism of BoNT action is similar regardless of exposure route, species or serotype.

The mechanism of BoNT action at the peripheral cholinergic nerve terminals is comprised of distinct steps which include receptor binding, internalization, membrane translocation, intracellular trafficking and proteolytic degradation of the target protein. The binding of BoNT to the peripheral neuromuscular junction is the first step of intoxication. The two independent determinants of BoNT binding are a ganglioside anchorage site and a protein receptor that dictates serotype specificity (two-receptor model) [Montecucco et al., 2004]. Upon receptor binding, botulinum neurotoxins are internalized into sensitive cells *via* receptor-mediated endocytosis. Exposure of the botulinum neurotoxin-receptor complex to the acidic environment of the endosomes induces a conformational change, leading to the insertion of the heavy chain (HC) portion of the BoNT molecule into the endosomal membrane, forming a transmembrane protein-conducting channel that translocates the light chain (LC) portion of the BoNT molecule to the cytosol [Montal 2010]. Following BoNT internalization, the LC portion of the BoNT binds to Soluble *N*-ethylmaleimide Sensitive Factor Attachment Receptor Protein (SNARE). The proteolytic region of the LC cleaves the SNARE protein complex preventing docking of the acetylcholine transport vesicle on the inner surface of the cellular membrane. This results in the inhibition of acetylcholine release. By blocking acetylcholine release, BoNT causes the characteristic flaccid paralysis associated with botulism [Montal 2010] (Figure 3). Given the irreversible cleavage of these proteins in the presynaptic terminal, it is important for any specific BoNT treatment to be introduced as soon as possible after exposure.

Figure 3: Mechanism of Action of Botulism Neurotoxin



Arnon et al., 2001. SNAP-25 = Synaptosomal-associated Protein 25 kDa; SNARE = Soluble *N*-ethylmaleimide Sensitive Factor Attachment Receptor Protein.

Humans are susceptible to all seven BoNT serotypes; most cases are caused by serotypes A, B, E and F [Hatheway 1995]. Human botulism displays neurological symptoms that are characteristically bilateral and symmetric, typically presenting with the cranial nerves, followed by anatomical descending weakness or paralysis.

Common initial symptoms and signs include dry mouth, diplopia, ptosis, loss of accommodation and diminished or total loss of pupillary light reflex. Symptoms of bulbar paresis (e.g., dysarthria, dysphagia, dysphonia, a flaccid facial expression) develop with dysphagia leading to aspiration pneumonia in many patients. Muscles of the extremities, trunk, and of respiration progressively weaken in an anatomical descending pattern.

There are no sensory disturbances. Fever is absent, and pulse remains normal or slow unless a secondary infection develops. Routine studies of blood, urine and cerebral spinal fluid are usually normal. Constipation is common after neurologic impairment appears. Major complications include respiratory failure caused by diaphragmatic paralysis and pulmonary infections [Arnon et al., 2001; Hughes et al., 1981; Robinson & Nahata 2003; Shapiro et al., 1998]. The clinical signs may vary slightly depending on the route of exposure [Robinson & Nahata 2003]. For instance foodborne botulism may have gastrointestinal symptoms, including nausea, vomiting and less commonly diarrhea [Cherington 2004; Robinson & Nahata 2003], which are not seen with all forms of botulism.

Five forms of botulism have been described in the literature as defined by the route of exposure consisting of foodborne, wound, infant/adult intestinal colonization, inhalation and iatrogenic. The classical form of botulism is food poisoning caused by the ingestion of preformed toxin produced in food by *C. botulinum* or related Clostridial species. The most frequent source is home-canned foods, in which spores that survive an inadequate cooking and canning process germinate, reproduce and generate BoNT in the anaerobic environment of the canned food.

Infant intestinal colonization by Clostridial bacteria follows ingestion of spores with subsequent *in vivo* BoNT production [Arnon 2004]. It usually occurs in infants younger than one year of age, with 95% of the cases occurring in patients younger than six months [Arnon 2004]. It is believed that at this age, the normal intestinal flora have not developed to a degree that would prevent colonization by these bacteria [Arnon 2004]. In most infant cases (85%) the source of the spore ingestion is unknown; however, honey is suspected in about 15-20% of cases [Arnon et al., 1979; Chin et al., 1979; Morris et al., 1983; Spika et al., 1989]. Adult intestinal botulism is rare, but does occur [Chia et al., 1986; Fenicia et al., 1999; McCroskey & Hatheway 1988].

Wound botulism, previously a relatively rare form of botulism, is now being increasingly diagnosed among injecting drug abusers due to the use of contaminated needles or impure heroin [Akbulut et al., 2005; Brett et al., 2004; Galldiks et al., 2007; Passaro et al., 1998; Sandrock & Murin, 2001; Werner et al., 2000]. Wound botulism occurs when anaerobic conditions within an abscessed wound allow germination of *C. botulinum* spores, subsequent multiplication of the organism, followed by production and absorption of the BoNT *in vivo*.

Only one instance of inhalational botulism has been reported in the literature [Holzer 1962]. This case involved the accidental exposure of three veterinary personnel to BoNT re-

aerosolized from animal fur. Inhalational botulism is, however, a potential route of exposure for a bioterrorist attack and has been demonstrated in a number of animal models [Arnon et al., 2001; Franz et al., 1993; Gelzleichter et al., 1999; Park & Simpson 2003].

Iatrogenic botulism is an unintended consequence of treatment with BoNT. Botulinum neurotoxin serotypes A and B are commonly used for the treatment of focal dystonia and localized muscle spasticity [Jankovic 2004; Sethi et al., 2012; Truong 2012], cerebral palsy [Balaban et al., 2012], overactive bladder syndrome [Duthie et al., 2011], migraines [Lipton et al., 2011] as well as for cosmetic purposes [Cather et al., 2002; Flynn 2012; Jandhyala 2012]. When used at therapeutic doses, the neurotoxin is relatively safe and effective. Systemic adverse effects are rare but can include flu-like symptoms and fatigue [Brin 1997]. However, in rare cases, injections of BoNT have been reported to cause generalized weakness, dysphagia and respiratory distress [Bakheit et al., 1997; Bhatia et al., 1999; Coban et al., 2010; Onley et al., 1988; Yamasaki et al., 1994].

For four of the five forms of botulism intoxication (i.e., foodborne, wound, inhalational, and iatrogenic), the initial symptoms of the disease are anatomically proximal to the site of administration. For example, foodborne intoxication initially presents with gastrointestinal symptoms (including nausea, vomiting, abdominal cramps and diarrhea), while wound or iatrogenic botulism shows paralysis proximal to the site of neurotoxin exposure that subsequently spreads distally [Sobel 2005]. However, the pathophysiology of the disease is the same with absorption of toxin into the circulation and uptake into neuromuscular junctions that inhibits release of acetylcholine. Therefore, however irrespective of the mode of contracting the toxin, the disease symptoms are very similar and disease progression follows the same descending path.

Botulinum neurotoxins have significant potential to be used as biological weapons. The two most likely forms of botulism that could arise from the deliberate release of BoNT are foodborne and inhalational botulism. The deliberate release of BoNT in a civilian population would cause significant morbidity and mortality with substantial disruption in societal function. Based upon models, a point source aerosol release of BoNT could lead to significant morbidity or kill 10% of persons within a geographical location of 0.5 km down-wind of the intentional release [Arnon et al., 2001]. The Centers for Disease Control (CDC) has classified botulism as a category A biological warfare agent (i.e., with great potential to adversely affect public health) [Rotz et al., 2002].

Given the importance of intervention timing (as early as possible) and the inability to rapidly identify serotype intoxication in an emergency clinical situation, having a product available and licensed that can treat all seven serotypes is a critical unmet need.

Various licensed and unlicensed equine botulism antitoxins have been manufactured and used over the years. In North America, equine-based botulism antitoxins first became available in the 1960's. The original botulism antitoxin products (manufactured by Lederle and Connaught) were equine-derived immune globulin fragments that were licensed based on quality information and testing against international and United States (US) standards however, no clinical data was initially provided [Advisory Panel Report, 1979]. Subsequently, a limited amount of mostly retrospective clinical data has become available to

support the effectiveness of botulism antitoxins in a clinical setting [Konig et al., 1964; Dolman et al., 1963; Arnon et al., 2006; Vanella de Cuetos et al., 2011; Tacket et al., 1984; Kongsaengdao et al., 2006; Chang et al., 2003; Offerman et al., 2009; Sandrock & Murin, 2001].

Currently in North America, there are no available licensed products to treat botulism except for BabyBIG, which is a human immune globulin product used to treat infant botulism (less than one year of age) caused by BoNT serotypes A and B. BabyBIG is manufactured in smaller quantities from individuals vaccinated with an investigational botulinum toxoid vaccine. Given the limited availability of licensed product and the unmet need for all seven botulinum serotypes, the US government requested medical countermeasures to treat botulism. To address this unmet public health need, Cangene Corporation (hereafter Cangene) has developed H-BAT for the treatment of symptomatic botulism following documented or suspected exposure to BoNT serotype A, B, C, D, E, F or G.

3 H-BAT PRODUCT ATTRIBUTES

3.1 H-BAT Production

Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) – (Equine) (H-BAT) is a sterile solution of purified equine immune globulin fragments containing antitoxin activity to BoNTs A, B, C, D, E, F and G for intravenous (IV) administration. H-BAT is prepared from plasma obtained from horses vaccinated with one of the seven serotypes of BoNT. These vaccinated horses are well-maintained, used for repeat plasmapheresis to supply the plasma and are under the regular care of veterinary doctors. Plasma is collected by plasmapheresis, manufactured as individual serotypes, which are then pooled into an overall blended product of seven antitoxin serotypes.

The manufacturing process for each antitoxin serotype is identical and includes cation-exchange chromatography to purify the immune globulin fraction, digestion with pepsin to produce $F(ab')_2$ plus Fab and $F(ab')_2$ related fragments, anion exchange chromatography to remove the pepsin and other impurities and finally sterile filtration and bulk formulation. To obtain the final heptavalent product, the seven antitoxin serotypes bulks are blended to meet the target potency for each serotype and filled into vials. In addition, the manufacturing process includes two viral inactivation/removal steps: solvent/detergent (S/D) treatment and virus nanofiltration.

The S/D treatment is a well-established virus clearance step, and has been shown to be highly effective for the inactivation of lipid-enveloped viruses. Lipid enveloped viruses include several equine viruses including equine encephalitis, equine arteritis, West Nile virus (WNV), equine infectious anemia, equine herpes viruses, rabies and equine influenza viruses.

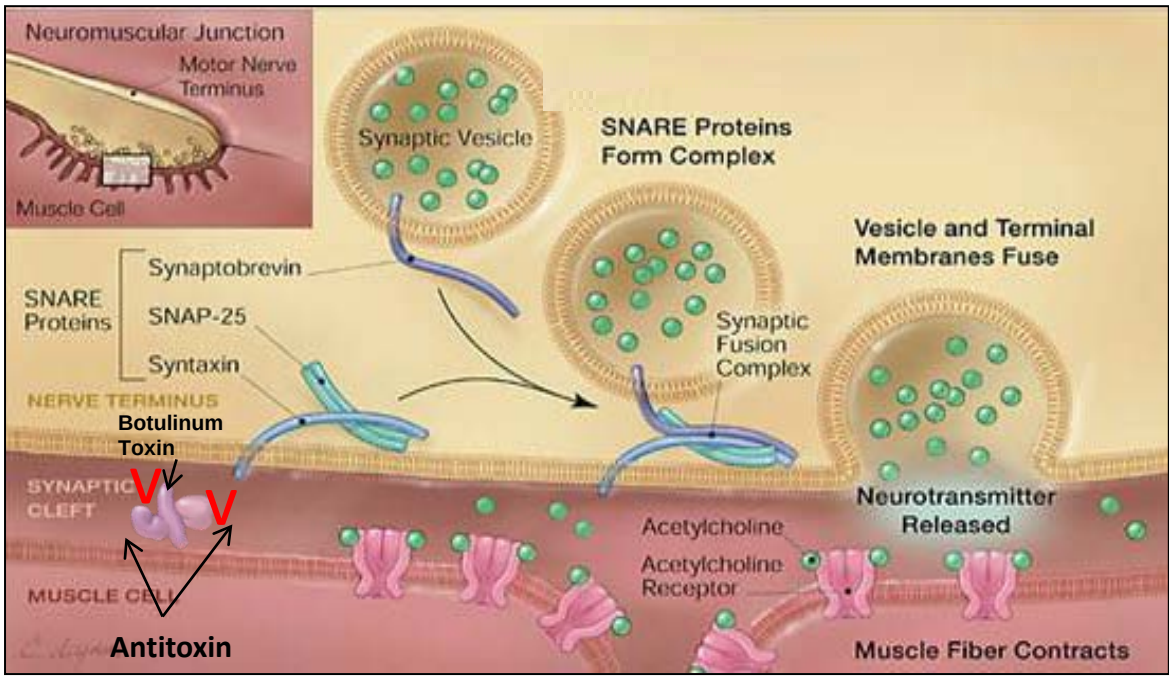
H-BAT manufacturing also includes filtration through a 20N nanofilter, which is a physical method that effectively reduces the levels of some lipid-enveloped viruses as well as non-enveloped viruses, including equine rhinovirus, equine adeno- and adeno-associated viruses, and equine parvovirus. This virus removal step has been validated for the effective removal of porcine parvovirus (PPV), with a size of approximately 18-24 nm. Therefore, the virus filtration step is effective for all of the above lipid-enveloped viruses, which range in size from 40-430 nm. The virus filtration is also effective against non-lipid enveloped viruses, including equine rhinovirus, equine adeno- and adeno-associated viruses, and equine parvoviruses, which range in size from 18-90 nm. Validation of the process for viral removal has been completed with model equine viruses including bovine viral diarrhea virus (BVDV), WNV, parainfluenza III virus, pseudorabies virus, PPV, adenovirus and xenotropic murine leukemia virus. These two viral inactivation and removal steps are robust and intended to address all known and potential viral contaminations to ensure end product safety for patients.

H-BAT is formulated with 10% maltose and 0.03% polysorbate 80. The formulated bulk material contains approximately 60 mg/mL protein. It contains no preservatives and is intended for single use by IV administration.

3.2 H-BAT Mechanism of Action

The mechanism of action of H-BAT is through passive immunization with equine polyclonal antibody fragments. In the circulation and extravascular compartment, H-BAT binds to free BoNT, preventing it from interacting with cholinergic nerve endings. H-BAT does not have an effect on BoNT once it has entered its target cell population. Therefore, H-BAT is not expected to reverse paralysis in botulism patients but instead reduces the severity and progression of symptoms by neutralizing unbound BoNT in the circulation (Figure 4).

Figure 4: Mechanism of Action of H-BAT



Modified from Arnon et al., 2001.

4 OVERVIEW OF H-BAT DEVELOPMENT

4.1 Regulatory History

During the course of 2003 to 2012, a series of meetings was held between Cangene and the Food and Drug Administration (FDA) to support the overall development program.

Two pre-pre-Investigational New Drug (IND) meetings were held between Cangene and FDA on March 26, 2003 and June 17, 2003, followed by two additional pre-IND meetings on March 24, 2004 and September 23, 2004 to discuss the proposed development plans for H-BAT. The IND application for H-BAT was submitted on November 5, 2004 and included information on the proposed clinical studies BT-001 and BT-002.

Cangene requested Fast Track designation, which was granted on January 28, 2007, for the investigation of H-BAT for the treatment of symptoms following documented or suspected exposure to BoNT serotypes A, B, C, D, E, F or G. Orphan Drug designation for the treatment of botulism was granted on June 29, 2011.

With completion of the required animal and human studies in the spring of 2012, Cangene submitted its Biologic License Application (BLA) to FDA in September 2012, which was granted priority review.

4.2 Justification for Guinea Pig and Rhesus Macaque Models

Controlled clinical trials to examine the efficacy of H-BAT in humans are not feasible since there is no appropriate comparator to allow an assessment of efficacy. It is unethical to withhold treatment with BAT in patients with botulism; therefore a placebo-controlled study is not possible. A historical untreated reference group approach is not feasible due to limited information in the literature on mortality rates and severity of botulism in patients who receive supportive care using current technologies. There are no other products available that provide protection against all seven serotypes that could be used as an active comparator for H-BAT. Thus, because it is not feasible or ethical to perform controlled clinical trials in humans, the development program for H-BAT was designed to meet the four criteria described in the “Animal Rule” regulation “Subpart I 21 CFR Part 314 and subpart H to 21 CFR 600” (Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible). These are the four essential elements:

1. *Reasonably well-understood pathophysiological mechanism for the toxicity and its amelioration or prevention by the product.*
2. *Demonstration of effectiveness in more than one animal species expected to react with a response predictive for humans.*
3. *Animal study endpoint is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity.*

4. *Pharmacokinetics and pharmacodynamics (PK/PD) of the product in animals and humans is sufficiently well understood to allow selection of an effective dose in humans and dosing is safe in humans.*

Key elements in the development program were selecting animal models that are expected to react with a response predictive for humans and identifying the definitive signs for use as a trigger for antitoxin administration. Another key element was determining the extent and appropriate supportive care to administer in the non-human primate studies. These issues are discussed in more detail in the following sections.

4.2.1 Comparative Pathophysiology of Botulism in Guinea Pig, Rhesus Macaque and Humans

Guinea pigs, Rhesus macaques and humans are susceptible to all seven known BoNT serotypes and the pathophysiology of the disease is generally similar across all three species, irrespective of BoNT exposure route or toxin serotype. The time to onset of disease following BoNT intoxication and the time course of botulism disease progression regardless of species are dependent on the BoNT serotype, dose and route of exposure. In all three species BoNT intoxication follows a typical pattern, starting with mild to moderate symptoms related to neuromuscular dysfunction, progressing to more severe symptoms (respiratory distress and paralysis) and eventually death.

The guinea pig model has been used for botulism research for well over 100 years with a large body of data using this model to investigate the efficacy of botulism vaccines and antitoxins [An Epitomes of Current Medical Literature 1898; Cardella 1964; Gelzleichter et al., 1999; Lewis & Metzger, 1980; Task 1997; Task 1998b, Task 2001]. The Rhesus macaque model has also been used previously in botulism research [Franz et al., 1993; Herrero et al., 1967; Middlebrook & Brown 1995; Oberst 1968; Sanford et al., 2010] and is considered well characterized, with a demonstrated sensitivity to all seven toxin serotypes [Boles et al., 2006; Ciccarelli et al., 1977; Department of Defense 2002; Dolman & Murakami 1961; Herrero et al., 1967; Middlebrook & Franz 1997; Oberst et al., 1968; Scott & Suzuki 1988].

While the pathophysiology of botulism caused by circulating toxin being taken up into neuromuscular junctions is very similar across species, the initial signs/symptoms and the course of the disease vary by species. These factors are important considerations in designing the therapeutic studies that must use a trigger for therapeutic antitoxin administration. In the guinea pig, the clinical signs include, right hind limb weakness, weak limbs, noticeable change in breathing sounds or pattern, salivation, lacrimation, forced abdominal respirations and total paralysis. As affirmed by Cangene (BBRC 670, BBRC 964), the first clinical sign observed after intramuscular (IM) BoNT exposure in the guinea pig is typically right hind limb weakness with some animals exhibiting a change in breathing rate that occurred either in conjunction with right hind limb weakness or immediately afterwards (Table 4).

Table 4: Median Time to Event (95% CI) for Botulinum Toxin Serotype A in Characterization Studies

Symptom	Guinea Pig* (hours; 95% CI)	Rhesus Macaque** (hours; 95% CI)	Human***
Ptosis (monkeys) 5Ds: dysphagia, diplopia, dysarthria, dysphonia, dry mouth (humans)	–	51 (39, 59)	3 days post exposure (12-72 hours)
Right hind limb weakness (guinea pigs) Muscular weakness (monkeys/humans)	20 (18, 21)	47 (41, 50)	
Respiratory distress (animals) Dyspnea, tachypnea, hypoxia (humans)	40 (30, 43)	56 (51, 63)	
Total paralysis (Guinea pigs) Extreme weakness (humans)	102 (100, 111)	–	Day 4, Variable
Death	115 (103, 118)	65 (55, 74)	Variable (if left untreated)

* Study: BBRC 1124; ** LBERI FY09-114; *** Arnon et al., 2001, Chertow et al., 2006.

Rhesus macaques present with an anatomical descending paralysis, which is consistent with the progression that defines the disease in humans. Typically, monkeys are first observed with ptosis either alone or in combination with muscular weakness or respiratory distress, and the disease quickly progresses with all three main clinical signs (ptosis, muscular weakness and respiratory distress) developing within a relatively short time frame. Additional clinical signs of oral/nasal discharge are frequently observed, but they are not universal. The disease progresses with the severity of these three signs increasing until animals are severely debilitated and either die or are euthanized for humane reasons.

For humans, it is difficult to define the clinical course as it is dependent upon BoNT exposure levels, route of BoNT administration, and timing and type of treatment intervention. For the guinea pigs and Rhesus macaques, there is a defined BoNT exposure level through a parenteral route, with close observation and defined treatment interventions upon symptom presentation. Table 4 provides a general timeline of symptom progression in humans based upon published literature, which demonstrates similarities to the non-clinical situation. While the absolute timeframe of symptom presentation and progression may vary, the sequence of symptom presentation is consistent across species.

4.2.2 Symptoms Leading to Antitoxin Treatment

Developing a well-defined set of symptoms that trigger treatment with antitoxin is a key component for licensure under the animal rule as it enables differentiation between prophylaxis and therapeutic intervention. It is critical that animals present overt clinical signs for these to be therapeutic treatment models; however, disease progression to severe symptoms significantly decreases the potential benefit of H-BAT in these models.

Cangene classified the clinical signs by species and severity. In the guinea pig, the most specific signs that allow for treatment before severe disease develops are right hind limb weakness (site of BoNT injection) and change in breathing. In the Rhesus macaque, BoNT exposure by IV route results in ptosis, muscular weakness, and respiratory distress as mild to moderate signs. The signs that initiated treatment in the guinea pig and Rhesus macaque studies were discussed with the FDA prior to pivotal study initiation.

In humans, triggering symptoms for antitoxin treatment include dysphagia, diploplia, dysarthria, dysphonia, and dry mouth. The presence of all five symptoms acutely developing in a patient is specific for botulism.

In conclusion, these initial studies confirmed that trigger symptoms were specific and readily identifiable by trained observers, which enabled the start of treatment and could be used in the efficacy studies to measure survival benefit.

4.2.3 H-BAT Dose used in Guinea Pig and Rhesus Macaque Studies

In the animal efficacy studies, the dose of H-BAT was adjusted by scaling based on body weight (BW). Since the adult human dose of H-BAT is one vial, the dose for a one kilogram animal would be 1/70 of a vial, based on the assumption that the average human BW is 70 kilograms. This was expected to result in a similar maximum concentration across each species following IV administration. This is a simple approach to ensuring that the dose used in humans will have at least the same or greater overall H-BAT exposure following administration. The dose selected as equivalent to the human dose and used in the animal studies is referred to as a “scaled human dose” throughout this document.

The pharmacokinetic and efficacy data attained from the guinea pig and Rhesus macaque models were then incorporated into a population PK and exposure-response model to support the human dose (see Section 9.7). In this translational work, animal to human scaling is incorporated to account for differences between species, in terms of body size, shape and physiology.

4.2.4 Extent of Supportive Care in Guinea Pig and Rhesus Macaque Models

In designing efficacy studies in the guinea pigs and Rhesus macaques to approximate human experience, the expected clinical course in each species is also important to consider. In humans the availability and sophistication of full supportive care, which can consist of long-term respiratory support and nutritional support [Arnon et al., 2001] significantly impact outcome. Such a high level of supportive care is difficult to implement routinely in large Good Laboratory Practice (GLP) studies with intoxicated animals.

Cangene studied the use of respiratory supportive care in the Rhesus macaque (LBERI 137) and found significant interference of respiratory care. The respiratory support was very invasive and required the animal to be completely sedated, making direct monitoring of clinical signs impossible.

Although the results of the study suggested extended mean time to survival with H-BAT treatment, the monkeys receiving respiratory supportive care had more severe pneumonia with partial atelectasis compared to animals not receiving respiratory support. The atelectasis was presumed to be due to artificial ventilation and considered to have significantly enhanced the pulmonary congestion. It was concluded that due to numerous complications involved in performing a study with extended respiratory support, its use in nonhuman primates would not be feasible in a study where the support would be needed for greater than 48 hours.

Monkeys can be maintained with minimal (nutritional) and fluid support, which allows for the direct assessment of clinical signs. This supportive care is sufficient to ensure an animal is receiving adequate nutrition and fluids to avoid becoming immediately emaciated or dehydrated and consequently requiring euthanasia for humane reasons.

Such a level of chronic care is simply not practical to replicate in the guinea pig. In addition to this lack of support, current best practices [Guide for the Care and Use of Laboratory Animals, 2011] require guinea pigs to be euthanized for humane reasons if severe clinical signs are present for extended periods of time and the animal is judged to be moribund and unlikely to recover.

Regardless, these studies are representative of the human situation based on the symptom progression and relevant endpoints. This is especially true when considering an event where there is a mass intentional release of BoNT, in which availability of supportive care measures may be limited.

4.3 Animal and Clinical Studies in H-BAT Development

In the H-BAT development program, Cangene used two animal models of botulism (guinea pigs and Rhesus macaques) to evaluate the efficacy of the heptavalent drug product. These animals were used in model development, PK, dose ranging efficacy studies and therapeutic efficacy studies. To demonstrate the product's safety in humans, Cangene conducted two clinical trials in normal healthy human subjects. These studies also examined the PKs and PDs of H-BAT. Clinical experience was also gained under a CDC EAP in which patients with suspected or confirmed botulism were treated with H-BAT. All studies are listed in Table 5 with more discussion in the following sections.

Table 5: H-BAT Development Program: Summary of Studies

Study Type	Study Identifier	Study Objectives
Guinea pig		
Model development	BBRC 621 (GLP)	To determine the appropriate intoxication challenge dose for BoNT serotypes A through G for use in subsequent studies.
	BBRC 670 (GLP)	To determine the clinical course of intoxication following challenge with BoNT serotypes A through G at 4x $GPIMLD_{50}$.
	BBRC 964	To establish and compare the difference in duration between onset of moderate clinical signs (moderate or severe) and death at a range of BoNT challenge doses for BoNT serotypes A and E.
PK in un-intoxicated animals	BBRC 684 (GLP)	To determine the PK profile of H-BAT when administered at 1x and 0.1x the scaled human dose.
Dose ranging	BBRC 731 (GLP)	To determine the efficacy of H-BAT at various doses when administered either 12 hours (BoNT serotypes A, B, C, D, F and G) or six hours (BoNT serotype E) after intoxication with 4x $GPIMLD_{50}$ for all seven BoNT serotypes.
Efficacy	BBRC 843 (GLP)	To determine the efficacy of H-BAT at 1x scaled human dose when administered after onset of clinical signs (moderate or severe) following intoxication with 4x $GPIMLD_{50}$ for all seven BoNT serotypes.
	BBRC 993	To determine the efficacy of H-BAT at 1x scaled human dose when administered after the onset of clinical signs (moderate or severe) following intoxication with 1.5x $GPIMLD_{50}$ for BoNT serotypes A and E.
	BBRC 1005	To determine the efficacy of H-BAT at 1x scaled human dose when administered after the onset of clinical signs (moderate or severe) following intoxication with 1.5x $GPIMLD_{50}$ for BoNT serotypes B, C, D, F and G.
	BBRC 1124	To demonstrate the efficacy of H-BAT at 1x scaled human dose when administered after the onset of clinical signs (moderate or severe) following intoxication with the revised 1.5x $GPIMLD_{50}$ for all seven BoNT serotypes.
	BBRC 1180 (GLP)	To determine the efficacy of H-BAT at 1x scaled human dose when administered after the onset of clinical signs (moderate or severe) following intoxication with 1.5x $GPIMLD_{50}$ for all seven BoNT serotypes.
Rhesus Macaques		
Model development	LBERI 027 (GLP)	To confirm the LD_{50} and to determine the clinical profile and onset of intoxication for BoNT serotype A following IV administration.
PK in un-intoxicated animals	LBERI 056 (GLP)	To assess the PK of serotype A botulism antitoxin following IV administration at two different dose levels.

Study Type	Study Identifier	Study Objectives
PK and Dose-Ranging Efficacy in intoxicated animals	LBERI 061 (GLP)	To demonstrate that H-BAT administered four hours post-intoxication with BoNT serotype A prevents the occurrence of mortality and clinical signs and also to assess the PK of H-BAT following administration of BoNT serotype A.
Efficacy	LBERI 137	To determine the feasibility and effectiveness of long-term respiratory support in combination with botulism antitoxin therapy and to determine whether administration of H-BAT at the onset of clinical signs decreases the progression of clinical signs and mortality.
	LBERI 016	To demonstrate that H-BAT administration to Rhesus macaques in combination with minimal supportive care after onset of clinical signs following exposure to 1.7x $NHPLD_{50}$/kg of BoNT serotype A prevents mortality and clinical signs of intoxication.
	LBERI 114 (GLP)	To confirm that H-BAT administered to Rhesus macaques in combination with minimal supportive care at the onset of clinical signs following exposure to 1.7x $NHPLD_{50}$/kg of BoNT serotype A enhances survival compared to placebo control.
	LBERI 066 (GLP)	To demonstrate that H-BAT administered to Rhesus macaques in combination with minimal supportive care at the onset of clinical signs following exposure to 1.7x $NHPLD_{50}$/kg of BoNT serotype A enhances survival compared to placebo control.
Humans		
Safety and PK in healthy volunteers	BT-001(GCP)	Primary – to evaluate the safety of H-BAT based upon clinical observations, AEs and laboratory assessments. Secondary – to assess the PK of the seven botulism antitoxin serotypes in H-BAT following IV administration.
Safety and PD in healthy volunteers (Connaught BAT AB product)	BT-002 Stage A (GCP)	Primary – to evaluate the effect of bivalent BAT (Aventis Pasteur) in preventing paralysis of the EDB muscle following Botox® or Myobloc® administration. Secondary – to evaluate the safety of bivalent BAT (Aventis Pasteur) in healthy subjects.
Safety and PD in healthy volunteers (H-BAT)	BT-002 Stage B (GCP)	Primary – to evaluate the effect of H-BAT in preventing paralysis of the EDB muscle following Botox® or Myobloc® administration. Secondary – to evaluate the safety of H-BAT in healthy subjects.
Safety and efficacy in patients with suspected or confirmed botulism	CDC IND 6750 (expanded access program)	To enable the use of investigational H-BAT for treatment of individuals with botulism toxin poisoning as a result of a naturally-occurring outbreak or in cases of isolated, unintentional incidents.
Interspecies		
Modeling and simulation	BT-008	To develop a model-based understanding of H-BAT PK/PD properties to extrapolate animal dose regimen to the clinical scenario.

Note: Studies in bold type were pivotal.

Note 2: Placebo control used in animal studies was a purified despeciated (pepsin treated) equine IgG.

4.3.1 Guinea Pig and Rhesus Macaque Development Programs

The guinea pig was chosen as the primary model and the Rhesus macaque as the non-human primate model of botulism for evaluating the therapeutic efficacy of H-BAT. The guinea pig model was used to determine the efficacy of H-BAT against all seven BoNT serotypes and the Rhesus macaque model used serotype A as representative.

The guinea pig and Rhesus macaque possess several characteristics that make it ideal candidates as the botulism animal models. These characteristics include:

- The pathophysiology and clinical course of BoNT intoxication (muscular weakness, progression from mild to severe signs) is similar to that seen in humans.
- Unlike several other rodent species (rabbit and rat) [Greenbaum & Anderson 2006], guinea pigs are sensitive to intoxication by all seven BoNT serotypes [Cardella 1964; Ciccarelli et al., 1977, Harvey et al., 2002], which is similar for the non-human primates (NHPs) (serotype A [Herrero et al., 1967]; serotype B [Boles et al., 2006]; serotype C [Department of Defense 2002]; serotype D [Middlebrook & Franz 1997]; serotype E [BB-IND 7451]; serotype F [Dolman & Murakami 1961]; serotype G [Ciccarelli et al., 1977]).
- The guinea pig and Rhesus macaque are well-established and extensively used models for botulinum antitoxin and vaccine efficacy evaluation [Cardella 1964; Gelzleichter et al., 1999; Lewis & Metzger, 1980; Task 1998a; Herrero et al., 1967; Oberst et al., 1968; Scott & Suzuki 1988].
- The observed clinical signs of botulism in the animal models are relatively easy to recognize and were reliably identified by trained technicians:
  - Guinea pig: weak limbs including right hind limb weakness, noticeable change in breathing, salivation, lacrimation, forced abdominal respiration, total paralysis.
  - Rhesus macaque: ptosis, muscular weakness, respiratory distress, oral and nasal discharge.
- The clinical course of BoNT intoxication is reproducible.
- The guinea pig provides a small animal model in which a statistically significant treatment effect against all seven BoNT serotypes can be demonstrated.
- Minimal supportive care is feasible in the Rhesus macaque [Oberst et al., 1968].

The guinea pig development program included model development studies to examine the IM route of BoNT exposure, level of BoNT exposure (lethal dose 50%, LD_{50}) for each BoNT serotype, clinical course and disease progression, dose response, and trigger for treatment (moderate or severe clinical signs of botulism) (Table 5). The model development information was then used to design the efficacy studies. The guinea pig development program also included a PK study in un-intoxicated animals, a dose-ranging efficacy study to vary antitoxin doses and several efficacy studies including the pivotal study BBRC 1180.

The Rhesus macaque development program included model development studies to determine the level of exposure (LD_{50}) for BoNT serotype A, clinical course and disease

progression, dose-range efficacy, and trigger for treatment (moderate or severe clinical signs of botulism) (Table 5). This information was then used to design the efficacy studies. The Rhesus macaque development program also included PK studies in un-intoxicated and intoxicated animals, a dose ranging study and several efficacy studies including pivotal study LBERI 066.

4.3.2 Clinical Development Program

The focus of the clinical development program was to provide clinical safety data in humans to support the use of H-BAT for the treatment of botulism (see Table 6). In this regard, safety data in humans was obtained from two clinical trials, BT-001 and BT-002 Stage B, which included a total of 56 normal healthy subjects, administered either one or two vials of H-BAT. The FDA indicated that it is usual for safety studies to include 50 to 60 subjects and suggested Cangene enroll this number to establish an informative database (FDA pre-IND meeting September 23, 2004).

The clinical development program also provided PK data to bridge animal efficacy data to human clinical use of H-BAT through modeling and simulation studies. A PD study was conducted to demonstrate the ability of H-BAT to neutralize BoNT clinically in humans following the administration of commercially available Botox® (serotype A toxin) and Myobloc® (serotype B toxin). In addition, the CDC provided supporting clinical safety and effectiveness data on the use of H-BAT in humans with suspected or confirmed botulism.

4.3.3 H-BAT Development Summary

The development program covers all the required elements of the animal rule and ensures that the animal efficacy results translate into a clinically meaningful outcome. At the cellular level, the BoNT mechanism of action is identical across species for the same serotype and the presentation of clinical signs is consistent, irrespective of BoNT serotype. While there are different BoNT absorption kinetics from different potential routes of exposure, the BoNT levels in circulation and subsequently in the extravascular space that determine the extent of neuromuscular uptake and symptom progression. Additionally, the botulism antitoxin mechanism of action is conserved across species and serotypes and is dependent upon BoNT in the circulation and extravascular compartment. Thus, demonstration of H-BAT efficacy in animal models of botulism that used a parenteral exposure of BoNT administration is expected to translate to the human setting, regardless of BoNT exposure route. The following sections will provide detailed information regarding the specific studies in each species and the integration of the animal and human development studies.

5 EFFICACY IN GUINEA PIG MODEL

5.1 Guinea Pig Efficacy Summary

The guinea pig pivotal efficacy study demonstrated a significant survival benefit and reduced the incidence of severe signs and clinical severity scores for H-BAT-treated animals compared to placebo controls.

- Treatment started only after symptoms appeared (similar to treatment decision in humans).
- Significant improvement in survival of guinea pigs treated with H-BAT was demonstrated against all seven serotypes (97-100% survived in the H-BAT group compared to 0-50% in the placebo control group).
- The incidence of severe botulism symptoms was lower in the H-BAT group compared to the placebo control group, suggesting that disease progression was halted.
- The severity scores gradually declined in the majority (99%) of the H-BAT-treated animals towards study end, indicating the full recovery of animals to a normal clinical state.

5.2 Pivotal Study Design

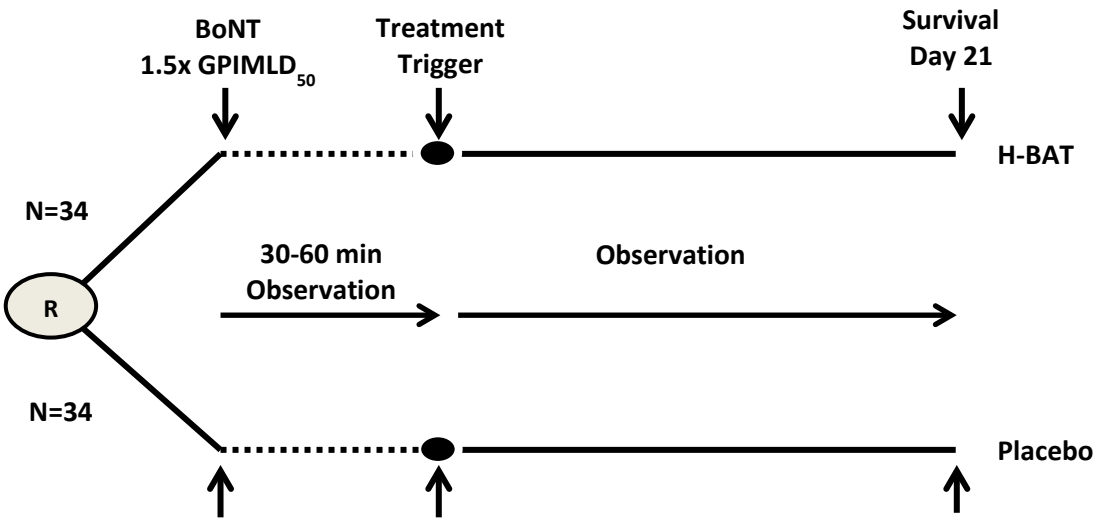
The study protocol was approved by the Institutional Animal Care and Use Committee prior to animal receipt. All study personnel reviewed the protocol and attended a pre-study meeting. Qualified clinical observers attended a clinical observations training session prior to performing clinical observations on the study.

Groups of 14-20 animals per sex were randomized into 14 groups of 34 animals by BoNT serotype (A, B, C, D, E, F or G) and study treatment (H-BAT or placebo). A BoNT dose equivalent to 1.5x the guinea pig intramuscular LD_{50} ($GPIMLD_{50}$) was administered as a single IM injection to the right hind limb. The BoNT dose was based upon results from a blinded and controlled non-GLP study (BBRC 964).

Following BoNT administration, study personnel made hourly observations for animals in serotypes A-D and G and every 30 minutes for animals in serotypes E and F due to the rapid disease progression (Figure 5). Personnel blind to assignment of study treatment made all observations. Identification of triggering symptoms (included right hind limb weakness and change in breathing) prompted blinded study drug administration. By protocol, animals were treated with either the scaled human dose of H-BAT or placebo after the onset of four consecutive moderate clinical signs. Moderate clinical signs included weakness of the right hind limb, noticeable change in breathing sounds rate or pattern, lacrimation, salivation and weak limbs. Following treatment, clinical observations were continued for 21 days or until death (every 3-6 hours until day 14 and every 12 hours from day 14-21). Decisions on sacrifice following standard laboratory practices were made by personnel blind to study treatment assignment.

Therapeutic administration of H-BAT or placebo was initiated if any one of the moderate signs was observed over four consecutive periods. This ensured that animals were therapeutically treated (similar to treatment decision in humans) and that bias from observers would be minimized or eliminated.

Figure 5: Study Design for H-BAT Guinea Pig Efficacy Model



Note: Shown for one serotype.

5.2.1 Pivotal Study Endpoints

The primary endpoint was the proportion of animals surviving to day 21 post-intoxication.

Secondary endpoints included time to death, incidence of clinical signs, and clinical severity scores.

Clinical severity scores were computed for each sign as defined in Table 6 at each observation time point. The sum of clinical severity scores were calculated for each individual animal at a given observation time point and summarized for each treatment group (Section 5.3.4).

Table 6: Clinical Sign, Definition and Clinical Severity Score for Observations

Severity	Clinical Sign	Clinical Severity Score Assigned
Mild	**Lethargy**: general appearance of tiredness or fatigue	1
Moderate	**Salivation**: presence of excessive salivation, or drooling, causing wetness outside of the mouth, muzzle or chin.	2
	Lacrimation: an overproduction of tears, causing wetness around the eye(s), or pooling of tears at eyelid edges or at the corners of the eye(s). Tears may appear milky or cloudy.	2
	Right hind limb weakness: slowness, abnormal position, dragging, trembling, or awkward movement of the hind limb only; the affected rear foot may splay out at an angle instead of being held straight under the body	2
	Weak limbs: weakness of multiple limbs; a weak limb may be slow, awkward upon movement, may be held in an abnormal position, or may drag, tremble, splay outward or otherwise be associated with an abnormal gait when the animal moves forward	2
	Noticeable change in breathing sounds or pattern: change in breathing sounds or pattern	2
Severe	**Forced Abdominal Respirations**: an exaggerated, deep spasmodic movement of the flank muscles during inspiration, including gasping (open the mouth wide during inspiration) and/or arch or stretch neck out	3
	Total Paralysis: animal is recumbent and unable to right itself when placed on its back	3
Death	Animal found dead or euthanized for humane reasons due to poor and deteriorating physical condition as determined by the Study Director or Clinical Veterinarian.	20

Euthanasia Criteria

Any animals meeting a criterion for euthanasia were pre-terminally euthanized. The three criteria were:

1. Any animal having a 25.0% or greater weight loss (when compared to last pre-intoxication body weight) in conjunction with any concurrent severe sign of intoxication;
2. Any animal that has two consecutive observations of total paralysis.
3. Any animal that did not meet either of the first two criteria but was judged to be moribund. Only the Study Director (who was a veterinarian) or (in their absence), a Battelle staff veterinarian determined if an animal was moribund.

5.2.2 Statistical Methodology

The primary objective of this study was to evaluate the effect of H-BAT on the overall survival. For each treatment and placebo control group, the survival rate at 21 days post-

intoxication was calculated, along with an exact 95% confidence interval using the Clopper-Pearson method. Two-tailed Fisher's exact tests were used to determine if there was a statistically significant difference between survival rates for each serotype. All statistical testing was performed at an alpha level of 0.05 with no adjustment for multiple comparisons, since each serotype was considered independent.

A secondary objective of the study was to evaluate the effect of H-BAT on the time to death. Kaplan-Meier curves along with log-rank tests were used to compare the time to death for each serotype. The median time to death was determined along with a two-sided 95% CI for each group using the product limit method.

Additional secondary objective of the study were to evaluate the effect of H-BAT on the incidence and time to onset of clinical signs. For each treatment and control group, the incidence of clinical signs was calculated, along with an exact 95% CI using the Clopper-Pearson method. Two-tailed Fisher's exact tests were then used to compare the incidence of clinical signs for each serotype.

Kaplan-Meier curves along with log-rank tests were used to compare the time to onset of clinical signs for each serotype. The median time to onset of clinical signs was determined along with a two-sided 95% CI for each group using the product-limit method. Summary statistics (number of animals, arithmetic mean, standard deviation (SD), standard error, median, minimum, and maximum) for the duration of each clinical sign and the grouped clinical signs were also calculated.

Summary statistics (number of animals, arithmetic mean, standard deviation, standard error, median, minimum, and maximum) for time to trigger for treatment were calculated for each group, by serotype.

The final secondary objective was to monitor the time to resolution of clinical signs. The duration of clinical signs and clinical severity scores contributed to this objective. Summary statistics (number of animals, arithmetic mean, standard deviation, standard error, median, minimum, and maximum) for the duration of each clinical sign and the grouped clinical signs, were calculated for each group, by serotype.

Study Populations

All animals that were intoxicated with BoNT and who survived to receive H-BAT or placebo were included in the intent to treat (ITT) analysis set, in the treatment group to which they were assigned. When animals were replaced with spares, the spare animal was included in the ITT analysis set and the original animal was excluded. The ITT analysis set was used to assess all the endpoints defined in the Study Endpoints section of this Briefing Document and to generate the study conclusions.

5.3 Efficacy Findings

5.3.1 Primary Endpoint: Survival

In the guinea pig efficacy study, a statistically significant difference in survival was observed with most animals treated with H-BAT surviving (97-100%) compared to substantial

mortality in the control group. The observed survival benefit with H-BAT was independent of serotype: 100% for serotypes A, B, E, F and G and 97% for serotypes C and D (each $p < 0.0001$ vs. placebo; Table 7).

Table 7: Survival by Treatment Group and Serotype in Pivotal Guinea Pig Efficacy Study BBRC 1180

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

5.3.2 Time to Death

The Kaplan-Meier median time to death could not be estimated for any of the H-BAT-treated groups since few or no deaths were observed. The time to death was censored at the end of the study (day 21 post-intoxication) for all surviving animals. The H-BAT-treated groups had significantly (log-rank test, $p < 0.0001$) longer time to death compared to the placebo control groups for all seven serotypes. The median time to death in placebo control groups varied for each serotype, ranging from 29 to 168 hours (serotypes E and G, respectively). The Kaplan-Meier median times to death and their 95% CI are presented in Table 8. The Kaplan-Meier curves are presented in Figure 6.

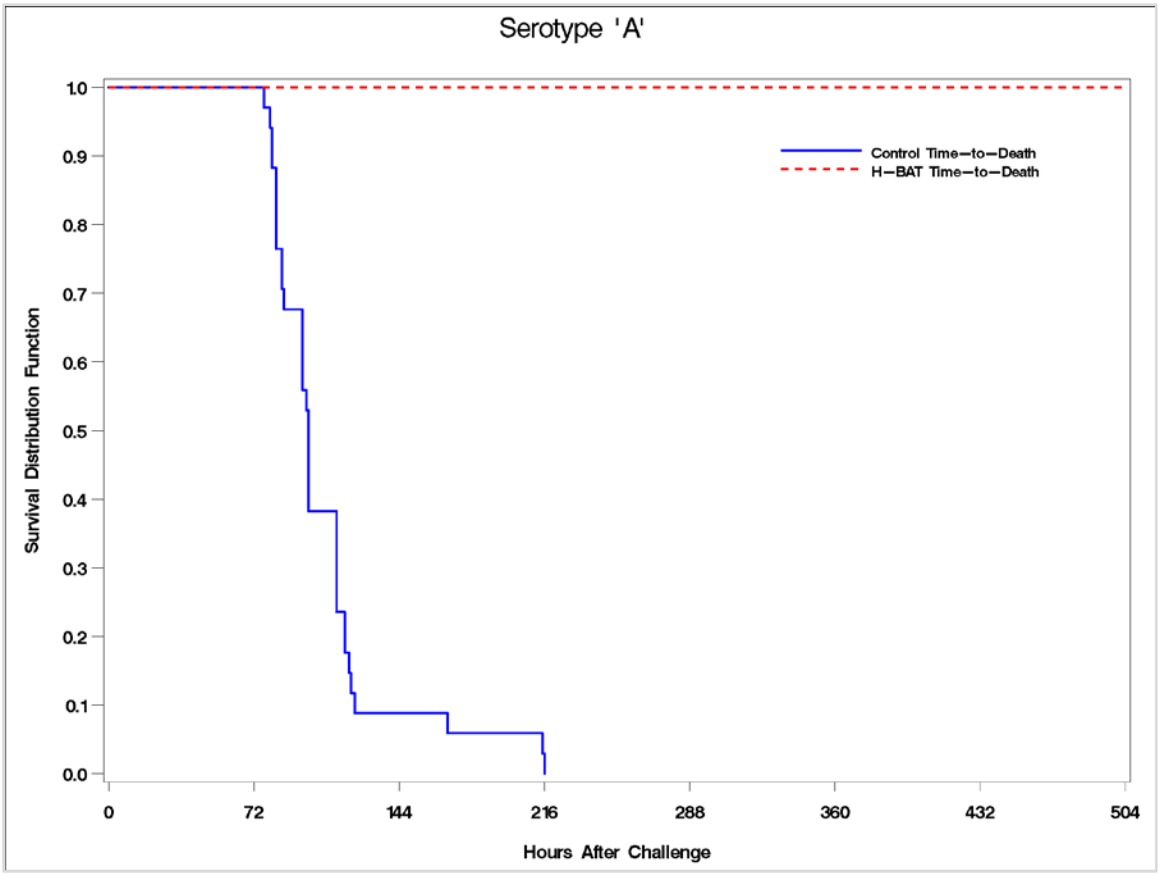
Table 8: Kaplan-Meier Median Time-to-Death in Pivotal Guinea Pig Efficacy Study BBRC 1180 (ITT Analysis Set)

Serotype	Group	Time-to-Death (Hours) Kaplan-Meier Median (95% Confidence Interval)	Log-Rank Test Time-to-Death Comparison (p-value)
A	H-BAT	--(--)	0.0001
	Placebo control	99 (87, 113)	
B	H-BAT	--(--)	0.0001
	Placebo control	94 (94, 112)	
C	H-BAT	--(--)	0.0001
	Placebo control	114 (111, 141)	
D	H-BAT	--(--)	0.0001
	Placebo control	156 (141, 180)	
E	H-BAT	--(--)	0.0001
	Placebo control	29 (27, 30)	
F	H-BAT	--(--)	0.0001
	Placebo control	58 (45, 68)	
G	H-BAT	--(--)	0.0001
	Placebo control	168 (143, --)*	

--The Kaplan-Meier median time-to-death could not be estimated since animal death among H-BAT-treated animals was not observed for serotypes A, B, E, F and G and only one death was observed for serotypes C and D.

* The upper bound of the 95% CI interval could not be estimated due to the high incidence of censoring in the data.

Figure 6: Survival Rate for H-BAT and Placebo Control Groups for Serotype A in Pivotal Guinea Pig Efficacy Study BBRC 1180



Graphical representations of the survival rates for Serotypes B-G are presented in Section 13.5.

5.3.3 Incidence of Clinical Signs of Botulism

The incidence of clinical signs (percentage of animals that showed each sign at least once) was also evaluated as a measure of treatment effect on the progression of clinical signs in H-BAT-treated groups compared to placebo controls for all seven serotypes. Despite immediate intervention after the onset of clinical signs, almost all (~99%) H-BAT-treated animals developed change in breathing rate/sound or pattern in addition to right hind limb weakness (the trigger in majority of the cases) at rates consistent with placebo control animals (Table 9). However, the incidence of other moderate clinical signs (e.g., lacrimation, salivation, weak limbs) was significantly reduced in the H-BAT-treated groups compared to their respective placebo controls.

Most importantly, a decreased incidence of severe signs of botulism was observed in the H-BAT-treated animals compared to placebo groups. For example, 0.8% (2/238) of the animals that received H-BAT exhibited forced abdominal respirations compared to 49% in the

placebo control groups (117/238). Similarly, 0% (0/238) in the H-BAT-treated groups progressed to total paralysis (severe sign requiring euthanasia) compared to 75% (178/238) in the placebo groups. These results suggest that H-BAT treatment halted disease progression compared to placebo control groups that exhibited continued progression of the disease from mild to moderate to severe for all serotypes.

Table 9: Incidence of Botulism Signs in H-BAT and Placebo Treatment Groups in Pivotal Guinea Pig Efficacy Study BBRC 1180

	Number of Animals Showing Each Clinical Sign by Group													
	Serotype A		Serotype B		Serotype C		Serotype D		Serotype E		Serotype F		Serotype G	
Clinical Sign	**H-BAT N=34**	**Placebo N=34**	**H-BAT N=34**	**Placebo N=34**	**H-BAT N=34**	**Placebo N=34**	**H-BAT N=34**	**Placebo N=34**	**H-BAT N=34**	**Placebo N=34**	**H-BAT N=34**	**Placebo N=34**	**H-BAT N=34**	**Placebo N=34**
Lethargy	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Right hind limb weakness	34	34	34	34	34	34	34	34	34	34	34	34	34	34
Change in breathing sounds or pattern	34	34	34	34	33	34	30	34	34	34	34	34	34	34
Weak limbs	5	34	10	34	6	34	3	34	16	34	26	34	14	34
Salivation	0	22	0	24	0	25	0	29	0	3	6	24	4	31
Lacrimation	0	3	0	11	0	12	0	17	0	5	0	5	1	15
Forced abdominal respirations	0	9	0	15	1	6	0	6	0	32	1	33	0	16
Total paralysis	0	34	0	31	0	26	0	25	0	34	0	21	0	7

5.3.4 Severity Score for Botulism Signs

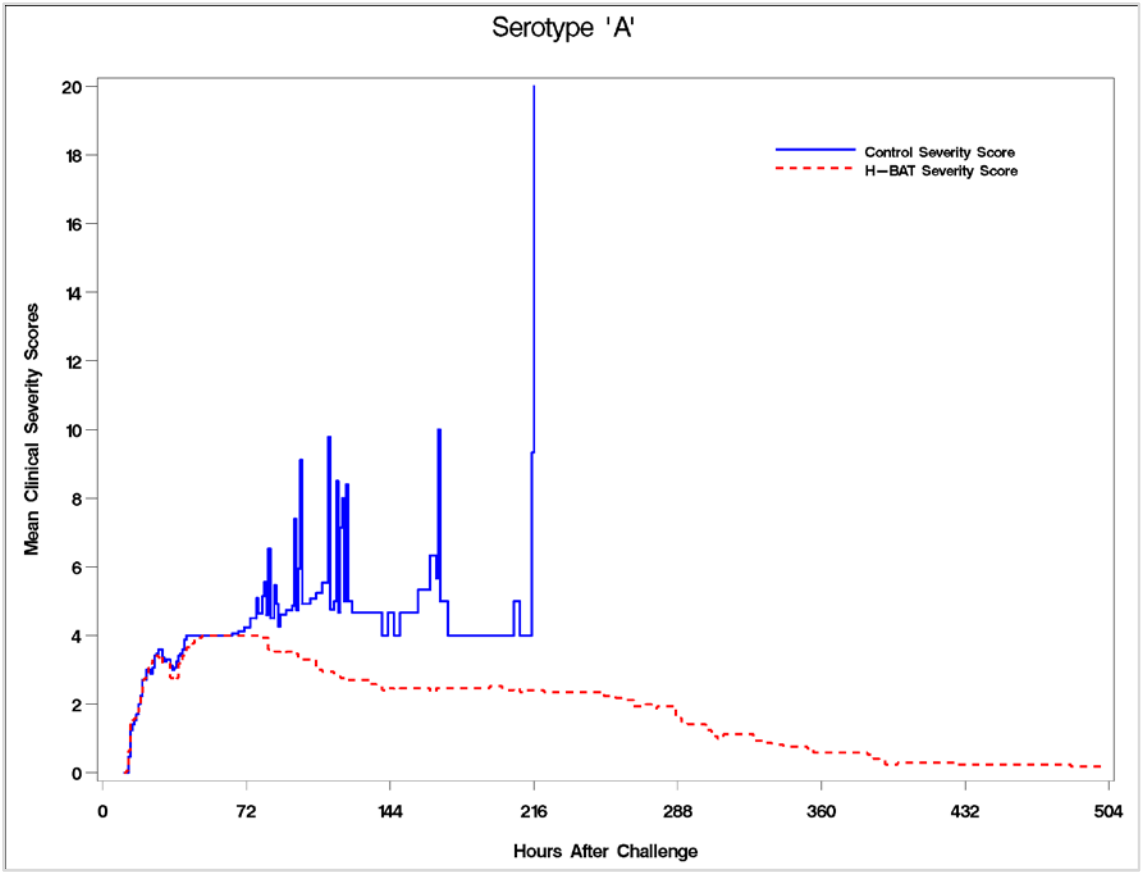
The mean sum of clinical severity scores for both H-BAT-treated and placebo control groups were comparable from toxin challenge to the treatment time and also for a period of time following treatment as would be expected due to the toxin already taken up into neuromuscular tissue; however, subsequently a steady decline in the sum of severity scores to normal in the H-BAT-treated groups compared to placebo control across all seven serotypes (Figure 7). The worsening of clinical signs in the placebo treated arms began to diverge from the H-BAT-treated animals ~16-48 after treatment initiation (Table 10 and Figure 7). The sum of severity scores gradually declined in the majority (99%) of the H-BAT-treated animals towards study end, indicating the recovery of animals to a normal clinical state. The majority of the placebo controls died or were euthanized due to severe disease conditions. The spikes in the severity curves for the placebo groups represent scores of 20 assigned to animals that died or were euthanized at that observation. A small percentage (13%) of placebo controls survived the intoxication and recovered to a normal clinical state.

Table 10: Median Time to Treatment per Serotype for H-BAT and Placebo Groups in Pivotal Guinea Pig Efficacy Study BBRC 1180

Serotype	H-BAT Hours (range)	Placebo Hours (range)
A	17 (15-23)	17 (16-29)
B	26 (20-29)	25 (19-29)
C	22 (12-26)	22 (12-26)
D	24 (22-37)	24 (22-37)
E	9 (7-16)	8 (8-10)
F	15 (11-20)	15 (10-20)
G	23 (15-28)	22 (16-29)

As anticipated from the guinea pig model development studies, serotypes A, E and F were the fastest to treatment trigger presentation, while the remaining serotypes developed signs approximately 24 hours after BoNT exposure. Once treated, animals were closely monitored for clinical sign progression or regression as a key measure of H-BAT potential benefits (see Figure 7).

Figure 7: Clinical Severity Scores for H-BAT and Placebo Control Groups for Serotype A in Pivotal Guinea Pig Efficacy Study BBRC 1180



Graphical representations of the survival rates for Serotypes B-G are presented in Section 13.6.

5.4 Guinea Pig Conclusion

The guinea pig pivotal efficacy study demonstrated a significant survival benefit associated with H-BAT treatment compared to placebo controls. This survival benefit was statistically significant and consistent across the seven serotypes. In addition, there was substantial improvement in clinical signs and H-BAT decreased in the incidence and severity of clinically meaningful symptoms. Overall, these results clearly demonstrate the benefit of H-BAT and fulfill one of the essential elements of the ‘Animal Rule’.

6 EFFICACY IN RHESUS MACAQUES

6.1 Rhesus Macaque Efficacy Summary

The Rhesus macaque pivotal efficacy study demonstrated a significant survival benefit and marked improvement in clinical severity scores for H-BAT-treated animals.

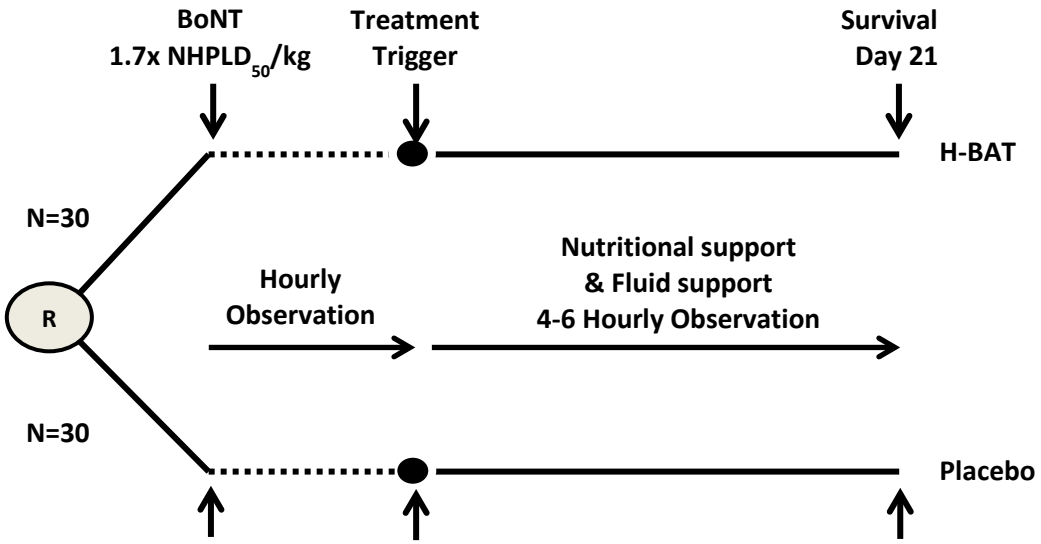
- A significant improvement in survival was observed in Rhesus macaques intoxicated with BoNT serotype A and then treated with H-BAT, administered in combination with minimal supportive care, after development of botulism symptoms.
- Overall, 47% (14/30) in the H-BAT treatment group survived to 21 days with 0% (0/30) surviving in the placebo control group (Fisher's exact test, $p < 0.0001$).
- Time to death was significantly longer in the H-BAT group and the incidence of severe botulism symptoms was lower in the H-BAT group compared to the placebo control group.

6.2 Pivotal Study Design

The Rhesus macaque efficacy study (LBERI 066) was a randomized, blinded, placebo-controlled study. Two groups of 30 Rhesus macaque monkeys were intoxicated with 1.7x the non-human primate intravenous LD_{50} per kilogram ($NHPLD_{50}$/kg) of serotype A. Following administration of BoNT, clinical observations were focused on identifying pre-defined symptoms that would trigger treatment. At the onset of moderate clinical signs, monkeys were treated with IV administration of either H-BAT or placebo and all animals received supportive care (nutritional and fluid support). Observation continued until day 21 post intoxication or death. All personnel were blind to study treatment (Figure 8).

The study protocol was approved by the Institutional Animal Care and Use Committee prior to animal receipt. All study personnel reviewed the protocol and attended a pre-study meeting. Qualified veterinarians performed the clinical observations on the study.

Figure 8: Rhesus Macaque H-BAT Efficacy Study Design



Assessment of all seven toxin serotypes would require more Rhesus macaques than is ethically acceptable. If the Rhesus macaque development program needed to be expanded to include the remaining six BoNT serotypes, an additional 900 non-human primates would have been required to conduct this program. As all seven serotypes have comparable mechanisms of action and are lethal to Rhesus macaques, evaluation of a single serotype was selected to be representative. Serotype A is the most prevalent serotype found in cases of foodborne botulism in adults in the US and is the second most prevalent serotype (after serotype B) reported worldwide [Hatheway 1995]. In addition, serotype A produces a more severe clinical course in humans when compared to serotypes B and E [Hughes et al., 1981; Woodruff et al., 1992]. Serotype A was selected as representative of the seven serotypes and included in the H-BAT Rhesus macaque studies.

A total of 63 Rhesus macaques were surgically implanted with an indwelling central venous catheter, with 60 of them randomly allocated to either the H-BAT (n=30, 14 male, 16 female) or placebo group (n=30, 15/sex). Three animals were maintained as spares. 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 botulinum intoxication in each animal (ptosis, muscular weakness, and/or respiratory distress), H-BAT at the scaled human dose or placebo was administered IV *via* the central venous catheter. Study personnel were blinded to treatment group allocations. Minimal supportive care (nutrition and fluid support) was initiated in all animals following 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 a nutritional liquid supplement containing proteins, lipids and carbohydrates. Animals were observed for 21 days.

6.2.1 Study Endpoints

The primary endpoint was the proportion of animals surviving to day 21 post-intoxication.

Secondary efficacy endpoints included time to death, time to onset of clinical signs, time from onset of clinical signs to recovery and clinical severity scores.

Table 11 shows the clinical signs observed and the associated protocol defined score for each sign. The sum of clinical severity scores were calculated for each individual animal at a given observation time point and summarized for each treatment group.

Table 11: Clinical Severity Scores in Rhesus Macaque Model

Clinical Sign	Observation	Clinical Severity Score Assigned
Ptosis	Absent	0
	Slight Drooping of one or both upper eyelids so that less than ½ of the eye globe is covered	1
	Marked Drooping of one or both upper eyelids so that ½ or greater of the eye globe is covered	2
Muscular weakness	Normal	0
	Hunched posture	1
	Prostrate but able to rise	2
	Prostrate unable to rise	3
Respiratory distress	Closed mouth and rhythmic (thoracic)	0
	Open mouth shallow (thoracic)	1
	Gasping and jerky (abdominal)	3
Oral discharge	Absent	0
	Present	1
Nasal discharge	Absent	0
	Present	1

Euthanasia Criteria

When a Rhesus macaque severity score was greater than or equal to seven (≥ 7), euthanasia was considered for that animal; however, animals with severe clinical signs or a poor and deteriorating clinical condition were also considered for euthanasia regardless of severity score, as determined by the Study Director or another veterinarian associated with the study.

6.2.2 Statistical Methodology

The proportion of animals in the efficacy analysis set for each group that survived to 21 days post-toxin exposure was calculated along with two-sided 95% CIs, using the exact binomial distribution. The proportion of animals that survived to 21 days post-toxin exposure in each

group was compared using a two-sided Fisher's exact test, with the probability of type one error set at $\alpha = 0.05$.

The median time to death was calculated along with a two-sided 95% CIs for each treatment group, using the product-limit method. The survival curves were compared between the treatment and placebo groups using the log-rank test.

Time to onset of clinical signs and time from onset of clinical signs to recovery were analyzed using the same techniques as described for the time to death endpoint. The duration of each clinical sign was also summarized by treatment group.

6.3 Efficacy Findings

6.3.1 Primary Endpoint: Survival

Treatment with the scaled human dose of H-BAT, administered in combination with basic supportive care at the onset of clinical signs, resulted in a statistically significant improvement in survival of Rhesus macaques intoxicated with BoNT serotype A, as compared to placebo control (Table 12).

Table 12: Survival in Rhesus Macaque Efficacy Study LBERI 066

Treatment	Survival (%)	Fisher's Exact Test (p-value)
H-BAT	14/30 (47%)	< 0.0001
Placebo control	0/30 (0%)	

The survival rate observed in Rhesus macaques was lower in comparisons to survival rates observed in guinea pigs. The reason for this may be attributed to the difference in the disease onset times and treatment time between the animal models. The average time to onset of symptoms is shortest in guinea pigs (median trigger was between 17-24 hours post intoxication for majority of the serotypes), and thus were treated at an early stage of the disease progression resulting in higher survival rate. For the monkeys, median time to onset was 58-64 hours post intoxication (more than 2-fold delayed compared to guinea pig) and treatment with H-BAT was able to prevent progression of the disease and death in approximately half the animals, likely due to internalization of lethal doses of the toxin before the therapy was administered. Additionally, the progression from onset to death was very rapid compared to guinea pigs. In dose range studies where the time of BoNT exposure and H-BAT treatment is controlled, guinea pigs and Rhesus macaques were similar in that all animals survived at the scaled human dose. The observed survival rate in monkeys was consistent with the other investigational antitoxin treatment data available in published literature [Franz et al., 1993]. Therefore, H-BAT treatment was effective in both animal models and difference in survival is likely due to the fact that Rhesus macaques were treated at a later time point in the disease progression and less likely to benefit from H-BAT administration due to the irreversible toxin-mediated damage.

6.3.2 Secondary Endpoints

6.3.2.1 Time to Onset of Clinical Symptoms

Clinical signs in all animals in both groups were consistent with BoNT intoxication. Since the onset of the pre-defined signs (Table 13) triggered the H-BAT administration, the time to onset of these signs were comparable between the two groups in both treatment and placebo groups. Overall, there was little difference for ptosis, muscular weakness and respiratory distress, symptoms that correlate with specific botulism symptoms in humans. The time to onset of nasal discharge tended to be longer in the H-BAT group, suggesting halting of symptom progression.

Table 13: Estimated Median Time to Onset of Clinical Signs for Rhesus Macaques in Efficacy Study LBERI 066

Clinical Sign	Kaplan Meier Median Time to Onset (95% Confidence Intervals) in Hours		Log-Rank Test (p-value)
	Placebo Control	H-BAT	
Ptosis	64 (55, 67)	62 (55, 66)	0.294
Muscular weakness	59 (52, 63)	60.5 (53, 63)	0.129
Respiratory distress	58 (53, 63)	59.5 (55, 63)	0.347
Oral discharge	56 (53, 60)	61.5 (58, 65)	0.072
Nasal discharge	84 (67, 110)	107 (90, –) [a]	0.048

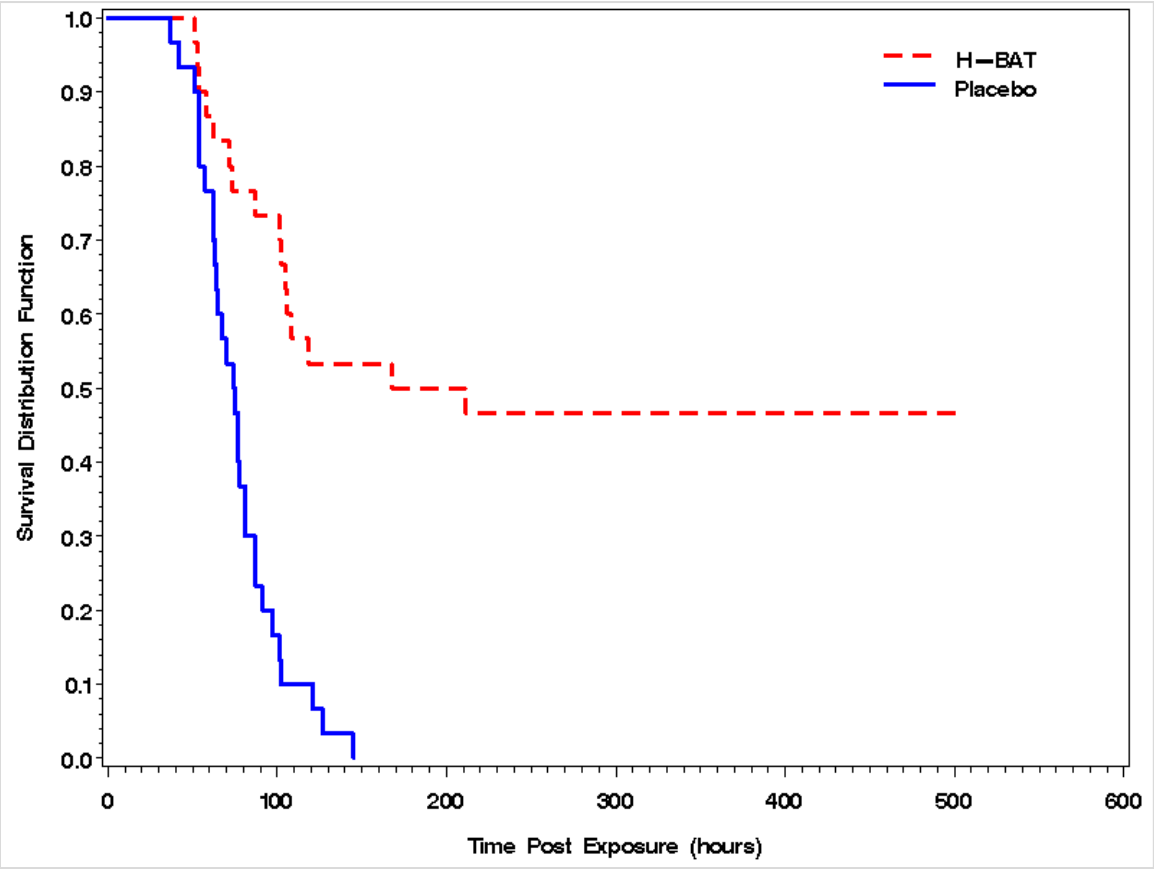
[a] The upper bound of the CI could not be estimated due to the limited number of events.

These results indicate that the blind was maintained and no bias was introduced into the study as animals were treated similarly regardless of treatment group allocation.

6.3.2.2 Time to Death

The time to death was defined as the time from BoNT exposure to death. For animals that were alive at study end (day 21 post-intoxication), time to death was censored at the end of the study. A statistically significant difference in the time to death between treatment groups was noted ($p < 0.0001$, log-rank test) (Figure 9).

Figure 9: Survival Rate in Rhesus Macaques for H-BAT and Placebo Control Groups in Efficacy Study LBERI 066



6.3.2.3 Time to Recovery

Time from onset of clinical signs to recovery was calculated for those animals that survived to the end of the study (Table 14). Recovery was defined as the time when the sum of scores returned to zero. The median time to recovery was 137 hours (95% CI: 118, 222) in H-BAT-treated animals. Animals that survived to the end of the study without recovery were censored at the last time point that they were assessed. Since no animals in the placebo group survived to the end of the study, the calculation of median time to recovery was not possible.

Table 14: Analysis of Time from Onset of Clinical Signs to Recovery for Rhesus Macaques in Efficacy Study LBERI 066

Treatment	Median Time From Clinical Onset to Recovery in Hours	95% CI	Log-Rank Test (p-value)
H-BAT	137	(118, 222)	N/A
Placebo control	N/A [a]	N/A [a]	

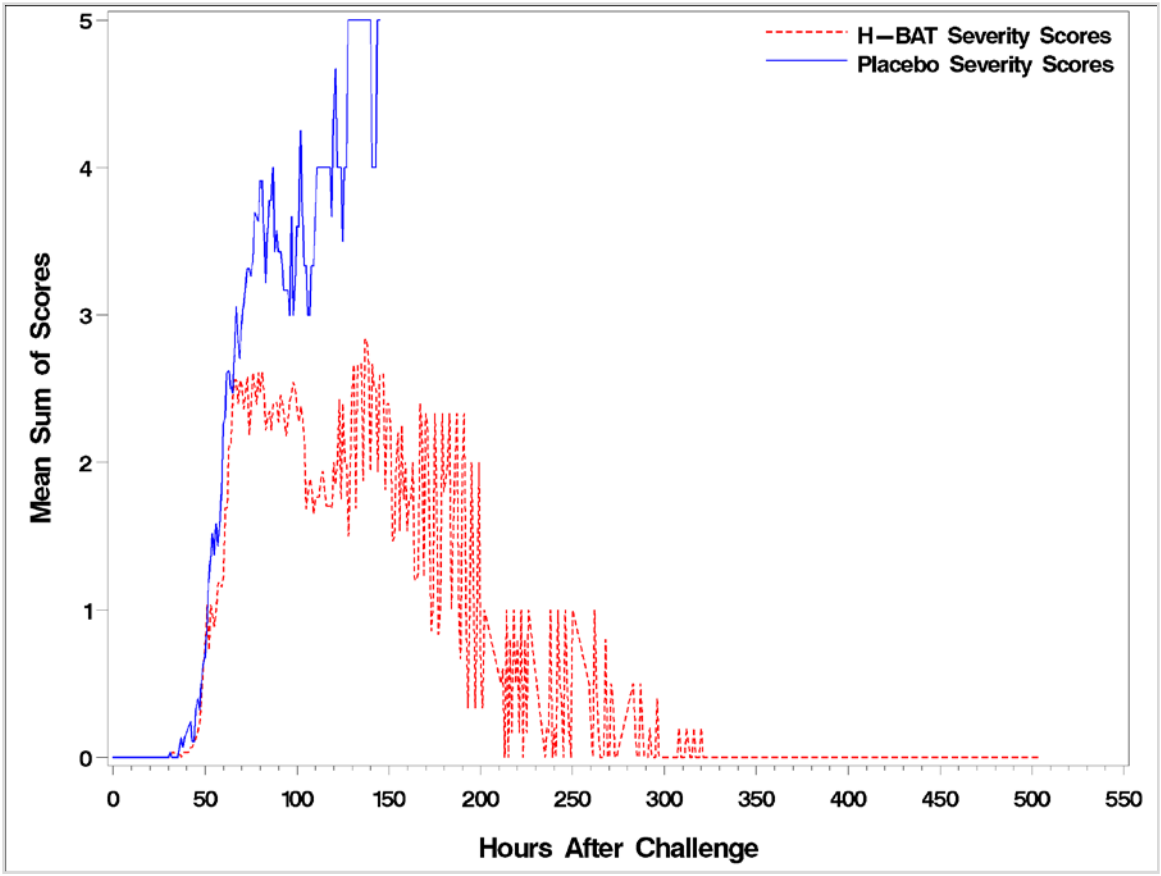
[a] Median and 95% CI not calculated for placebo group since no animals survived to the end of the study.
N/A = Not Applicable.

The delayed time to death and recovery from symptoms demonstrated that H-BAT is able to halt sign progression. This delay may provide opportunity for intervention with enhanced supportive measures to provide additional potential benefit in a clinical setting.

6.3.2.4 Severity Score for Botulism Signs

Clinical severity scores were calculated for each individual animal at a given observation time point. The clinical severity score is calculated as the sum of scores assigned to each of the clinical signs based on severity (Table 11). The maximum sum of scores is 10, with no score assigned at the time of death. A graph of the average severity score sum over time by treatment group is provided to visually display the treatment effect over time (Figure 10).

Figure 10: Average Sum of Severity Scores over Time by Treatment Group in Rhesus Macaque Efficacy Study LBERI 066



Both groups showed similar profiles in the development of clinical signs for up to 69 hours following intoxication. Thereafter the curve diverged. Since the median time to treatment was 53 hour (range 37-75 hours), this is very quickly after treatment start. Subsequently, the H-BAT-treated animals that survived became stable and gradually recovered, while the clinical scores of placebo controls (and of H-BAT-treated animals that died) continued to increase until death/euthanasia (Figure 10).

6.4 Rhesus Macaque Conclusions

The study demonstrated that a single scaled human H-BAT dose administered to symptomatic Rhesus macaques resulted in a significant survival benefit and improved clinical symptoms compared to placebo controls. The statistically significant improvement in survival and severe signs are expected to translate into a clinical benefit of H-BAT.

7 SAFETY FINDINGS ACROSS NONCLINICAL DEVELOPMENT

7.1 Summary of Safety

Overall H-BAT was well-tolerated in animals, with no histological signs of anaphylaxis.

- The H-BAT was well tolerated in normal guinea pigs and Rhesus macaques following IV administration.
- In intoxicated Rhesus macaques the following gross pathology and histology findings were observed:
  - There was no difference in the incidence of the gross findings (lung discoloration) between animals found dead/sacrificed due to moribund state in H-BAT-treated (12/16, 75%) or placebo (21/29, 72%) group.
  - In surviving animals with H-BAT treatment, there were fewer incidence of the lung discoloration (2/14,14%), indicating that H-BAT prevented BoNT mediated lung damage in surviving animals.
  - No histopathology changes suggestive of death resulting from anaphylaxis in animals.
  - All histological findings were related to secondary effects expected with botulinum intoxication.

7.2 General Safety in Animals

Nonclinical toxicological studies were not conducted because the active ingredient of H-BAT is equine immune globulin fragments. The general safety of equine immune globulins is well established due to their long experience in clinical practice; therefore, nonclinical evaluation of toxicity in animal models was not warranted for H-BAT as per International Conference on Harmonization (ICH) S6 Guideline: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. Similarly, the safety of the excipients and impurities in H-BAT is also well established in the published literature. The tolerability of H-BAT was assessed in normal guinea pigs as well as normal and intoxicated Rhesus macaques.

7.3 Safety in Guinea Pigs

A single dose PK study was performed with H-BAT in guinea pigs (BBRC 684). Although not designed as a safety study, basic toxicology endpoints (i.e., body weight, clinical observations) were assessed for tolerability. H-BAT was well tolerated in normal guinea pigs following IV administration.

7.4 Safety in Rhesus Macaques

A single dose PK study was performed with H-BAT in Rhesus macaques (LBERI 056). Basic toxicology endpoints (i.e., body weight, clinical observations) were assessed for

tolerability. H-BAT was well tolerated in normal Rhesus macaques following IV administration.

In the Rhesus macaque efficacy study, complete necropsy was performed on 59 Rhesus macaques. The spectrum of gross pathology did not vary substantively among animals receiving H-BAT or placebo. The primary gross findings, observed in both H-BAT-treated and placebo control animals, were irregularly shaped areas of red discoloration visible on the surface of the lungs of some animals. Such areas were grossly interpreted as congestion and or atelectasis with the possibility of underlying pneumonia. Though some degree of these pulmonary changes are not uncommon in animals found dead or euthanized, the gross changes were likely exacerbated in these animals due to hypoventilation resulted from secondary to BoNT A intoxication. None of these changes are expected to be related to treatment with H-BAT. There was no difference in the incidence of these gross findings between animal that found dead/euthanized in the H-BAT-treated 75% (12/16) or placebo 72% (21/29) groups. However, fewer of the H-BAT-treated animals (2/14, 14%) that survived until study end had lung discoloration. The majority of the gross findings observed were expected with BoNT intoxication.

Histopathologic examination was performed on selected tissues and gross lesions in 11 Rhesus macaques based on two separate, somewhat unusual clinical courses. In one clinical course, the clinical condition of three animals (one H-BAT and two placebo animals) rapidly declined, leading to euthanasia of these three animals within approximately one hour of treatment. Histopathological examination was performed on tissue from these three animals and that from four comparison animals (two in each treatment group) Tissues examined were not remarkable for markers of anaphylaxis such as pulmonary (alveolar and peribronchovascular) edema, laryngeal edema, marked generalized visceral congestion and edema within tissues. Additionally, the changes observed were not substantively different from the animals selected for comparison.

In addition, two H-BAT-treated animals appeared to have been recovering from BoNT intoxication, but subsequently died or declined. Tissues from those two animals and two comparison animals (one placebo and one H-BAT-treated) were examined. In one of these animals that had apparently recovered, histopathology analysis documented moderate widespread mixed pulmonary inflammation with associated foreign material. This “aspiration pneumonia” was likely the cause of death in this animal. In the other animal, gross and histopathology findings were characteristic of systemic septicemia/endotoxemia. It is likely that long-term ileus allowed for bacterial imbalance/overgrowth, mucosal permeability, and endotoxemia/septicemia. Tissues from animals selected for comparison were generally not remarkable but had substantive pulmonary inflammation.

In conclusion, for all Rhesus macaques from which tissues were examined microscopically (i.e., animals from both clinical courses of interest and their respective comparisons) the principal gross lesion of lung discoloration was correlated histologically to pulmonary congestion and inflammation, which is often associated with associated foreign (food) material. Scattered animals of both groups had histological evidence of pulmonary inflammation associated with aspiration. These findings were due to secondary effects

expected with BoNT intoxication rather than H-BAT related since they were consistent between placebo and H-BAT-treated groups.

8 HUMAN EXPERIENCE

8.1 Clinical Summary

The clinical trials and CDC EAP demonstrated that H-BAT is safe and well-tolerated in healthy subjects as well as patients with suspected or confirmed botulism. The efficacy of H-BAT was demonstrated by maintaining muscle function in the extensor digitorum brevis (EDB) model and the CDC EAP suggests that when patients are treated earlier they have better outcomes.

- In healthy subjects H-BAT was safe and well-tolerated with the common side effects of headache and somnolence, and less frequently hypersensitivity symptoms such as pruritus and urticaria.
- In the CDC EAP only 12% of patients reported adverse drug reactions (ADRs), with pyrexia, chills and rash reported as most frequent. There were two serious adverse events (SAEs), and one patient reported serum sickness.
- No anaphylaxis or anaphylactoid reactions were reported in healthy subjects or patients.
- H-BAT was efficacious in maintaining muscle function in an EDB model; similar to the previously marketed Aventis Pasteur (Connaught) AB BAT and significantly better than placebo.
- The data from the CDC EAP suggest that H-BAT treatment given within two days from symptom onset results in shorter duration of hospitalization, shorter stay in ICU and less mechanical ventilation, compared to treatment delayed beyond two days.

8.2 Clinical Safety

The primary safety concern with heterologous immune globulins is immunologic reactions to foreign proteins. These reactions include anaphylactic and anaphylactoid reactions, delayed allergic reactions (serum sickness) and possible febrile responses to immune complexes formed from animal antibodies. These safety concerns correspond to the adverse reactions listed in the prescribing information for the previously marketed Aventis Pasteur (Connaught) BAT AB product [BAT AB Prescribing Information], which included anaphylactic reactions, thermal reactions and serum sickness.

During an 11-year period from 1967 to 1977, the CDC monitored hypersensitivity reactions in patients receiving equine botulism antitoxins. Black and Gunn [1980] reported that of the 268 people receiving botulism antitoxin, 24 (9.0%) had nonfatal hypersensitivity reactions, with 5.3% of the reactions being acute and 3.7% of the reactions being delayed. The overall reaction rates did not differ with age or sex of the recipient or with the botulism antitoxin type (previously marketed Aventis Pasteur (Connaught) BAT AB product or BAT ABE). However, serum sickness occurred significantly ($p < 0.02$) more frequently in persons receiving more than 40 mL of botulism antitoxin.

The focus of the H-BAT clinical development program was to provide clinical safety information to support the use of H-BAT for the treatment of symptomatic botulism following documented or suspected exposure to BoNT serotypes A, B, C, D, E, F or G. The clinical safety information for H-BAT was obtained during two clinical trials (BT-001 and BT-002 Stage B), which included a total of 56 healthy subjects administered either one or two vials of H-BAT.

Limited but relevant safety information was also gathered from patients treated with H-BAT under a CDC EAP.

8.3 Treatment-Emergent Adverse Events in Healthy Subjects

Clinical Trial BT-001

Study BT-001 was a Phase 1, single-center, randomized, double-blind, parallel arm study. H-BAT was administered IV to healthy, male and female volunteers between the ages of 19 and 52 years. Forty subjects were randomized 1:1 to receive either one or two vials of H-BAT, representing a single or double dose. Subjects checked into the study center the day before and remained at the center 24 hours after administration of H-BAT (on Day 0). Subjects returned for follow-up visits on Days 1, 3, 7, 14, 21 and 28. Safety of H-BAT was evaluated based on clinical observations, AEs, laboratory assessments and human anti-equine antibody testing.

Overall, adverse events were reported for 45% (18/40) of subjects. The most frequently reported AEs (i.e., reported by ≥ 10% of total subjects) were headache and somnolence. Five subjects (three (15%) and two (10%) in the one and two vial groups, respectively) reported seven headaches, two were considered moderate and five were mild (Table 15). Somnolence was reported for four subjects (10%), with all events mild.

Table 15: Adverse Events Reported by ≥ 10% of the Total Subjects in Clinical Trial BT-001

Adverse Event Preferred Term *	H-BAT 1 Vial	H-BAT 2 Vials	H-BAT Combined
	N = 20 n (% of subjects)	N = 20 n (% of subjects)	N = 40 n (% of subjects)
Headache	3 (15%)	2 (10%)	5 (13%)
Somnolence	3 (15%)	1 (5%)	4 (10%)

* Adverse events are classified according to Medical Dictionary for Regulatory Activities (MedDRA) version 11.0.

Drug-related AEs (those assessed as possibly or probably related) included: dysphagia, flatulence, nausea, throat irritation, feeling cold, pain, pyrexia, swelling, pharyngolaryngeal pain, hyperhidrosis, pruritus, pruritus generalized, skin disorder and urticaria.

Clinical Trial BT-002

Study BT-002 was an exploratory PD study that was divided into two stages. Stage A was designed to evaluate the ability of the previously marketed Aventis Pasteur (Connaught) BAT AB product to neutralize BoNTs in the EDB muscle model of paralysis in healthy subjects versus placebo.

Stage B of this study evaluated the ability of H-BAT to neutralize BoNTs in the same model. In this stage, 26 healthy adult subjects, aged between 19-48 years were randomized 8:5 to receive either a single IV infusion of H-BAT or placebo (0.9% saline solution) one day prior to administration of BoNT serotypes A (Botox®) and B (Myobloc®) into the EDB muscle of the left and right foot, respectively.

Overall, adverse events were reported for 92% (24/26) of subjects in study BT-002 Stage B. The most frequently reported AEs included contusions (25%), tonsillar hypertrophy (25%), lymphadenopathy (19%), and skin laceration (19%) (Table 16). All events were considered mild. Other AEs reported in > 10% of subjects included dysmenorrhea (13%), headache (13%), pain in extremities (13%), rhabdomyolysis (13%), and somnolence (13%).

Table 16: Adverse Events Reported by ≥ 10% of the Subjects Receiving either H-BAT or Placebo Control in Clinical Trial BT-002 Stage B

Adverse Event Preferred Term*	H-BAT 1 Dose	Placebo Control
	N = 16 n (%) [a]	N = 10 n (%)
Acne	0 (0%)	1 (10%)
Aphthous stomatitis	0 (0%)	2 (20%)
Breast mass	0 (0%)	1 (10%)
Contusion	4 (25%)	1 (10%)
Diarrhoea	0 (0%)	1 (10%)
Dysmenorrhoea	2 (13%)	0 (0%)
Excoriation	1 (6%)	1 (10%)
Haematoma	0 (0%)	1 (10%)
Headache	2 (13%)	3 (30%)
Lymphadenopathy	3 (19%)	2 (20%)
Myalgia	1 (6%)	1 (10%)
Nasal congestion	1 (6%)	1 (10%)
Pain in extremity	2 (13%)	1 (10%)
Palpitations	0 (0%)	1 (10%)
Pulmonary granuloma	0 (0%)	1 (10%)
Rhabdomyolysis	2 (13%)	2 (20%)
Skin laceration	3 (19%)	1 (10%)
Somnolence	2 (13%)	2 (20%)

Adverse Event Preferred Term*	H-BAT 1 Dose	Placebo Control
	N = 16 n (%) [a]	N = 10 n (%)
Tonsillar hypertrophy	4 (25%)	1 (10%)
Upper respiratory tract infection	1 (6%)	2 (20%)
Viral upper respiratory tract infection	0 (0%)	2 (20%)

* Adverse events are classified according to Medical Dictionary for Regulatory Activities (MedDRA) version 11.0.

[a] The common AEs reported by ≥ 10% of H-BAT-treated subjects.

These results, from blinded observations, indicated that H-BAT is relatively well tolerated compared to saline administration to normal healthy subjects.

The most common AEs reported in more than 5% of all subjects in BT-001 and treatment groups from BT-002 Stage B, were headache, somnolence, contusion, tonsillar hypertrophy, nausea, pharyngolaryngeal pain, pruritus, skin laceration and urticaria. The most common related AEs were headache (9%), nausea (5%) and urticaria (5%).

8.3.1 Adverse Events Leading to Premature Discontinuation in Healthy Subjects Trials

One subject from each clinical trial had study drug discontinued prematurely because of AEs.

The infusion of one subject in study BT-001 was stopped 52 minutes after the start of the administration of H-BAT due to events of skin disorder (soft, subcutaneous bumps, occipital scalp) and urticaria, which the investigator judged to be probably treatment-related. There was no other skin rash present and the subject's lungs were clear. Of note, this subject did not react to either a horse dander IgE test or skin sensitivity test during screening. Human anti-H-BAT antibody testing before and 28 days after dosing was also negative.

One subject in study BT-002 Stage B developed urticaria within five minutes following the start of H-BAT administration. The subject also experienced chest discomfort, elevated body temperature and elevated fibrinogen levels. Study drug administration was immediately terminated. Although this subject appeared to have a moderate allergic reaction to the study drug infusion, they did not react to the skin sensitivity test during screening. Anti-H-BAT antibody testing before and 28 days after dosing was also negative.

8.3.2 Serious Adverse Events and Deaths in Healthy Subjects Trials

No SAE or deaths were reported during clinical trials BT-001 and BT-002.

8.3.3 Immunogenicity

All exogenous proteins, including H-BAT, have the potential to cause antibody formation. The development of anti-therapeutic antibodies (ATA) has the potential to impact the clinical

safety (hypersensitivity reactions) and efficacy of protein therapeutics; therefore, the immunogenicity of H-BAT was examined during clinical trials BT-001 and BT-002 Stage B (Section 13.3.1).

Of the 56 healthy subjects receiving H-BAT during these clinical trials, 17 (30%) subjects had anti-H-BAT antibodies at baseline before receiving the study drug. The existence of anti-H-BAT antibodies in these subjects was likely due to previous environmental exposure to equine antigens. At the completion of the clinical trials following exposure to H-BAT, 28 (50%) subjects had anti-H-BAT antibodies. Therefore, 11 subjects (20%) developed anti-H-BAT antibodies (IgG, IgA, IgM) during the course of these clinical trials.

The immunogenicity of a previously marketed Aventis Pasteur (Connaught) BAT AB product was examined during clinical trial BT-002 Stage A. In clinical trial BT-002 Stage A, three subjects receiving a single IV infusion of BAT AB were found to be positive for anti-BAT AB antibodies post-dosing (of 5, 60%). One subject in the control group was originally found reactive at baseline (of 5, 20%). Of the four subjects with anti-BAT AB antibodies, all had IgG antibodies. None of the subjects had antibodies for IgA, IgE or IgM.

The number of healthy subjects developing anti-equine antibodies after receiving either H-BAT or previously marketed Aventis Pasteur (Connaught) BAT AB product appeared to be similar for both products (Table 17).

Table 17: Summary of Immunogenicity Testing Results

Study Identifier	Treatment	Time Point	Screening Assay	
			Reactive	Difference
Overall	H-BAT	Baseline	30%	20%
		Day 28	50%	
BT-001	H-BAT	Baseline	35%	18%
		Day 28	53%	
BT-002 Stage B	H-BAT	Baseline	19%	25%
		Day 28	44%	
BT-002 Stage A	Previously marketed Aventis Pasteur (Connaught) BAT AB	Baseline	0%	60%
		Day 28	60%	

One subject each from clinical trials BT-001 and BT-002 Stage B experienced a moderate allergic reaction during the administration of H-BAT. Both subjects were negative for anti-H-BAT antibodies at baseline and at the end of their respective studies (28 days). Therefore, the allergic reactions experienced by these subjects did not correspond to the presence of anti-H-BAT antibodies at baseline before H-BAT administration and they did not appear to develop anti-H-BAT antibodies in response to H-BAT administration.

The immunogenicity results (Table 17) suggest that pre-existing antibodies to H-BAT do not alter the tolerability or pharmacokinetic profile. While there is some degree of antibody development following H-BAT administration, this did not correlate with adverse event risks

in these individuals. This immunogenic response may increase the risk of anaphylactic/anaphylactoid reactions or serum sickness with repeat doses of H-BAT, similar to other equine derived products.

8.4 Safety in CDC Expanded Access Program Patients

H-BAT is currently available through a CDC expanded access program (EAP), which is intended to enable the investigational use of H-BAT for treatment of individuals with botulism as a result of naturally-occurring outbreaks or in cases of isolated, unintentional incidents. In this program, a CDC botulism duty officer or his/her designee makes the decision to release H-BAT based upon receipt of a product request and clinical determination in consultation with the treating physician through CDC's botulism treatment program. Once the product is released for clinical use, the treating physician, acting as site investigator for this protocol, is responsible for informing the patient/guardian of the risks and benefits of investigational H-BAT and obtaining informed consent for the administration of H-BAT. The final decision of whether or not to administer H-BAT is based on the treating physician's judgment.

The treating physician is also responsible for monitoring AEs and responsiveness to therapy and providing data on the clinical course and outcome of patients to CDC *via* the case report forms, documenting signs, symptoms, physical exam findings, and reactions to H-BAT. Patient information obtained from case report forms, medical records, or other pertinent sources has been de-identified and reported to FDA, Cangene, and the US agency responsible for advanced development of H-BAT, the Department of Health and Human Services/Biomedical Advanced Research & Development Authority (BARDA).

As the CDC EAP is designed to provide access to a drug not otherwise available commercially, the focus of the data collection was on investigational product safety and botulism surveillance; however, because of the nature of the data collected, it was also used to assess the effectiveness of H-BAT in the target botulism population. The CDC provided Cangene with datasets developed from the case reports of patients treated with H-BAT up to December 31, 2011.

A total of 148 patients were treated with H-BAT between January 15, 2008 and December 31, 2011. The patients ranged in age from 10 days to 88 years (median age 47 years). Most of the patients, 105 (70.9%), were male and 43 (29.1%) were female. Of those with information on race (86 of 148), 71% were white. The most common symptoms and physical findings at presentation or hospital admission included: alert and orientated (92%), subjective weakness (85%), blurred vision (82%), dysphagia (81%), slurred speech (75%), ptosis (74%) diplopia (71%), fatigue (70%), change in sound of voice (65%), shortness of breath (61%), palatal weakness (57%), dizziness (54%), impaired gag reflex (54%), thick tongue (52%), extraocular palsy (51%) and dry mouth (50%).

The majority of patients (143/148; 96.6%) received one dose of H-BAT, including one patient who also received a dose of previously marketed Aventis Pasteur (Connaught) BAT AB product as well as their investigational monovalent BAT E prior to H-BAT administration. Five patients received two H-BAT doses. A 10 day-old male patient received

two infant doses of H-BAT eight hours apart (patient was treated with BabyBIG prior to receiving H-BAT) and a three year-old male received two pediatric doses seven hours apart. The treatment plan for these two young children included two doses, with an interval of 7-8 hours between doses; administration of the second dose for each was not triggered by worsening of signs or symptoms. A 46 year-old male was given two adult doses approximately one month apart. A 78 year-old female was given two adult doses eight days apart. During a food borne botulism outbreak in Mexico in 2010, a 29 year-old female patient was given two adult doses of H-BAT four days apart; however, this was a protocol deviation because the Mexican doctors did not seek CDC's approval prior to administering the second dose of H-BAT.

Most of the patients treated with H-BAT had a final diagnosis of botulism (97/148; 65.5%; Table 18). However, botulism was only laboratory confirmed for 52.6% (51/97) of those cases. Since early intervention has been shown to improve outcomes, there is the risk that H-BAT will be given empirically to patients with neurologic signs and symptoms similar to botulism but who are subsequently found to have other illnesses. This was reported in 39/148 patients in the CDC EAP, and there was no apparent difference in the tolerability profile in these patients compared to patients with confirmed or suspected botulism.

Table 18: Clinical Discharge Diagnosis of CDC Patients Receiving H-BAT

Clinical Discharge Diagnosis	**N = 148** **n (%)**
Botulism	97 (65.5%)
Guillain-Barre Syndrome	14 (9.5%)
Myasthenia Gravis	5 (3.4%)
Tick Paralysis	1 (0.7%)
Other	21 (14.2%)
Missing/Unknown	10 (6.8%)

Includes patients treated with H-BAT between January 15, 2008 and December 31, 2011. Information based on case report datasets.

Wound botulism was the most common route of exposure for these patients, followed by foodborne botulism (Table 19).

Table 19: Botulism Route of Exposure of CDC Patients Receiving H-BAT

Route of Exposure	All Suspected Cases at Clinical Presentation N = 148 n (%)	Confirmed or Suspected Cases at Discharge N = 109 n (%)
Wound	70 (47.3%)	58 (53.2%)
Foodborne	40 (27.0%)	33 (30.2%)
Iatrogenic	1 (0.7%)	1 (0.9%)
Infant	1 (0.7%)	1 (0.9%)
Other	7 (4.7%)	4 (3.7%)
Undetermined	29 (19.6%)	12 (11.0%)

Includes patients treated with H-BAT between January 15, 2008 and December 31, 2011. Information based on case report datasets.

Serotype A was the most commonly identified BoNT serotype isolated from patients with confirmed botulism (Table 20). Patients with serotype F, B and E botulism were also reported.

Table 20: Botulinum Neurotoxin Serotype of Confirmed CDC Botulism Patients Receiving H-BAT

Serotype	N = 51 n (%)
A	33 (64.7%)
B	3 (5.9%)
C	0 (0.0%)
D	0 (0.0%)
E	3 (5.9%)
F	4 (7.8%)
G	0 (0.0%)
Indeterminate	8 (15.7%)

Includes patients treated with H-BAT between January 15, 2008 and December 31, 2011. Safety Analysis Population – CDC Confirmed Cases (n = 51).

Adverse reaction (AEs related to H-BAT) information from patients treated with H-BAT under the CDC's EAP (BB-IND 6750) was collected on 146 of 148 patients being treated for suspected of confirmed botulism.

As of December 31, 2011, 18 patients (12.3%) reported 31 adverse reactions following administration of H-BAT. The most common adverse reactions (>1%) included: pyrexia (5.5%), chills (2.1%), rash (2.1%), edema (1.4%), and nausea (1.4%). Other adverse reactions (< 1%) included: tachycardia, vomiting, chest discomfort, feeling jittery, agitation, anxiety, urinary retention, bronchospasm, erythema, hyperhidrosis, hemodynamic instability,

and hypotension. No patients experienced anaphylaxis however, one patient experienced serum sickness.

A 64 year-old male, experienced mild serum sickness (with myalgia, arthralgia and hematuria) 12 days after H-BAT administration. The patient did not experience rash and the investigator considered the serum sickness to be mild.

A 72 year-old female, experienced rebound toxicity after receiving H-BAT. Six days after H-BAT treatment, the patient had substantial recovery of upper and lower extremity muscle strength and was weaned from the ventilator. Afterwards, she experienced recurrence of botulism symptoms (e.g., proximal and distal upper and lower extremity weakness, dyspnea, partial ptosis, and bilateral facial paralysis) from intestinal colonization with Clostridia and ongoing toxin production, and required re-intubation and ventilator support. She improved after treatment with metronidazole and vancomycin for two weeks and was described as "back to normal" at last report.

Two CDC botulism patients experienced SAEs, one during and one after H-BAT administration. One SAE occurred during H-BAT treatment. A 10 year-old Hispanic male patient experienced hemodynamic instability characterized by tachycardia, bradycardia and asystole during infusion of H-BAT. The events were rechallenge positive, with symptom improvement after initial interruptions of H-BAT infusion, and reappearance of symptoms after the infusion restarted. H-BAT infusion was stopped permanently at 70% of the planned dose. The patient recovered from both hemodynamic instability and botulism. The clinical team treating the patient assessed the hemodynamic instability as serious and unexpected and possibly related to H-BAT administration.

The second SAE patient was a 27 year-old male with a history of Fanconi's anemia and myelodysplastic syndrome who underwent an allogeneic stem cell transplant and was rehospitalized for cytomegalovirus (CMV) viremia. The patient was diagnosed with botulism and treated with H-BAT. Approximately 28 hours after completion of the H-BAT infusion, during a tracheostomy change, the patient experienced respiratory and subsequently cardiac arrest, resulting in a pulse-less status for approximately 25 minutes. The patient had completed the infusion without any interruption and no evidence of an allergic reaction to H-BAT was observed during or after completion of the infusion. At the request of the family, ventilator support was removed 17 days after the cardiac arrest, and the patient subsequently died. The medical team assessed the cardiac arrest as being due to severe hypoxia from a mucus plug during a tracheostomy change and unrelated to H-BAT administration.

The CDC reported six deaths, for patients treated with H-BAT under the EAP. Four patients were diagnosed with botulism, of which two patients died due to progression of botulism toxicity and two patients of unknown causes. The other two patients were diagnosed with other diseases (Miller-Fisher variant of the Guillain-Barre Syndrome and "not botulism") and died of comorbidity complications. The investigators considered all deaths unrelated to H-BAT. Brief summaries of the six deaths are provided below:

- A 64 year-old male with metastatic prostate cancer, ongoing mucosal bleeding for which he required transfusions and pneumonia received H-BAT for botulism. The

patient died 45 days after H-BAT administration due to respiratory failure and metastatic cancer.

- A 64 year-old male, with unremarkable history, received H-BAT for the treatment of botulism. He experienced mild diaphoresis during H-BAT infusion that resolved within a few hours and later experienced mild serum sickness (described above). The patient died of unknown causes 52 days after H-BAT administration. The investigators considered the death unrelated to H-BAT and not directly related to botulism.
- A 77 year-old male with a history of morbid obesity and sleep apnea received H-BAT for treatment of botulism. The patient died of unknown causes 94 days after H-BAT administration.
- An 82 year-old female with dementia received H-BAT for a disorder other than botulism (no definitive diagnosis). The patient died of respiratory failure and pneumonia three days after H-BAT administration, which she tolerated well, but had no improvement of encephalopathy. At her family's request, life support was terminated and the patient subsequently died.
- An 88 year-old female with stable myelodysplastic syndrome received H-BAT for a Miller-Fisher variant of the Guillain-Barre Syndrome, the cause of her death seven days after H-BAT administration.
- A 27 year-old male with a history of Fanconi's anemia and myelodysplastic syndrome died 18 days after H-BAT administration. See above for more information (one of the SAE patients).

Overall H-BAT was safe and well-tolerated in healthy subjects as well as in patients with suspected or confirmed botulism, with hypersensitivity reactions similar to the previously available BATs.

8.5 Clinical Efficacy

8.5.1 Pharmacodynamics in Healthy Subjects

Two human PD studies were completed during the Clinical Development Program. Clinical trial BT-002 Stage A was designed as a proof of concept study using the previously marketed Aventis Pasteur (Connaught) AB product [BAT AB Prescribing Information] to evaluate the EDB muscle model. Clinical trial BT-002 Stage B was designed to evaluate H-BAT's ability to neutralize BoNT serotypes A [Botox® Prescribing Information, 2004] and B [Myobloc® Prescribing Information, 2004] in the human body prior to uptake by the cholinergic nerve endings. Since the principle effect of exposure to botulism is muscle paralysis, inhibition of muscle paralysis induced by BoNT serotypes A or B, was used as a surrogate endpoint to demonstrate the effectiveness of H-BAT in humans.

In clinical trial BT-002 Stage A, previously marketed Aventis Pasteur (Connaught) BAT AB product [BAT AB Prescribing Information] was administered 24 hours prior to BoNT exposure and prevented subjects from experiencing a decrease in muscle function following

injection of BoNT serotypes A and B. Subjects receiving the placebo control (0.9% saline) demonstrated a loss of muscle function within three days of exposure to both BoNT serotypes A and B. In the group treated with previously marketed Aventis Pasteur (Connaught) BAT AB product, muscle function was stable over time, indicating that the antitoxin was effective at preserving muscle function for up to 28 days following BoNT exposure. The results of this clinical trial indicated that the EDB muscle model could be used to examine the effects of botulism antitoxins in humans.

In clinical trial BT-002 Stage B, H-BAT was administered 24 hours prior to BoNT exposure and prevented subjects from experiencing a decrease in muscle function following injection of BoNT serotypes A and B. Subjects receiving the placebo control (0.9% saline) demonstrated a loss of greater than 50% muscle function within three days of exposure to both BoNT serotypes A (Figure 11) and B (Figure 12). In the treatment arm, muscle function was stable over time, indicating that H-BAT was effective at preserving muscle function for up to 28 days following exposure to both BoNTs. A longitudinal analysis of variance model was fitted to the data and demonstrated a significant decrease in the percent muscle function of EDB muscle for the placebo arm as compared to the treatment arm over time ($p < 0.05$). Essentially, BoNT serotypes A and B inhibited action potential development and muscle contraction in the presence of placebo treatment but no loss of action potential or muscle function was seen when H-BAT was administered.

Figure 11: Percent Muscle Function Over Time (CMAP M Wave Amplitudes) of the EDB Muscle Following Exposure to Botox® (BoNT Serotype A) and H-BAT

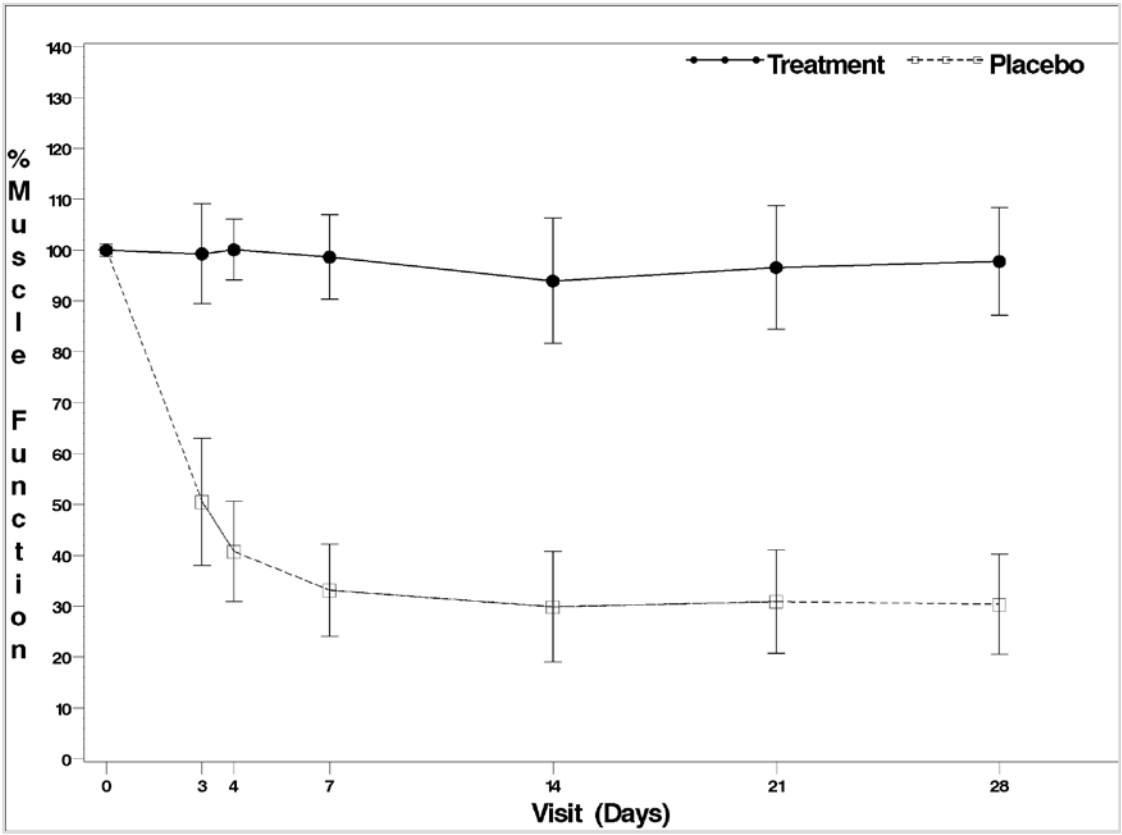
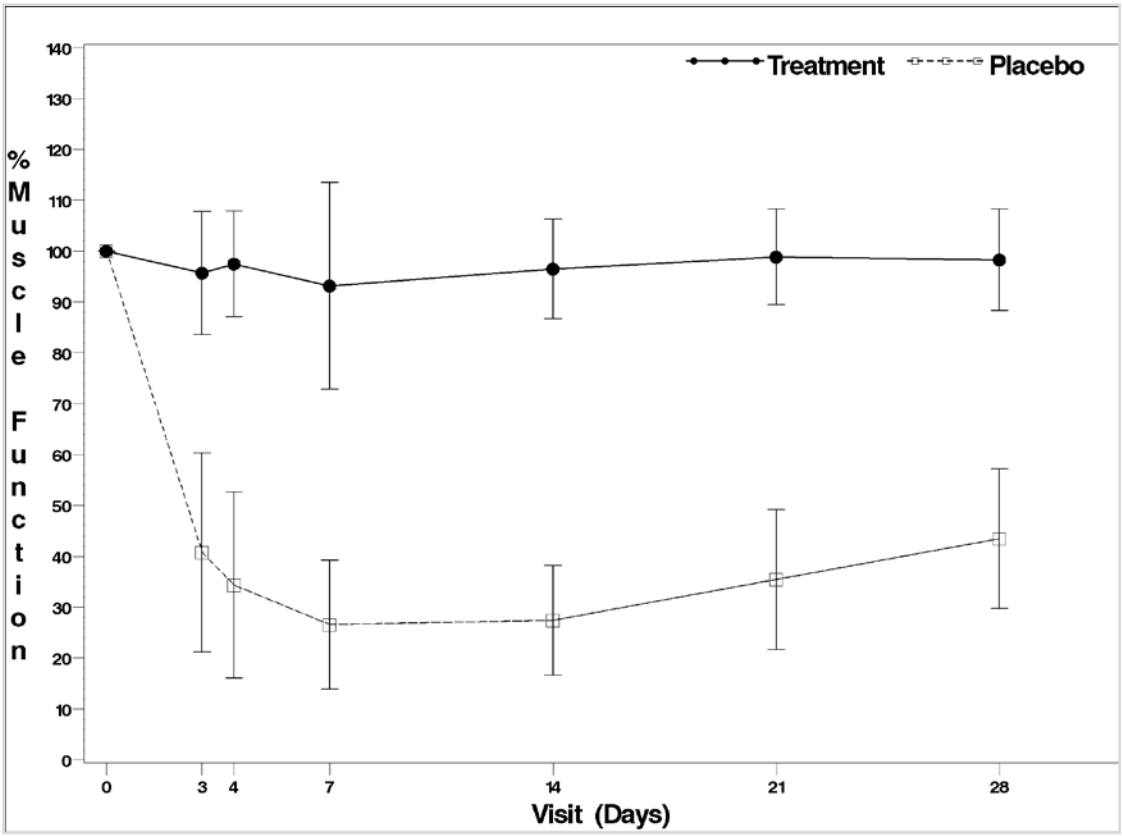


Figure 12: Percent Muscle Function Over Time (CMAP M Wave Amplitudes) of the EDB Muscle Following Exposure to Myobloc® (BoNT Serotype B) and H-BAT



This study demonstrated that H-BAT is capable of blocking toxin from internalizing into nerve cells, and therefore preventing the further progression of disease.

8.5.2 Efficacy of H-BAT in CDC Expanded Access Program

To evaluate the efficacy of H-BAT in patients with botulism, Cangene performed a *post-hoc* statistical analysis of the CDC patient data. Patients treated early after symptom onset (≤ 2 days) versus later (> 2 days) were compared to determine the effect of H-BAT on the duration of hospitalization. The rationale behind this analysis was that early treatment with H-BAT would decrease or prevent symptom progression and reduce disease duration; however, later treatment would be less effective since H-BAT does not have an effect on BoNT once it has acted upon the cholinergic nerve endings. Previous studies have shown that early administration of the antitoxin is critical for a positive patient outcome [Tacket et al., 1984; Kongsaengdao et al., 2006; Chang et al 2003; Offerman et al., 2009; Sandrock et al., 2001].

Survival was not a suitable endpoint for this study due to the limited patient sample size and the lack of information on an appropriate comparator population or historical reference rate in patients receiving modern supportive care.

Duration of ICU stay and duration of mechanical ventilation were also considered as primary endpoints; however, not all patients required care in the ICU or mechanical ventilation.

Duration of hospitalization was selected in order to maximize the available sample size since all patients included in the CDC program required hospitalization.

The primary efficacy analysis population (109 patients, 73.6%) included patients with a final diagnosis of suspected or confirmed botulism, or where the final diagnosis was unknown. Two patients with a clinical discharge diagnosis of "other" were also included in the primary efficacy analysis since additional information confirmed that botulism was suspected. A total of 39 patients (26.4%) were diagnosed with a condition other than botulism after treatment with H-BAT.

Across the 148 patients treated with H-BAT, duration of hospital admission ranged from 1 to 104 days, with a median value of 15 days. Duration of ICU stay ranged from 1 to 95 days, with a median value of 11.9 days. Over two-thirds of the patients received mechanical ventilation (92/148; 62.2%), ranging from 1 to 83 days, with a median value of 16 days. Of the patients who received mechanical ventilation, 56 (60.9%) received tracheostomy.

The mean ± SD duration of hospitalization for patients with botulism was 12.4 ± 9.3 days for the patients who were treated with H-BAT within two days of symptom onset, as compared to 26.1 ± 26.4 days for the patients who were treated more than two days after symptom onset (Table 21).

Table 21: Summary of Incidence and Duration of Hospitalization for CDC Patients Treated with H-BAT

		Incidence		Duration (days)	
		N	**Count (%)**	**N**	**Mean (SD)**
Overall		107 [a]	107 (100%)	95	23.2 (24.9)
Route of exposure	Foodborne	33	33 (100%)	31	21.7 (27.2)
	Iatrogenic	1	1 (100%)	1	57 (-)
	Infant	1	1 (100%)	0	- (-)
	Wound	56	56 (100%)	51	20.5 (19.8)
	Other	4	4 (100%)	3	56.7 (40.5)
Age	< 60 years	86	86 (100%)	79	24.2 (26.7)
	≥ 60 years	21	21 (100%)	16	18.3 (12.5)
Time from symptoms to H-BAT treatment	≤ 2.0 days	15	15 (100%)	14	12.4 (9.3)
	> 2.0 days	81	81 (100%)	72	26.1 (26.4)

Includes patients treated with H-BAT between January 15, 2008 and December 31, 2011. Information based on case report datasets.

[a] Hospitalization information is missing for two patients.

H-BAT treatment relative to symptom onset had a similar impact on duration of ICU stay. Early administration of H-BAT reduced the mean ± SD duration of ICU stay from 15.8 ±

18.8 days for the late H-BAT administration patients to 9.2 ± 7.4 days for early H-BAT administration patients (Table 22).

Table 22: Summary of Incidence and Duration of ICU Stay for CDC Patients Treated with H-BAT

		Incidence		Duration (days)	
		N	**Count (%)**	**N**	**Mean (SD)**
Overall		105	93 (88.6%)	90	14.1 (17.2)
Route of exposure	Foodborne	33	30 (90.9%)	29	19.2 (24.5)
	Iatrogenic	1	1 (100%)	1	56 (-)
	Infant	1	1 (100%)	0	- (-)
	Wound	54	46 (85.2%)	48	8.7 (8.4)
	Other	4	4 (100%)	3	26.7 (11.6)
Age	< 60 years	84	74 (88.1%)	74	13.6 (18.1)
	≥ 60 years	21	19 (90.5%)	16	16.1 (12.6)
Time from symptoms to H-BAT treatment	≤ 2.0 days	15	14 (93.3%)	13	9.2 (7.4)
	> 2.0 days	80	73 (91.3%)	70	15.8 (18.8)

Includes patients treated with H-BAT between January 15, 2008 and December 31, 2011. Information based on case report datasets.

Similar findings were observed with early treatment of patients with H-BAT and duration of mechanical ventilation: The mean ± SD duration of mechanical ventilation was 11.6 ± 7.8 days for early and 23.4 ± 21.1 days for the late H-BAT administration patients (Table 23).

Table 23: Summary of Incidence and Duration of Mechanical Ventilation for CDC Patients Treated with H-BAT

		Incidence		Duration (days)	
		N	**Count (%)**	**N**	**Mean (SD)**
Overall		106	72 (67.9%)	54	21.0 (19.8)
Route of exposure	Foodborne	33	24 (72.7%)	18	20.7 (19.8)
	Iatrogenic	1	1 (100%)	1	56 (-)
	Infant	1	1 (100%)	0	- (-)
	Wound	55	32 (58.2%)	26	18.5 (20.2)
	Other	4	4 (100%)	2	27.5 (16.3)
Age	< 60 years	85	57 (67.1%)	44	20.9 (21.5)
	≥ 60 years	21	15 (71.4%)	10	21.8 (10.6)
Time from symptoms to H-BAT treatment	≤ 2.0 days	15	10 (66.7%)	9	11.6 (7.8)
	> 2.0 days	81	56 (69.1%)	41	23.4 (21.1)

Includes patients treated with H-BAT between January 15, 2008 and December 31, 2011. Information based on case report datasets

The CDC EAP data suggest that H-BAT is effective in reducing duration of hospitalization and ICU stay as well as need for mechanical ventilation, especially when given early after symptom onset.

8.6 Other Experience

H-BAT has not been licensed in any country. A limited amount of H-BAT has been distributed for emergency use in other countries; however, to date, Cangene has received no reports about its use in patients outside of the US.

9 JUSTIFICATION FOR PROPOSED HUMAN DOSE

9.1 Summary

A single vial of H-BAT of H-BAT is the proposed adult human dose. The specifications for this single vial dose were predefined in the program based on historical information. The effectiveness of the proposed dosing dose was confirmed through multiple animal efficacy trials, clinical experience and PK/PD modeling. The proposed dose is justified based upon the following lines of reasoning.

- Clinical data support the effectiveness of previously marketed and unlicensed equine-derived botulinum antitoxin products, which had comparable levels of antitoxin [Arnon et al., 2006; Chang & Ganguly 2003; Dolman & Iida 1963; Kongsaengdao et al., 2006; Offerman et al., 2009; Tacket et al., 1984; Vanella de Cuetos 2011].
- The theoretical neutralizing capacity of H-BAT is 2-19 times in excess of the estimated maximum historical exposure level in humans.
- Pharmacokinetic data for H-BAT from studies in guinea pig, Rhesus macaque and human demonstrates that the maximum serum concentration (C_{max}) is similar between species and that the overall exposure (i.e., area under the curve (AUC) is the highest in humans.
- Efficacy data from dose-ranging efficacy studies and therapeutic efficacy studies demonstrated that the scaled human dose was effective in significantly enhancing survival in both animal models.
- *Post-hoc* analysis of information collected from botulism patients treated with H-BAT under the CDCs EAP supports that early treatment with H-BAT (within two days of symptoms onset) shortens the duration of hospitalization compared to treatment more than two days after symptom onset.
- Population PK/PD modeling and simulation was completed to integrate data from guinea pig, Rhesus macaque and human data (theoretical neutralizing capacity, PK, and dose-ranging efficacy in animals and PD and EAP data in humans). The translational modeling established that the human adult dose of one vial is the appropriate dose.
- Sensitivity analysis based on the label claim (i.e., lowest potency allowable over the shelf-life) have shown that even at the minimum allowable potency sufficient neutralizing capacity is available to protect against botulism toxin serotype A-G.

9.2 Dosing

A single vial of H-BAT is the proposed adult human dose and each vial is filled based on potency and contains sufficient potency to neutralize each of the seven BoNT serotypes.

The infant dose (less than one year) for H-BAT is 10% of the adult dose (based on volume in vial) regardless of body weight. The pediatric dose (1-16 years) is adjusted based on body weight according to the "Salisbury Rule" [Lack & Stuart-Taylor 1997], which states:

- For pediatric patients < 30 kg, double their body weight in kilograms to determine the percentage of the adult dose to administer. For example, for a 14 kg child, the pediatric dose would be 28% of the adult dose (14 x 2 = 28%, based on volume of vial).
- For pediatric patients ≥ 30 kg but less than 70 kg, add 30 to their body weight in kilograms to determine the percentage of the adult dose to administer. For example, for a 60 kg child the pediatric dose would be 90% of the adult dose (60 + 30 = 90%, based on volume of vial).
- The dose should not exceed one vial, regardless of body weight.

Development of the H-BAT product was built on the assumption that this dose should be targeted based on the neutralization potency of previously available botulinum antitoxin products.

9.3 Potency of H-BAT

The potency of H-BAT is determined based on its BoNT neutralizing capacity using a validated mouse neutralization assay (MNA). The MNA is a well-established *in vivo* potency assay for quantification of neutralizing antitoxins against BoNT serotypes A, B, C, D, E, F, and G [Cardella 1964]. To determine the neutralizing antibody concentration, a series of antitoxin test article dilutions are mixed with a fixed quantity of BoNT, incubated and injected intraperitoneal into mice. Lethality is determined over a 96 ± 2 hour time period. The antibody concentration in the sample is determined relative to a standard curve constructed from antitoxin reference standards. The median effective dose (ED_{50}) is calculated for the reference standard and the sample curves by separate probit analysis. The neutralizing antibody concentration for a sample (U/mL) is calculated as the ED_{50} for the reference standard curve divided by the ED_{50} for the sample curve, multiplied by the known neutralizing potency concentration of the reference standard. The end measure of the MNA is a mouse intraperitoneal lethal dose 50% ($MIPLD_{50}$) of toxin, which is then used to determine antitoxin neutralization capacity.

Four sources of botulism antitoxin reference standards have been used to evaluate the potency of botulism antitoxins over the years and include World Health Organization (WHO), US, PerImmune, and Cangene reference standards. The WHO reference standards against serotypes A, B, C, D and E were originally produced in horses at the Microbiological Research Establishment in the United Kingdom (UK) and were later considered suitable as WHO International Standards in the 1960s [Bowmer 1963; Jones et al., 2006]. In 1964, an antitoxin against serotype F raised in rabbits was added as an international standard [Jones et al., 2006; WHO 1964].

The PerImmune reference standards (A, B, C, D, E, F and G) were prepared by PerImmune Inc. in the 1990's under contract with the US Department of Defense (DoD) and were

supplied to Cangene by the CDC. The potency of the PerImmune reference standards was based on the definition of one unit (U) of antitoxin in which each unit of antitoxin against serotype A, B, C, D, F, and G neutralizes 10,000 $MIPLD_{50}$ of toxin, while each unit of antitoxin to serotype E neutralizes 1,000 $MIPLD_{50}$.

Cangene's manufacturing process is based on achieving target potency as outlined in Table 24. The labeling claim for H-BAT is based on maintaining a minimum potency per vial over the shelf-life of the product.

Table 24: Potency of One Vial of Cangene's H-BAT Product

Serotype	Target Potency U/vial	Labeling Claim U/vial
A	9,375	> 4,500
B	6,875	> 3,300
C	6,250	> 3,000
D	1,250	> 600
E	10,625	> 5,100
F	6,250	> 3,000
G	1,250	> 600

Since the MNA used to determine potency is an *in vivo* bioassay with intrinsically high variability, Cangene's targeted potency was established at a higher potency than the labeling claim. The overage accounts for variability in the MNA and the manufacturing process, which involves combining the individually bulk antitoxin serotypes into the finished heptavalent product. The labeling claim was set in order to account for both MNA variability, as well as the need to maintain potency over the product shelf life for all serotypes, in all product lots (i.e., seven different potency measurements in a single dose vial).

The potency of H-BAT was initially established based on antitoxin levels in other licensed and investigational botulism antitoxin products. The target potency is set at the higher levels of the bivalent and trivalent products and the labeling claim potency is similar to those of the DoD Heptavalent product. A summary of these BAT products and their potencies can be found in Table 25.

Table 25: Antitoxin Potency Levels in Various Botulism Antitoxin Products

Serotype	Equine Based Products					Human Based Products	
	Aventis Pasteur (Connaught) Trivalent Product (IU/vial) Licensed	Aventis Pasteur (Connaught) Bivalent Product (IU/vial) Licensed	Unlicensed Aventis Pasteur (Connaught) Monovalent Product (IU/vial)	Unlicensed DoD Heptavalent Products (IU/vial)	Lederle Bivalent Product (US U/vial) Licensed	Unlicensed Pentavalent Product (IU/vial)	BabyBIG Bivalent Product (mg/kg) Licensed
A	7,500	7,500	-	> 4,000	10,000	> 900	75
B	5,500	5,500	-	> 4,000	10,000	> 90	75
C	-	-	-	> 4,000	-	> 1,000	-
D	-	-	-	> 500	-	> 1,600	-
E	8,500	-	8,500	> 4,000	-	> 600	-
F	-	-		> 4,000	-	-	-
G	-	-	-	> 500	-	-	-

The potency of H-BAT was also set to optimize the limited amount of heptavalent botulism antitoxin product available for shipment to the Strategic National Stockpile (SNS). Following the terrorist attacks of September 11, 2001 and the release of anthrax spores through the US postal service in October 2001, there was a need to increase the available stockpile of BAT in response to heightened national and international concern about the potential use of biological warfare agents, including BoNTs. As there is significant uncertainty surrounding the levels of BoNT that clinical cases of botulism involve and the timeframe from exposure to antitoxin treatment, there is a balance between a human dose containing too much and too little antitoxin for each clinical situation. Using the antitoxin levels in the historical equine-derived antitoxin products, Cangene developed a product for the SNS that is expected to provide sufficient neutralization capacity to treat the majority of anticipated clinical circumstances.

9.4 Theoretical Neutralizing Capacity of H-BAT

Each vial of H-BAT contains the neutralization capacity outlined in Table 26 based on the reference standard definition for antitoxin unit neutralization mentioned on the previous page.

Table 26: Potency and Neutralizing Capacity of One Vial of H-BAT

Serotype	Target Potency		Proposed Label	
	Potency U/Vial	Neutralizing Capacity ($MIPLD_{50}$)	Potency U/Vial	Neutralizing Capacity ($MIPLD_{50}$)
A	9,375	93,750,000	> 4,500	45,000,000
B	6,875	68,750,000	> 3,300	33,000,000
C	6,250	62,500,000	> 3,000	30,000,000
D	1,250	12,500,000	> 600	6,000,000
E	10,625	10,625,000	> 5,100	5,100,000
F	6,250	62,500,000	> 3,000	30,000,000
G	1,250	12,500,000	> 600	6,000,000

The potency and neutralizing capacity of H-BAT is well above the amount required to neutralize the highest documented BoNT exposure levels ever reported in the literature. The highest serum level of BoNT reported in the US following foodborne intoxication was 32 $MIPLD_{50}$/mL. Assuming that BoNT is equally distributed throughout the extracellular fluid (approximately 15 liters in the average adult), this serum level would correspond to a total body BoNT load of approximately 480,000 $MIPLD_{50}$. The highest serum level of BoNT ever reported in the world was 160 $MIPLD_{50}$/mL of serotype E BoNT; this corresponds to a total body BoNT load of approximately 2,400,000 $MIPLD_{50}$. These serum concentrations [Hatheway et al., 1984; Ball et al., 1979] are an estimate of the maximum amount of BoNT a patient is likely to be exposed to following foodborne intoxication. Currently there are no

estimates available on the expected potential exposure levels from wound, inhalational or intestinal colonization botulism patients.

Based on the H-BAT label claim, Table 27 shows the theoretical neutralizing capacity of H-BAT against the highest known serum concentrations of BoNT reported in the US and rest of world. According to these numbers, the adult dose of H-BAT (1 vial) should provide sufficient antitoxin to neutralize the highest serum levels of BoNT.

Table 27: Capacity of H-BAT to Neutralize Maximum Known Serum Concentrations of BoNT Reported in the Literature

Serotype	Label Claim (U/vial)	Toxin Neutralization Capacity ($MIPLD_{50}$)	Theoretical Number of Times Antitoxin Could Neutralize BoNT Levels	
			480,000 $MIPLD_{50}$ (US [a])	2,400,000 $MIPLD_{50}$ (World [b])
A	> 4,500	45,000,000	93.8	18.8
B	> 3,300	33,000,000	68.8	13.8
C	> 3,000	30,000,000	62.5	12.5
D	> 600	6,000,000	12.5	2.5
E	> 5,100	5,100,000	10.6	2.1
F	> 3,000	30,000,000	62.5	12.5
G	> 600	6,000,000	12.5	2.5

[a] Hatheway et al., 1984; [b] Ball et al., 1979.

The human lethal dose for each BoNT serotype has not been determined. However, it has been estimated from studies carried out in monkeys (Rhesus macaques and squirrel) to be approximately 40 $MIPLD_{50}$/kg [Herrero et al., 1967; Scott and Suzuki et al., 1988] for BoNT serotype A.

Assuming that the average human weighs 70 to 100 kg, the lethal human dose for BoNT serotype A would be approximately 2800 to 4000 $MIPLD_{50}$. Based on these estimates, the adult dose of H-BAT (1 vial) contains enough antitoxin to neutralize the estimated human lethal BoNT level thousands of times over based on the products label claim (Table 28).

Table 28: Capacity of H-BAT to Neutralize the Estimated Human Lethal Dose of BoNT Serotype A

Serotype	Label Claim (U/vial)	Toxin Neutralization Capacity ($MIPLD_{50}$)	Theoretical Number of Times Antitoxin Could Neutralize BoNT Levels	
			2,800 $MIPLD_{50}$ (Estimated Lethal Dose [a, b])	4,000 $MIPLD_{50}$ (Estimated Lethal Dose [a, b])
A	> 4,500	45,000,000	16,071	11,250
B	> 3,300	33,000,000	11,786	8,250
C	> 3,000	30,000,000	10,714	7,500
D	> 600	6,000,000	2,143	1,500
E	> 5,100	5,100,000	1,821	1,275
F	> 3,000	30,000,000	10,714	7,500
G	> 600	6,000,000	2,143	1,500

[a] Herrero et al., 1967; [b] Scott and Suzuki, 1988.

Therefore, the antitoxin quantities contained within Cangene's H-BAT are expected to be sufficient meet the intoxication levels seen in the majority of sporadic or intentional clinical botulism situations. It is important to have a sufficient level of antitoxin for most clinical situations as there are no available diagnostic tools to help aid diagnosis or determination of botulism exposure levels. Thus, the antitoxin levels in H-BAT provide confidence that a single dose is sufficient in most situations.

9.5 Pharmacokinetic Data from Guinea Pig, Rhesus Macaque and Humans

Pharmacokinetic studies were conducted in the guinea pig, Rhesus macaque and humans. PK parameters were estimated using standard non-compartmental modeling methods. Table 29 summarizes the findings for serotype A across the three species. The C_{max} was similar across species while the overall exposure as measured by AUC was highest in humans. The similarity of C_{max} in humans and the higher AUC support the generalizability of the guinea pig and Rhesus macaque efficacy data and exposure response findings to humans.

Table 29: Pharmacokinetic Parameters (Mean) for Antitoxin Serotype A in Male Guinea Pigs, Rhesus Macaque Monkeys and Humans Following Intravenous Administration of 1.0x the Scaled Human Dose of H-BAT

Species	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/kg)	Vd (mL/kg)
Guinea pig	10.00	10.30	2.92	0.17	6.24	14.47	130.20
Rhesus macaque	N/A	9.7	2.86	0.50	3.02	13.10	57.20
Human	21.40	26.00	2.69	0.70	8.64	3.99	49.48

Human data from BT-001; Guinea pig data from BBRC 648; Rhesus macaque data from LBERI FY07-056.

The comparable maximum concentration suggests that the peak and immediate toxin neutralization will be similar across species while the greater overall exposure in humans indicates that equal or greater toxin neutralization will occur compared to the animal models. Taken together, the human equivalent dose is expected to have similar if not greater efficacy than seen at a similar botulinum exposure as was demonstrated in animals.

9.6 Dose-Ranging Efficacy Data from Guinea Pig and Rhesus Macaque

Cangene sought to further confirm the proposed human dose by performing dose-ranging studies in guinea pigs and Rhesus macaques. Both models involved multiple doses of antitoxin administered at a set time point following a robust botulinum toxin challenge.

9.6.1 Guinea Pig Dose-Ranging Study

In dose-ranging efficacy study BBRC 731, guinea pigs (10 per sex) were administered H-BAT IV either 12 (serotypes A, B, C, D, F and G) or six (serotype E) hours after IM administration of a 4x $GPIMLD_{50}$ of BoNT. As the study involved H-BAT treatment at a set time point following BoNT exposure rather than on an individual animal trigger based treatment as per the pivotal efficacy studies, a more robust BoNT exposure level was used in the dose ranging study. H-BAT was administered at 1x, 0.2x, 0.04x, or 0.008x the scaled human dose. An additional group of guinea pigs received BoNT and were treated with the placebo consisting of normal equine immune globulins (dose levels matching the highest dose of H-BAT).

Regardless of the BoNT serotype administered, all of the animals in the placebo control groups died. Treatment with H-BAT improved the survival rate as compared to the placebo groups. Improvement in survival rates were observed at all dose levels for serotypes A, B, C, F and G, and at ≥ 0.2x for serotype D and ≥ 0.04x for serotype E (Table 30).

Table 30: Summary of Guinea Pig Survival Data from Dose Ranging Study BBRC 731

Serotype	Treatment Group	Survival Rate n/n (%)	Fisher's Exact Test p-value
A	1x H-BAT	20/20 (100)	< 0.0001
	0.2x H-BAT	19/20 (95)	< 0.0001
	0.04x H-BAT	20/20 (100)	< 0.0001
	0.008x H-BAT	11/20 (55)	0.0009
	Placebo control	0/20 (0)	N/A
B	1x H-BAT	19/20 (95)	< 0.0001
	0.2x H-BAT	20/20 (100)	< 0.0001
	0.04x H-BAT	17/20 (85)	< 0.0001
	0.008x H-BAT	20/20 (100)	< 0.0001
	Placebo control	0/20 (0)	N/A
C	1x H-BAT	20/20 (100)	< 0.0001
	0.2x H-BAT	18/20 (90)	< 0.0001
	0.04x H-BAT	18/20 (90)	< 0.0001
	0.008x H-BAT	14/20 (70)	< 0.0001
	Placebo control	0/20 (0)	N/A
D	1x H-BAT	20/20 (100)	< 0.0001
	0.2x H-BAT	20/20 (100)	< 0.0001
	0.04x H-BAT	2/20 (10)	0.9744
	0.008x H-BAT	0/20 (0)	1.0000
	Placebo control	0/20 (0)	N/A
E	1x H-BAT	19/19 (100)	< 0.0001
	0.2x H-BAT	19/20 (95)	< 0.0001
	0.04x H-BAT	18/20 (90)	< 0.0001
	0.008x H-BAT	0/19 (0)	1.0000
	Placebo control	0/20 (0)	N/A
F	1x H-BAT	20/20 (100)	< 0.0001
	0.2x H-BAT	20/20 (100)	< 0.0001
	0.04x H-BAT	18/20 (90)	< 0.0001
	0.008x H-BAT	17/20 (85)	< 0.0001
	Placebo control	0/20 (0)	N/A
G	1x H-BAT	19/20 (95)	< 0.0001
	0.2x H-BAT	20/20 (100)	< 0.0001
	0.04x H-BAT	20/20 (100)	< 0.0001
	0.008x H-BAT	17/20 (85)	< 0.0001
	Placebo control	0/20 (0)	N/A

These data demonstrated that IV treatment of guinea pigs with H-BAT was highly effective in preventing the lethal effects of BoNT intoxication, even when administered at a fraction of the scaled human dose.

9.6.2 Rhesus Macaque Dose-Ranging Study

In dose-ranging efficacy study LBERI 061, two groups of Rhesus macaques (five per sex) were administered H-BAT intravenously four hours after IV exposure of BoNT serotype A (4x $NHPLD_{50}$/kg). Consistent with the guinea pig dose ranging study, a more robust BoNT exposure level was used in the Rhesus macaque dose ranging study as animals were treated at a set time point rather than an individual clinical sign. The first group received 1x the scaled human dose of H-BAT, while the second group received the 0.1x scaled human dose. A third group of Rhesus macaques (five per sex) were exposed to BoNT serotype A (4x $NHPLD_{50}$/kg) and received placebo treatment.

All of the animals in the H-BAT-treated groups survived until the end of the study, at which time they were sacrificed. All of the animals in the placebo group died during the study or were sacrificed after they began exhibiting severe signs of botulinum intoxication (Table 31).

Table 31: Summary of Rhesus Macaque Survival Data from Dose Ranging Study LBERI 061

Treatment Group	Survival Rate n/n (%)	Fisher's Exact Test p-value
1x H-BAT	10/10 (100)	< 0.001
0.1x H-BAT	10/10 (100)	< 0.001
Placebo control	0/10 (0)	N/A

All of the animals treated with H-BAT, regardless of dose, showed no clinical signs of intoxication after administration of BoNT serotype A, except for one animal with oral discharge. All 10 animals in the placebo group exhibited most of the typical signs of botulism toxicity. The median time to onset of clinical signs (ptosis) in these animals was 27.5 hours with a 95% CI of 23 to 29 hours. The median time to death or euthanasia for the animals exhibiting severe signs of botulism intoxication was 36.5 hours with a 95% CI of 28 to 39 hours. Thus, in Rhesus macaques the progression of clinical signs to death was very rapid at 4x $NHPLD_{50}$/kg.

These data show that IV treatment of Rhesus macaques with H-BAT was effective in preventing the lethal effects and severe clinical signs of botulinum intoxication at 4x LD_{50} when administered within four hours of exposure to BoNT serotype A, even when administered at one-tenth the scaled human dose.

9.7 Population Pharmacokinetic and Pharmacodynamic Modeling

To support Cangene's human dosing recommendations for H-BAT, a population pharmacokinetic and pharmacodynamic modeling and simulation study was undertaken which incorporated information from both nonclinical (guinea pig and Rhesus macaque) and

clinical studies. The modeling and simulation study entailed the development of two models, a compartmental PK model and an exposure-response model.

9.7.1 Compartmental Pharmacokinetic Model

A compartmental PK model was constructed to assess the PK of H-BAT for the three species (human, guinea pig and Rhesus macaque) used in the H-BAT development program, including nonclinical and clinical studies.

Population PK analysis of H-BAT for all of the serotypes were best fitted using a three-compartment model with the exception of serotype E, which was fitted using a two-compartment model. Serotype E profiles appeared to behave as a two-compartment model due to lack of information during the terminal phase and had minimal effect on the overall model. The apparent bi-exponential disposition may therefore, be an artifact of the MNA's lower limit of quantification (LLOQ) for serotype E.

Population PK parameters for H-BAT derived for all serotypes, for all species are summarized in Table 32. Mean systemic clearance ranged from 4.44 mL/hr/kg (serotype D) to 14.75 mL/hr/kg (serotype A) and the volume of distribution at steady-state (Vss) which is the sum of the central volume of distribution (Vc), the peripheral volume of distribution (Vp), and deep tissue volume of distribution (Vdt) of H-BAT ranged from 4.44 mL/kg (serotype E) to 1309 mL/kg (serotype C). The Vss were comparable to known volumes of serum (42.9 mL/kg; e.g., serotype D) and water (600 mL/kg; e.g., serotype B) in humans [Davis and Morris, 1993]. The residual variability derived with the proportional error model was low. For example, the unexplained error associated with a fitted concentration value of 100 U/mL would be approximately 24 to 45 U/mL [i.e., (100 × 0.24) U/mL]. The residual variability represents the sum of all variability that is not explained by the model, including bioanalytical variability, experimental noise, and variability that cannot be explained by the structural PK model.

Table 32: Population Pharmacokinetic Parameters All Serotypes of H-BAT

Serotype	Parameter (Geometric Mean)						
	CL (mL/hr/kg)	CLd (mL/hr/kg)	CLdt (mL/hr/kg)	Vc (mL/kg)	Vp (mL/kg)	Vdt (mL/kg)	Proportional Error %
A	14.75	2.70	8.15	31.88	21.17	23.38	24%
B	10.82	7.24	136.03	21.32	145.29	34.39	34%
C	8.08	16.17	4.56	56.45	25.44	1227.12	45%
D	4.44	5.29	2.63	0.66	14.93	5.44	30%
E	7.72	0.88	N/A	0.25	4.19	N/A	38%
F	12.25	43.40	2.53	32.44	44.23	40.94	35%
G	10.01	2.84	40.48	20.56	67.04	28.03	35%

The corresponding allometric exponent calculations for systemic clearance (CL) and Vc for each of the serotypes are presented in Table 33. For most serotypes there was a non-linear relationship between body weight (BW) and CL, only serotype E displayed a CL that was directly proportional to BW (slope of 1.033; Table 33). The remaining estimates were close to 0.75 or 0.66 in all cases but for serotype D clearance, which was significantly lower (0.376). Volume slope estimates were close to one for all serotypes except for serotypes A and D, where slopes were significantly different than one (0.460 for serotype A and 0.193 for serotype D). Results are biased by the lack of data points to perform the scaling (2 points form a straight line) for serotypes B-G, often widening the estimated 95% CI on the slopes.

These results indicate that using a slope of 0.75 for CL and 1.00 for volumes [Gabrielsson and Weiner, 2006], in the absence of other data, would be a reasonable alternative to the empirical scaling performed in this report across all species given that the CI described above typically include the value of 0.75 for CL and one for volumes.

Table 33: Allometric Exponent Values of Systemic Clearance and Central Volume of Distribution of H-BAT for All Serotypes

Serotype	Parameter	Slope	95% CI	
			Lower	Upper
A	CL	0.729	0.705	0.752
	Vc	0.460	0.099	0.822
B	CL	0.700	0.648	0.752
	Vc	1.129	0.344	1.913
C	CL	0.640	0.347	0.933
	Vc	0.847	0.704	0.990
D	CL	0.376	0.116	0.636
	Vc	0.193	-0.434	0.820
E	CL	1.033	0.714	1.352
	Vc	1.557	0.936	2.178
F	CL	0.659	0.621	0.696
	Vc	1.023	0.466	1.581
G	CL	0.645	0.510	0.780
	Vc	0.982	-0.273	2.236

The PK modeling demonstrated that H-BAT generally followed tri-exponential decline and that the pharmacokinetics could be extrapolated from animals to humans using an allometric function with slopes close to 0.75 for CL and 1.00 for volumes.

9.7.2 Exposure-response Model

Logistic regression was used to explore the relationship between H-BAT exposure measures predicted by the population PK model (area under the curve, AUC) and the probability of survival. Survival information from the dose-ranging efficacy studies was used in this model. Dose ranging study survival data were used to eliminate the confounding effects of the time of administration of H-BAT on the survival results. Parameter estimates (logit scale) for the regression model (slope and intercept) were estimated for each exposure-survival relationship. Figures of observed and fitted probabilities of survival as a function of H-BAT exposure were also derived.

Given a certain exposure to BoNT, the objective of the exposure-response model was to determine the risk of death following a human dose of H-BAT (one vial) based upon the non-clinical development studies. If H-BAT body burden is defined as a benchmark neutralizing the risk from BoNT exposure, any exposure to H-BAT greater than this benchmark will be a sufficient dose of H-BAT, because the exposure is greater than this benchmark.

For this model, the margin of efficacy (MOE) is defined as the ratio between H-BAT exposure and a defined minimum efficacy benchmark (MOE = $AUC_{HUMAN\ DOSE}/AUC_{MEE}$, where MEE is the minimum effective H-BAT exposure). The magnitude of this H-BAT body burden to benchmark ratio provides an assessment of the relative safety margin for antitoxin

levels. A ratio of 1,000 indicates significant distance from the negligible risk benchmark whereas a ratio of one indicates close proximity to the benchmark with minimal antitoxin buffer. A ratio of less than one indicates insufficient antitoxin levels to be protective in the majority of anticipated situations. This ratio, which we defined as MOE, provides a rapid assessment of the risk related to a scaled human dose of H-BAT.

In this modeling study, the body burden of H-BAT that would result in 80% survival was calculated and identified as the benchmark minimum efficacious exposure (MEE) that is used in the ratio for assessing risk. The lower confidence interval (based on 95% CI) of this calculated body burden was also calculated, but contrary to the minimum effective dose (MED), it was not used in establishing the MEE. The human body burden corresponding to administration of one or two vials of H-BAT was compared to the MEE and a MOE was estimated. Similar results are seen if 90% survival is used as the benchmark (data not shown).

The statistical significance of the exposure-survival relationship for the predicted probability of survival was tested at an alpha level of 0.05. The logistic slope of the AUC effect on survival was highly significant, suggesting a direct relationship between H-BAT body burden and the probability of survival in guinea pigs and Rhesus macaques.

Logistic regression was used to explore the relationship between H-BAT exposure measures predicted by the population PK model (AUC) and the probability of survival. The predicted probabilities for survival for serotype A are shown in Table 34**.**

Table 34: Estimated Survival and H-BAT AUC for Serotype A

Survival Probability		**H-BAT AUC (U*hr/mL)**	
Predicted	95% CI	Predicted	95% CI
58.3%	36.8-77.0%	0.064	0.034-0.12
70.3%	52.1-83.7%	0.094	0.054-0.164
80.1%	64.1-90.0%	0.137	0.076-0.246
90.2%	75.6-96.5%	0.249	0.114-0.545
95.1%	81.9-98.8%	0.429	0.15-0.226
99.0%	90.2-99.9%	1.415	0.25-7.996

A conservative MEE of 0.137 U*hr/mL was considered (the point estimate AUC associated to an 80% survival rate) as an initial benchmark for estimating efficacy. This benchmark value was used considering the critical nature of response (i.e., survival) and any possible model uncertainty due to low resolution of data creating a widening of the confidence intervals. The lower 95% CI of the observed AUC (conservative estimate) in the human PK study (proposed clinical dose of one vial), as estimated by non-compartmental analysis, was approximately 21.0 U*hr/mL. Overall, the resulting MOE (based on body burden across two species) for BoNT serotype A is presented below.

- MOE = $AUC_{HUMAN\ DOSE}/AUC_{MEE}$ = 21.0/0.137 = 153, for the proposed clinical dose of one H-BAT vial derived at 104 (Rhesus macaque) and 45 (guinea pig) $MIPLD_{50}$/kg BoNT exposure.

Since the body burden of H-BAT following administration of one vial in humans was 21.0 U*hr/mL (MOE of at least 153), more than 99.977% survival against serotype A toxin would be predicted for the one vial dose. This margin can provide a safety margin for any potential decrease in H-BAT body burden due to increases in BoNT exposure and any possible interaction between the doses of H-BAT and BoNT. Furthermore, by dividing the proposed human dose by the MOE (1/153 = 0.0065x), we find the human dose that will protect 80% of the population. Since H-BAT is expected to be in excess of BoNT, there is currently no evidence that increasing BoNT exposure would alter the MOE at least until units of BoNT exceed the ~100 million $MIPLD_{50}$ neutralizing units of serotype A available in the systemic circulation following the proposed clinical dose of H-BAT. As the antitoxin/BoNT toxin complex is inactive, additional clinical signs would not be expected to manifest until the free fraction of H-BAT significantly decreases.

The body burden following administration of one H-BAT vial in humans is expected to protect more than 99.977% of the population against serotype A neurotoxin. A similar analysis was undertaken with serotypes B-G. The MOE was estimated in the same manner as for serotype A. The MOE for serotypes A-G are summarized in Table 35.

Table 35: Margin of Efficacy for 80% Survival for Lot 2060401

Serotype	MEE (U*hr/mL)	Lower 95% CI for $AUC_{0-\infty}$ (U*hr/mL)	MOE	Probability of Survival (%)
A	0.137	20.96	153.0	99.977
B	0.085	17.44	205.2	96.436
C	0.249	23.88	95.9	98.549
D	1.413	4.15	2.9	99.971
E	0.794	5.25	6.6	99.860
F	0.072	18.54	257.5	99.754
G	0.088	4.86	55.2	98.063

H-BAT offered the greatest protection for serotypes B and F following a scaled human dose, with MOE values of 205.2 and 257.5, respectively. A lower MOE was observed for serotypes D and E, which resulted in a decreased predicted protection for a scaled human dose. Based on the data currently available, the probability of survival for all serotypes following a dose of one vial of H-BAT was > 96%.

Since Cangene plans on using the labeling claim to determine the shelf-life of H-BAT, additional sensitivity analyses were performed by determining the MOE of one H-BAT vial based on the labeling claim of the product, rather than the potency measured on the manufacturing date. Although the MOE were reduced, the probability of survival following administration of one vial of H-BAT based on the labeling claim was still 93.148% for serotype D and 98.952% for serotype E (Table 36).

All MOE values, whether based on the lot potency or label claim were above the threshold value of one, for each botulism antitoxin serotype (Table 36). This information indicates that even based on the labeling claim (lowest value), a minimum efficacious exposure level for humans was obtained after administration of a single vial of H-BAT.

Table 36: Margin of Efficacy for H-BAT Based on the Lot Potency or Label Claim of the Product

Serotype	Measured Lot Potency		Label Claim	
	MOE	Survival Probability	MOE	Survival Probability
A	153	99.977	> 66.2	99.926
B	205	96.178	> 95.8	95.589
C	96	98.195	> 40.3	97.535
D	2.9	99.704	> 1.21	93.148
E	6.6	99.737	> 3.15	98.952
F	258	99.695	> 118	99.540
G	55	97.880	> 27	97.352

The efficacy results in animals as well as humans, and the PK and the exposure-response models demonstrated that the one vial dose for adult humans is the appropriate dose.

10 BENEFIT RISK DISCUSSION

The development program has defined known and potential H-BAT benefits as well as potential risks, which clearly demonstrate that the benefit/risk profile favors H-BAT administration for patients with suspected or confirmed botulism.

Demonstrated Benefits: Animal Efficacy

The efficacy of H-BAT has been demonstrated in two well-characterized animal models of botulism (guinea pig and Rhesus macaque).

In the guinea pig efficacy model the findings were as follows:

- Marked improvement in survival of guinea pigs treated with H-BAT that was independent of BoNT serotype.
- Overall, 97-100% (236/238) survived in the H-BAT treated groups compared to 0-50% (31/238) in the placebo control group.
- The incidence of severe botulism symptoms was lower than in the H-BAT group.

In NHP efficacy model, the findings were as follows:

- Significant improvement in survival of Rhesus macaques inoculated with serotype A of BoNT and then treated with H-BAT after development of botulism symptoms.
- Overall, 47% (14/30) in the H-BAT treatment group survived to 21 days with 0 of 30 surviving in the placebo control group ($p < 0.0001$).
- As with survival, time to death was substantially longer in the H-BAT-treated animals that succumbed than placebo treated animals.
- The incidence of severe botulism symptoms was lower in the H-BAT group compared to placebo treatment.

Potential Benefits: Human Pharmacodynamics & Clinical Efficacy

The BoNT neutralizing ability of H-BAT has also been demonstrated in the controlled clinical study using the human EDB muscle model. H-BAT prevented subjects from experiencing a decrease in muscle function, following injection of BoNT serotypes A and B into the EDB muscle. And also in this study H-BAT showed to be as effective as the previously marketed Aventis Pasteur (Connaught) BAT AB product. This demonstrates that H-BAT can neutralize free botulinum toxin in the extravascular space, which clearly shows its potential benefits for human cases of botulism.

Post-hoc analysis of information provided by the CDC, from patients with suspected or confirmed cases of botulism, supports the positive impact of early treatment with H-BAT. Patients treated with H-BAT within two days of symptom onset experienced a shorter duration of hospitalization as compared to patients treated more than two days after onset of symptoms. Similar findings were also seen with duration of ICU stay and duration on mechanical ventilation. Historically, early treatment with botulism antitoxins has been the only effective therapy to mitigate the effects of this deadly neurotoxin.

Potential Risks: Safety in Humans

Based on historical data for human use of equine products, the primary safety concern with heterologous immune globulins is immunologic reactions to proteins from other species. These reactions include anaphylactic and anaphylactoid reactions, delayed allergic reactions (serum sickness) and possible febrile responses to immune complexes formed from animal antibodies. These safety concerns correspond to the adverse reactions listed in the prescribing information for the previously marketed Botulism Antitoxin Bivalent (Equine) Type A and B product, which included anaphylactic reactions, thermal reactions and serum sickness.

During an 11-year period from 1967 to 1977, the CDC monitored hypersensitivity reactions in patients receiving equine botulism antitoxins. Black and Gunn [1980] reported that of the 268 people receiving botulism antitoxin, 24 (9.0%) had nonfatal hypersensitivity reactions, with 5.3% of the reactions being acute and 3.7% of the reactions being delayed. The overall reaction rates did not differ with age or sex of the recipient or with the botulism antitoxin Connaught BAT AB or BAT ABE products. However, serum sickness occurred significantly ($p < 0.02$) more frequently in persons receiving more than 40 mL of botulism antitoxin.

The risk of serum sickness is also a concern, particularly in situations where re-dosing is a possibility (wound botulism or intestinal colonization). In potential re-dosing situations, the benefit-to-risk ratio should be carefully assessed on a case-by-case basis. The clinical trial results indicated that the skin sensitivity test was not predictive of anti-equine antibodies. Subjects with pre-existing anti-equine antibodies did not exhibit a different safety profile in terms of the frequency or type of adverse events. However, the risk-benefit should be carefully assessed on a case-by-case basis until the safety in a larger number of patients and a broader population is available.

Products made from equine plasma may contain infectious agents, such as viruses, that can cause disease. As part of the manufacture of H-BAT, controls at the equine plasma collection stage are in place to test for the presence of specific infectious agents prior to use including testing by 9CFR (i.e., Code of Federal Regulations - Title 9: Animals and Animal Products) standards. Additional controls are incorporated into the manufacturing process for H-BAT that includes two viral inactivation and removal steps. Validation of the process for viral clearance has been completed with several model viruses. The platforms used for viral inactivation and clearance are employed in the manufacture of Cangene's licensed human Hyperimmune products WinRho® and HepaGamB®. Despite these measures, blood-derived products can still transmit disease. No known cases of transmission of viral diseases have been associated with the use of H-BAT or with Cangene's other licensed products, WinRho, HepaGam B, VARIZIG or VIGIV.

The formulation for H-BAT contains 10% maltose, which can interfere with some types of blood glucose monitoring systems and result in false readings for elevated glucose [Gaines et al., 2008; Schleis 2007]. In such case, untreated hypoglycemia or inappropriate insulin administration could follow, resulting in life-threatening hypoglycemia. Only testing systems that are glucose-specific should be used in patients receiving H-BAT.

Risk to patients without botulism needs to be considered. Since early intervention has been shown to lead to improved outcomes, there is the risk that H-BAT will be given empirically

to patients with neurologic signs and symptoms similar to botulism who are subsequently found to have other illnesses such as Guillain-Barre syndrome. While this was known to occur in 39/148 patients in the CDC EAP, there was no appreciable difference in the tolerability of H-BAT in patients with botulism and those confirmed to not have botulism. Release of H-BAT will remain under the control of the CDC in consultation with the requesting health care provider to limit this potential risk.

The benefit-risk ratio for H-BAT is clearly positive, with a survival benefit and clinical symptom improvement shown in two animal species which are sensitive to all seven serotypes. The result of a study in healthy volunteers showed H-BAT to be safe and well-tolerated, and the CDC's EAP suggests similar findings. This positive benefit/risk profile applies to the sporadic natural cases of botulism and even more so to the scenario of a mass intentional exposure, where 100's to 1000's of individuals could develop botulism in a short period of time.

11 CONCLUSION

H-BAT is a sterile solution of purified $F(ab')_2$ plus Fab and $F(ab')_2$ related immune globulin fragments that has been developed to address a critical unmet medical need. The overall developmental dataset supports the proposed indication, which is "*for the treatment of symptomatic botulism following documented or suspected exposure to BoNT serotypes A, B, C, D, E, F or G*".

Based upon the regulatory path for approval that is described in the "Animal Rule", H-BAT has been demonstrated to meet the four Essential Elements for licensure.

1. *There is a reasonably well-understood pathophysiological mechanism of the toxicity of the (chemical, biological, radiological, or nuclear) substance and its prevention or substantial reduction by the product.*

The pathophysiology of BoNT has been well-characterized through the investigation of naturally occurring cases of botulism, the medical uses of the neurotoxins, and through *in vitro* and *in vivo* studies in animals. While there are several routes of exposure to BoNT, the pathophysiology is mediated by uptake of the toxin into the cholinergic nerve endings and inhibition of release of acetylcholine. Thus the binding of circulating toxin to prevent uptake into nerves is the well-established mechanism of action of H-BAT to reduce the severity and progression of symptoms from BoNT, and is consistent with the mechanism of other previously marketed BATs, such as the previously marketed Aventis Pasteur (Connaught) BAT AB product and investigational monovalent BAT E.

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 for predicting the response in humans.*

Guinea pigs and Rhesus macaques were selected as the models for demonstration of efficacy because the clinical progression of disease in these animal models is similar to that observed in humans, with each moving through stages from progressive muscle weakness to total paralysis and, ultimately, to death. These animals are well-characterized models for BoNT intoxication. In both animal models, H-BAT demonstrated a strong and robust survival benefit and reduction in clinical severity. In the CDC EAP, early treatment with H-BAT suggests a reduction of the morbidity associated with botulism intoxication in patients with suspected or confirmed botulism.

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.*

Improvement in survival along with reduction in morbidity, the important endpoints in humans, were the endpoints studied in the animal models. In both animal models, H-BAT demonstrated a significant and robust survival benefit compared to placebo.

4. *The data or information on the (pharmaco-) kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.*

Population PK/PD modeling and simulation work was completed to integrate data from guinea pig, Rhesus macaque and human studies that established the theoretical neutralizing capacity, PK/PD profile and efficacy in dose ranging and treatment studies. The translational modeling was used to justify that the proposed human dose of one vial is the appropriate dose.

Controlled safety studies in humans have affirmed safety for the proposed use. The CDC experience with H-BAT is consistent with a safe and well-tolerated product.

In conclusion, H-BAT is a safe and well-tolerated equine hyperimmune product with demonstrated efficacy in guinea pigs, Rhesus macaques and humans. H-BAT provides a favorable benefit-to-risk ratio, by reducing the morbidity and mortality associated with botulism intoxication in patients with suspected or confirmed botulism.

Therefore, H-BAT should be approved for the proposed indication as the overall development program meets the regulatory requirements, clinical benefit/risk expectations and addresses a critical unmet need.

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13 APPENDICES

13.1 Summary of Clinical Pharmacology Studies

A list of the pharmacology studies and their objectives is provided in Table 37. Brief summaries of the results of the individual studies are provided in the sections below.

Table 37: Summary of H-BAT Pharmacokinetic and Pharmacodynamic Studies in the Human, Guinea Pig and Rhesus Macaque

Species	Study Identifier	Study Type	Study Objectives
Guinea pig	BBRC 684-G005630	PK (un-intoxicated)	Primary – to assess the PK of the seven botulism antitoxin serotypes in H-BAT following IV administration Secondary – to determine the dose proportionality of the various PK parameters following IV administration of H-BAT
Rhesus macaque	LBERI FY07-056	PK (un-intoxicated)	Primary – to assess the PK of serotype A botulism antitoxin in H-BAT following IV administration at two dose levels
	LBERI FY08-061	PK (intoxicated)	Primary – to demonstrate that H-BAT administered 4 hours post-injection with BoNT serotype A prevents the occurrence of mortality and clinical signs Secondary – to assess the PK of H-BAT following administration of BoNT serotype A
Human	BT-001	Safety and PK (un-intoxicated)	Primary – to evaluate the safety of H-BAT based upon clinical observations, AEs, and laboratory assessments Secondary – to assess the PK of the seven botulism antitoxin serotypes in H-BAT following IV administration
	BT-002 Stage A	PD and Safety	Primary – to evaluate the effect of bivalent BAT (Aventis Pasteur) in preventing paralysis of the EDB muscle following Botox® or Myobloc® administration Secondary – to evaluate the safety of bivalent BAT (Aventis Pasteur) in healthy subjects
	BT-002 Stage B	PD and Safety	Primary – to evaluate the effect of H-BAT in preventing paralysis of the EDB muscle following Botox® or Myobloc® administration Secondary – to evaluate the safety of H-BAT in healthy subjects

Species	Study Identifier	Study Type	Study Objectives
Interspecies	BT-008	Modeling and Simulation	Primary - to develop a model-based understanding of H-BAT PK/PD properties to extrapolate animal dose regimen to the clinical scenario

13.1.1 Pharmacokinetic Studies

13.1.1.1 Study BBRC 684: Intravenous H-BAT Pharmacokinetics Determination in Guinea Pigs

Study BBRC 684 was a randomized study designed to assess the PK of the seven botulism antitoxin serotypes found in H-BAT at two dose levels (0.2x and 1.0x) following IV administration to un-intoxicated Hartley guinea pigs. The study was conducted under GLP conditions.

The PKs of the seven botulism antitoxin serotypes (A through G) was determined in male guinea pigs following a single, slow bolus IV injection of H-BAT at either 0.2x or 1.0x the scaled human dose (wt/wt based on an average human body weight of 70 kg). The dose levels examined in this study were based on the effective doses used in study BBRC 731. Blood samples were collected at defined time points during the study (baseline, 10 minutes, 4, 8, 12, 24, 48 hours, 3, 5, 8 and 12 days post-dose). The serum from 12 animals was collected at each time point. Equal volumes of serum from three animals were pooled to provide sufficient sample for the MNA. This resulted in four samples per time point. Serum H-BAT levels were measured using the guinea pig serum MNA method.

The following PK parameters were calculated from the serum drug concentration-time curves for H-BAT: AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, CL and Vd.

The pharmacokinetic parameters in guinea pigs varied based upon the antitoxin serotype measured (Table 38). Comparison of the seven antitoxins showed that the mean $AUC_{0-\infty}$ values ranged from 0.36 U*hr/mL (0.2x dose) and 1.73 U*hr/mL (1.0x dose) for serotype G to 1.46 U*hr/mL (0.2x dose) for serotype C and 10.30 U*hr/mL (1.0x dose) for serotype A. The mean C_{max} ranged from 0.11 U/mL (0.2x dose) and 0.48 U/mL (1.0x dose) for serotype G to 0.43 U/mL (0.2x dose) and 2.92 U/mL (1.0x dose) for serotype A. The half-lives of the different antitoxin serotypes varied with the serotype. H-BAT was undetectable in the serum samples of guinea pigs from the 0.2x group for botulism antitoxins serotypes D, E and F therefore; the various PK parameters could not be calculated for these groups.

A dose proportionality assessment was performed and it was found that there was no statistical evidence of a lack of dose-proportionality. Therefore, it is reasonable to assume dose-proportionality for the dose ranges tested in this study. Only male guinea pigs were used in this study as a result, gender related effects were not determined.

Table 38: Pharmacokinetic Parameters (Mean) for Antitoxin Serotypes A Through G in Male Guinea Pigs Following Intravenous Administration of 0.2x or 1.0x the Scaled Human Dose of H-BAT

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/kg)	Vd (mL/kg)
A	0.2x	1.30	1.39	0.43	0.17	2.11	21.31	65.00
	1.0x	10.00	10.30	2.92	0.17	6.24	14.47	130.20
B	0.2x	1.37	1.45	0.39	0.17	7.44	13.87	148.92
	1.0x	8.18	8.39	1.42	0.17	15.30	11.93	263.94
C	0.2x	1.30	1.46	0.26	0.17	3.76	14.05	76.19
	1.0x	6.97	7.98	1.86	0.17	4.84	12.80	89.29
D	0.2x	ND	ND	ND	ND	ND	ND	ND
	1.0x	3.77	3.97	1.17	0.17	3.31	5.25	25.09
E	0.2x	ND	ND	ND	ND	ND	ND	ND
	1.0x	2.15	2.25	0.56	0.17	2.81	67.12	271.88
F	0.2x	ND	ND	ND	ND	ND	ND	ND
	1.0x	7.68	7.83	1.79	0.17	10.40	11.94	179.47
G	0.2x	0.34	0.36	0.11	0.17	3.14	9.82	44.42
	1.0x	1.69	1.73	0.48	0.17	5.54	10.13	81.00

13.1.1.2 Study LBERI 056: H-BAT Antitoxin: Intravenous Pharmacokinetic Study in Rhesus Macaques

Study LBERI 056 was a randomized study designed to assess the PK of serotype A botulism antitoxin in H-BAT following IV administration to Rhesus macaques at two dose levels (1.0x and 5.0x). The study was conducted under GLP conditions.

The PK of botulism antitoxin serotype A was determined in male and female Rhesus macaques following a single, slow bolus IV injection of H-BAT at either 1.0x or 5.0x the scaled human dose (wt/wt scaled based on average human body weight of 70 kg). Botulism antitoxin serotype A, PK parameters were assessed because only the efficacy of botulism antitoxin serotype A was determined in the Rhesus macaque therapeutic efficacy model (LBERI 066).

Blood samples were collected at defined time points during the study (baseline, 30 minutes, 2, 4, 6, 8, 12, 18, 24 hours, 3, 6, 9, 12, 14 and 20 days post-dose) from three male and three female animals per group. Serum H-BAT levels were measured using the NHP serum MNA method.

The following PK parameters were calculated from the serum drug concentration-time curves for H-BAT: $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, CL and Vd.

The pharmacokinetic parameters for botulism antitoxin serotype A in the Rhesus macaque are summarized in Table 39. The $AUC_{0-\infty}$ for the combined NHP group was 9.7 U*hr/mL, the C_{max} was 2.9 U/mL, the half-life was 3.0 hours, the CL was 13.1 mL/hr/kg and the Vd was 57.2 mL/kg. Comparison of the PK parameters between male and female animals showed there were no gender-related differences following a single IV administration of H-BAT at either the 1.0x or 5.0x the scaled human dose. Assessment of dose proportionality was not planned as part of this study but the $AUC_{0-\infty}$ and C_{max} did not appear to be dose proportional over the dose range tested.

Table 39: Pharmacokinetic Parameters (Mean and SD) for Antitoxin Serotype A in Rhesus Macaques Following Intravenous Administration of 1.0x or 5.0x the Scaled Human Dose of H-BAT

Serotype	Treatment Group	$AUC_{0-\infty}$ (U*hr/mL)	C_{max} (U/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	CL (mL/hr/kg)	Vd (mL/kg)
A	All 1.0x	9.7 (1.6)	2.9 (0.6)	0.5 (0)	3.0 (0.8)	13.1 (3.2)	57.2 (21.9)
	All 5.0x	80.2 (14.2)	20.1 (8.3)	0.5 (0)	5.2 (1.3)	9.1 (1.5)	68.5 (21.9)
A	Male 1.0x	9.1 (2.2)	2.6 (0.9)	0.5 (0)	3.2 (1.2)	14.5 (4.4)	66.7 (30.1)
	Male 5.0x	82.8 (19.3)	24.6 (10.6)	0.5 (0)	5.2 (1.6)	8.9 (1.7)	69.7 (32.8)
A	Female 1.0x	10.3 (0.6)	3.1 (0)	0.5 (0)	2.8 (0.2)	11.8 (0.4)	47.8 (4.6)
	Female 5.0x	77.5 (10.4)	15.7 (0)	0.5 (0)	5.1 (1.3)	9.3 (1.5)	67.2 (10.5)

13.1.1.3 Study LBERI 061: Efficacy of Post-exposure Prophylactic Administration of H-BAT Botulinum Antitoxin Heptavalent in Rhesus Macaques (*Macaca mulatta*)

Study LBERI 061 was a randomized study designed to assess the efficacy and PK of two dose levels of H-BAT (0.1x and 1.0x) following IV administration to Rhesus macaques which had been exposed to BoNT serotype A. The study was conducted under GLP conditions.

The primary objective of this study was to demonstrate that H-BAT administered four hours post-intoxication with BoNT serotype A prevented mortality and clinical signs of intoxication. The secondary objective was to assess the PK of botulism antitoxin serotype A following BoNT serotype A intoxication.

The PK of botulism antitoxin serotype A was determined in Rhesus macaques challenged with BoNT serotype A at four times the NHP lethal dose 50% (4x $NHPLD_{50}$/kg) and treated four hours later with a single, slow bolus IV injection of H-BAT at either 0.1x or 1.0x the scaled human dose (wt/wt based on average human body weight of 70 kg). Blood samples were collected at defined time points during the study (4, 8, 12 and 24 hours post-H-BAT dose) from five male and five female animals per group. Serum H-BAT levels were measured using the NHP serum MNA method.

The following PK parameters were calculated from the serum drug concentration-time curves for H-BAT: $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, CL and Vd.

Serotype A antitoxin was only detectable in samples collected from animals treated at the 1.0x scaled human dose. The various PK parameters for the 1.0x H-BAT-treated group are

listed in Table 40. The highest concentration of antitoxin was detected at the first sample collection time point (4 hours post-H-BAT dose). The antitoxin level decreased quickly and was barely detectable at 24 hours post-BoNT dose.

Table 40: Pharmacokinetic Parameters (Mean and SD) for Antitoxin Serotype A Following Intravenous Administration of H-BAT in BoNT Serotype A Intoxicated Rhesus Macaques

Serotype	Treatment Group	$AUC_{0-\infty}$ (U*hr/mL)	C_{max} (U/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	CL (mL/hr/kg)	Vd (mL/kg)
A	0.1x	ND	ND	ND	ND	ND	ND
A	1.0x	11.1 (3.5)	0.9 (0.3)	4 (0)	4.7 (1.2)	13.5 (2.9)	94.2 (31.9)

13.1.1.4 Study BT-001: Pharmacokinetics of a Heptavalent Equine-derived Botulinum Antitoxin

Clinical trial BT-001 was a single-center, double-blind, randomized, parallel arm study to assess the safety and PK of H-BAT following IV administration of either one (n = 20) or two (n = 20) vials of H-BAT to normal healthy adults. The primary objective was to evaluate the safety of H-BAT based upon clinical observations, AEs and laboratory assessments. The secondary objective was to assess the PK of the seven botulism antitoxin serotypes contained in H-BAT following IV administration.

PK samples were collected at defined time points over 28 days (baseline, 0.5, 4, 8, 24 hours, 3, 7, 14, 21, and 28 days post-dose) and were analyzed using the human serum MNA method.

The following PK parameters were calculated from the serum drug concentration-time curves for H-BAT: AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, CL and Vd.

The PK parameters varied based upon the antitoxin serotype measured (Table 41). Comparison of the seven antitoxins showed that the mean $AUC_{0-\infty}$ values ranged from 7.05 U*hr/mL (1 vial) and 14.66 U*hr/mL (2 vials) for serotype G to 37.34 U*hr/mL (1 vial) and 86.25 U*hr/mL (2 vials) for serotype C. The mean C_{max} ranged from 0.59 U/mL (1vial) and 1.19 U/mL (2 vials) for serotype G to 2.69 U/mL (1 vial) and 6.23 U/mL (2 vials) for serotype A. 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 7.51 to 7.75 hours. Antitoxin serotypes C and B had the longest mean half-lives, ranging from 29.60 to 34.20 hours.

Table 41: Arithmetic Mean (% CV) of Pharmacokinetic Parameters for Serum Antitoxin Serotypes A-G in Subjects Following Intravenous Administration of One or Two Vials of H-BAT

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)	0.54 (9.4)	7.32 (23.9)	1110 (16.4)	11596 (22.4)

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)**
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)

Although no formal dose proportionality assessment was planned as part of clinical trial BT-001, AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values increased in a dose proportional fashion as H-BAT doses increased from one to two vials. In addition, mean clearance values appeared to be similar between both treatment groups for the seven antitoxin serotypes, suggesting dose linearity of H-BAT over the dose range studied. Comparison of the PK parameters between adult male and female subjects indicated that there did not appear to be any gender-related differences following IV administration of either one (Table 42) or two (Table 43) vials of H-BAT.

Table 42: Arithmetic Mean (% CV) of Pharmacokinetic Parameters for Serum Antitoxin Serotype A-G for Male and Female Subjects Following Intravenous Administration of One Vial of H-BAT

Serotype	Gender	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	Male	18.56 (24.3)	23.87 (14.5)	2.14 (33.1)	0.51 (1.9)	9.04 (14.6)	319 (13.6)	4130 (13.2)
	Female	23.96 (8.0)	27.42 (9.8)	3.18 (11.5)	0.87 (127.0)	8.37 (16.3)	276 (9.7)	3309 (13.2)
B	Male	26.04 (26.5)	29.58 (17.4)	1.96 (49.7)	1.29 (120.0)	33.50 (40.9)	191 (17.2)	9196 (45.3)
	Female	28.17 (20.5)	29.09 (21.5)	1.84 (36.8)	0.52 (5.0)	34.80 (42.3)	200 (28.8)	9926 (52.1)
C	Male	33.42 (31.4)	34.27 (32.5)	1.86 (40.9)	1.74 (150.0)	30.70 (48.5)	158 (28.9)	7066 (55.9)
	Female	39.51 (18.4)	40.92 (20.7)	2.63 (31.0)	2.03 (157.0)	28.40 (44.0)	127 (22.1)	4900 (34.6)
D	Male	5.69 (50.3)	6.19 (2.1)	0.81 (69.2)	0.91 (129.0)	6.54 (18.7)	161 (2.1)	1529 (20.9)
	Female	5.48 (49.4)	8.68 (19.1)	0.81 (42.2)	0.88 (127.0)	8.23 (24.5)	118 (16.6)	1418 (32.4)
E	Male	5.70 (36.2)	6.39 (16.4)	0.75 (38.7)	0.52 (3.9)	8.86 (15.6)	1360 (15.7)	17047 (8.6)
	Female	7.51 (25.9)	7.68 (25.5)	1.11 (36.1)	0.52 (4.1)	7.02 (15.4)	1180 (31.1)	12256 (40.7)

Serotype	**Gender**	**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)**
F	Male	28.80 (35.4)	31.81 (27.1)	2.26 (19.9)	0.90 (129.0)	13.50 (18.6)	169 (31.1)	3249 (33.7)
	Female	29.40 (24.3)	30.99 (25.3)	2.47 (29.9)	1.22 (119.0)	14.70 (14.9)	169 (20.6)	3577 (26.3)
G	Male	5.58 (25.4)	6.67 (25.4)	0.51 (28.2)	1.29 (120.0)	10.70 (41.4)	158 (25.5)	2327 (28.7)
	Female	7.01 (23.3)	7.26 (23.2)	0.66 (22.9)	1.27 (187.0)	12.20 (38.0)	144 (21.9)	2398 (31.5)

Table 43: Arithmetic Mean (% CV) of Pharmacokinetic Parameters for Serum Antitoxin Serotype A-G for Male and Female Subjects Following Intravenous Administration of Two Vials of H-BAT

Serotype	Gender	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	Male	44.62 (30.3)	48.38 (26.8)	5.60 (20.0)	0.52 (3.0)	9.60 (30.4)	329 (24.5)	4315 (18.1)
	Female	59.22 (18.9)	64.65 (14.4)	6.91 (19.1)	0.53 (5.0)	10.90 (38.6)	236 (13.3)	3636 (37.0)
B	Male	53.93 (19.9)	57.96 (21.0)	3.73 (23.6)	0.53 (3.2)	52.60 (72.1)	198 (22.1)	15309 (79.3)
	Female	63.12 (9.6)	66.63 (9.7)	4.83 (35.7)	0.55 (12.1)	61.10 (70.2)	166 (9.7)	14471 (68.0)
C	Male	64.94 (34.7)	74.64 (34.8)	3.95 (50.8)	0.52 (3.6)	45.20 (46.6)	148 (33.0)	9972 (57.7)
	Female	92.55 (14.1)	99.52 (16.3)	5.84 (28.7)	0.53 (4.4)	46.10 (14.0)	103 (15.9)	6786 (16.9)
D	Male	12.51 (33.6)	14.12 (34.4)	1.56 (50.1)	0.87 (128.0)	7.67 (20.5)	154 (27.5)	1695 (30.4)
	Female	14.03 (34.4)	15.62 (45.1)	1.65 (27.3)	0.54 (13.2)	7.90 (24.0)	148 (35.8)	1605 (32.5)
E	Male	12.63 (22.2)	15.70 (12.9)	1.58 (31.7)	0.53 (3.8)	7.03 (32.0)	1100 (14.1)	10978 (27.2)
	Female	14.31 (17.3)	15.63 (18.0)	1.91 (32.5)	0.54 (12.7)	7.52 (19.2)	1120 (18.4)	12008 (20.0)

Serotype	**Gender**	**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)**
F	Male	56.62 (30.6)	60.69 (26.7)	4.39 (33.9)	1.99 (129.0)	16.00 (37.9)	177 (29.6)	3838 (28.6)
	Female	62.64 (23.0)	65.68 (24.4)	4.19 (29.2)	1.27 (186.0)	20.40 (44.6)	160 (22.9)	4830 (60.9)
G	Male	11.93 (31.5)	13.74 (28.7)	0.91 (36.5)	0.88 (127.0)	14.90 (19.3)	155 (25.5)	3316 (30.3)
	Female	14.87 (20.5)	15.30 (20.4)	1.48 (36.4)	0.53 (5.4)	14.70 (15.3)	136 (21.7)	2886 (28.6)

13.1.2 Pharmacodynamic Studies

Clinical trial BT-002A was designed as a proof-of-concept study using the previously marketed BAT Bivalent (Equine) Types A and B, BAT AB product to evaluate the EDB muscle model. Clinical trial BT-002B was designed to evaluate H-BAT's ability to neutralize BoNTs serotypes A (Botox®) and B (Myobloc®) in the human body prior to uptake by the cholinergic nerve endings. Since the principle effect of exposure to botulism in humans is muscle paralysis, inhibition of muscle paralysis induced by BoNT serotype A (Botox®) or BoNT serotype B (Myobloc®) was used as a surrogate endpoint to demonstrate the effectiveness of H-BAT in humans.

13.1.2.1 Study BT-002 Stage A: Botulism Antitoxin Effects on Paralysis Induced by Type A and Type B Botulinum Neurotoxins in the Extensor Digitorum Brevis Muscle

Clinical trial BT-002 Stage A was a single-center study to assess the safety and PDs of one vial of the previously marketed Aventis Pasteur (Connaught) BAT AB product [BAT AB Prescribing Information] product (n = 5) or placebo control (0.9% saline) (n = 5) following IV administration in normal healthy adult subjects. The primary objective of the study was to evaluate the effect of Connaught BAT AB product in preventing paralysis of the EDB muscle following administration of BoNT serotypes A or B (Botox® or Myobloc®). This study acted as a proof-of-concept study for BT-002B. The secondary objective was to evaluate the safety of the previously marketed Aventis Pasteur (Connaught) BAT AB product in healthy human subjects.

The effect of the previously marketed Aventis Pasteur (Connaught) BAT AB product on paralysis induced by BoNTs serotype A and B was evaluated based on the ability of the previously marketed Aventis Pasteur (Connaught) BAT AB product to neutralize BoNT serotypes A and B in the EDB model. The EDB model is a well-characterized and standardized model for measuring the effect of IM administered BoNT [Hamjian & Walker, 1994; Sloop et al., 1996; Sloop et al., 1997]. In this model, the preservation of EDB muscle function in the subjects left and right foot is evaluated as percent muscle function based on the EDB CMAP M wave amplitude and area as recorded following bilateral peroneal motor nerve conduction.

The previously marketed Aventis Pasteur (Connaught) BAT AB product administered 24 hours prior to BoNT exposure prevented subjects from experiencing a decrease in muscle function following injection of BoNT serotypes A and B (Botox® and Myobloc®, respectively). Subjects receiving the placebo control demonstrated a loss of muscle function within three days of exposure to both BoNT serotype A (Table 44 and Figure 13) and serotype B (Table 45 and Figure 14).

In the group treated with the previously marketed Aventis Pasteur (Connaught) BAT AB product, muscle function was stable over time, indicating that the antitoxin was effective at preserving muscle function for up to 28 days following exposure to both BoNTs. By longitudinal analysis of variance model, there was a significant decrease of percent muscle

function of EDB muscle in the placebo group as compared to the H-BAT treatment group over time ($p < 0.05$).

Table 44: Mean of the Percent Muscle Function (CMAP M Wave Amplitude) of the EDB Muscle Following Exposure to Botox® (BoNT Serotype A) and Previously Marketed Aventis Pasteur (Connaught) BAT AB product

Study Visit	Previously Marketed Aventis Pasteur (Connaught) BAT AB Product			Placebo (0.9% Saline)		
	Number of Subjects	Mean Percent Muscle Function	SD	Number of Subjects	Mean Percent Muscle Function	SD
Day 3	5	100.6	9.1	5	41.6	11.8
Day 4	5	100.2	7.6	5	30.0	11.3
Day 7	5	104.0	8.2	5	20.8	10.8
Day 14	5	94.8	11.3	5	18.0	8.7
Day 21	5	104.0	5.8	5	20.6	10.0
Day 28	5	99.6	4.3	5	20.0	8.7

Figure 13: Percent Muscle Function Over Time (CMAP M Wave Amplitude) of the EDB Muscle Following Exposure to Botox® (BoNT Serotype A) and Previously Marketed Aventis Pasteur (Connaught) BAT AB Product

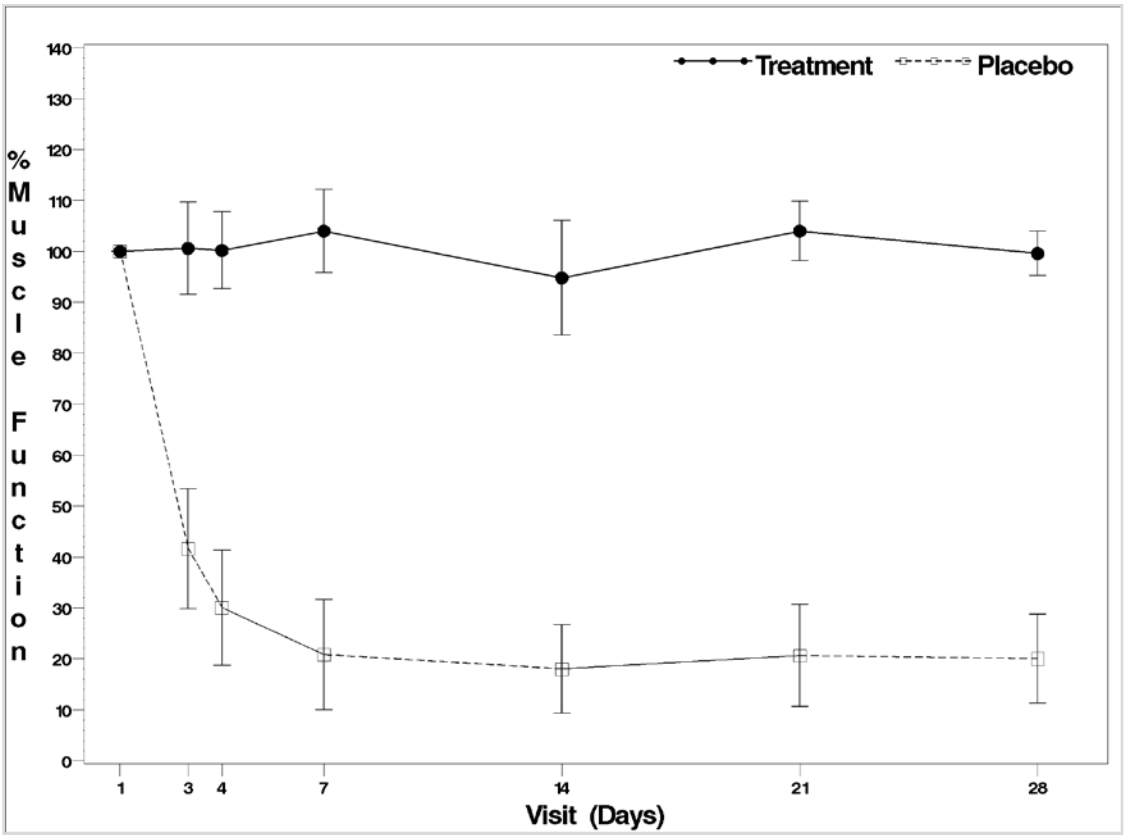
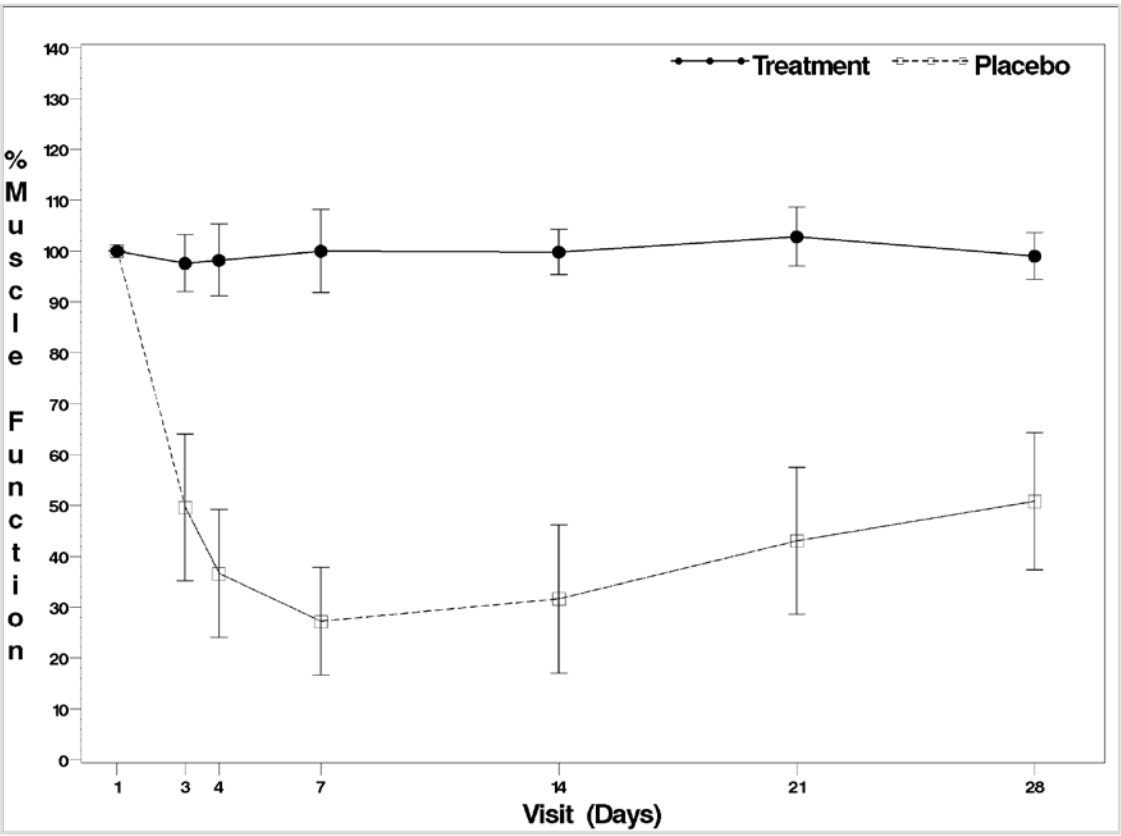


Table 45: Mean of the Percent Muscle Function (CMAP M Wave Amplitude) of the EDB Muscle Following Exposure to Myobloc® (BoNT Serotype B) and Previously Marketed Aventis Pasteur (Connaught) BAT AB product

Study Visit	Previously Marketed Aventis Pasteur (Connaught) BAT AB product			Placebo (0.9% Saline)		
	Number of Subjects	Mean Percent Muscle Function	SD	Number of Subjects	Mean Percent Muscle Function	SD
Day 3	5	97.6	5.6	5	49.6	14.4
Day 4	5	98.2	7.0	5	36.6	12.5
Day 7	5	100.0	8.2	5	27.2	10.6
Day 14	5	99.8	4.5	5	31.6	14.6
Day 21	5	102.8	5.8	5	43.0	14.4
Day 28	5	99.0	4.6	5	50.8	13.5

SD = Standard Deviation.

Figure 14: Percent Muscle Function Over Time (CMAP M Wave Amplitudes) of the EDB Muscle Following Exposure to Myobloc® (BoNT Serotype B) and Previously Marketed Aventis Pasteur (Connaught) BAT AB Product



13.1.2.2 Study BT-002 Stage B: Botulism Antitoxin Effects on Paralysis Induced by Type A and Type B Botulinum Toxins in the Extensor Digitorum Brevis Muscle

Clinical trial BT-002 Stage B was a single-center, double-blind, randomized, parallel arm study to assess the safety and PDs of one vial of H-BAT (n =16) or placebo control (0.9% saline) (n = 10) following IV administration in normal healthy adult subjects. The primary objective of the study was to evaluate the effect of H-BAT in preventing paralysis of the EDB muscle following administration of BoNT serotypes A or B (Botox® or Myobloc®, respectively). The secondary objective was to evaluate the safety of H-BAT in healthy human subjects.

The effect of H-BAT on paralysis induced by BoNTs serotype A and B was evaluated based on the ability of H-BAT to neutralize BoNT serotypes A and B in the EDB model. The principle effect of exposure to botulism in humans is muscle paralysis therefore, inhibition of muscle paralysis induced by BoNT serotype A (Botox®) or BoNT serotype B (Myobloc®) was used as a surrogate endpoint to demonstrate the effectiveness of H-BAT in humans. The preservation of function of the EDB muscle in the subjects left and right foot was evaluated as percent muscle function based on the EDB CMAP M wave amplitude and area as recorded following bilateral peroneal motor nerve conduction.

Administration of H-BAT 24 hours prior to BoNT exposure prevented subjects from experiencing a decrease in muscle function following injection of BoNT serotypes A and B (Botox® and Myobloc®, respectively). Subjects receiving the placebo control demonstrated a loss of > 50% muscle function within three days of exposure to both BoNT serotype A (Table 46; Figure 11) and serotype B (Table 47; Figure 12). In the H-BAT treatment arm, muscle function was stable over time, indicating that the antitoxin was effective at preserving muscle function for up to 28 days following exposure to both BoNTs. By longitudinal analysis of variance model, there was a significant decrease of percent muscle function of EDB muscle in the placebo arm as compared to the H-BAT treatment arm over time ($p < 0.05$).

Table 46: Mean of the Percent Muscle Function (CMAP M Wave Amplitudes) of the EDB Muscle Following Exposure to Botox® (BoNT Serotype A) and H-BAT

Study Visit	H-BAT			Placebo (0.9% Saline)		
	Number of Subjects	Mean Percent Muscle Function	SD	Number of Subjects	Mean Percent Muscle Function	SD
Day 3	15	99	10	10	50	12
Day 4	15	100	6	10	41	10
Day 7	15	99	8	10	33	9
Day 14	15	94	12	10	30	11
Day 21	15	97	12	10	31	10
Day 28	15	98	11	10	30	10

SD = Standard Deviation.

Table 47: Mean of the Percent Muscle Function (CMAP M Wave Amplitudes) of the EDB Muscle Following Exposure to Myobloc® (BoNT Serotype B) and H-BAT

Study Visit	H-BAT			Placebo (0.9% Saline)		
	Number of Subjects	Mean Percent Muscle Function	SD	Number of Subjects	Mean Percent Muscle Function	SD
Day 3	15	96	12	10	41	20
Day 4	15	97	10	10	34	18
Day 7	15	93	20	10	27	13
Day 14	15	96	10	10	27	11
Day 21	15	99	9	10	35	14
Day 28	15	98	10	10	43	14

13.2 Comparison and Analyses of Pharmacokinetic Results Across Studies

13.2.1 Non-compartmental PK Comparison and Analysis

A comparison of the non-compartmental PKs for H-BAT serotype A in un-intoxicated humans, guinea pigs and Rhesus macaques can be found in Table 48.

Table 48: Interspecies Comparison of Pharmacokinetic Parameters (Mean) for Botulism Antitoxin Serotype A after Administration of 1x the Scaled Human Dose of H-BAT

Serotype	Species	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/kg)	Vd (mL/kg)
A	Guinea pig	10.00	10.30	2.92	0.17	6.24	14.47	130.20
A	Rhesus macaque	N/A	9.70	2.86	0.50	3.02	13.10	57.20
A	Human	21.40	26.00	2.69	0.70	8.64	3.99	49.48

Human data from BT-001; Guinea pig data from BBRC 684; Rhesus macaque data from LBERI 056.

A comparison of the non-compartmental PKs for H-BAT serotypes B through G in un-intoxicated humans and guinea pigs can be found in Table 49.

Table 49: Interspecies Comparisons of Pharmacokinetic Parameters (Mean) for Botulism Antitoxin Serotype B through G after Administration of 1x the Scaled Human Dose of H-BAT

Antitoxin Serotype	Species	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/kg)	Vd (mL/kg)
B	Human	27.16	29.30	1.90	0.89	34.20	2.67	130.71
	Guinea pig	8.18	8.39	1.42	0.17	15.30	11.93	263.94
C	Human	36.63	37.34	2.26	1.90	29.60	1.96	82.53
	Guinea pig	6.97	7.98	1.86	0.17	4.84	12.80	89.29
D	Human	5.58	7.62	0.81	0.89	7.51	1.86	19.93
	Guinea pig	3.77	3.97	1.17	0.17	3.31	5.25	25.09
E	Human	6.65	7.16	0.94	0.52	7.75	17.01	192.82
	Guinea pig	2.15	2.25	0.56	0.17	2.81	67.12	271.88
F	Human	29.12	31.40	2.37	1.07	14.10	2.30	46.44
	Guinea pig	7.68	7.83	1.79	0.17	10.40	11.94	179.47
G	Human	6.33	7.05	0.59	1.28	11.70	2.03	32.27
	Guinea pig	1.69	1.73	0.48	0.17	5.54	10.13	81.00

Human data from BT-001; Guinea pig data from BBRC 684.

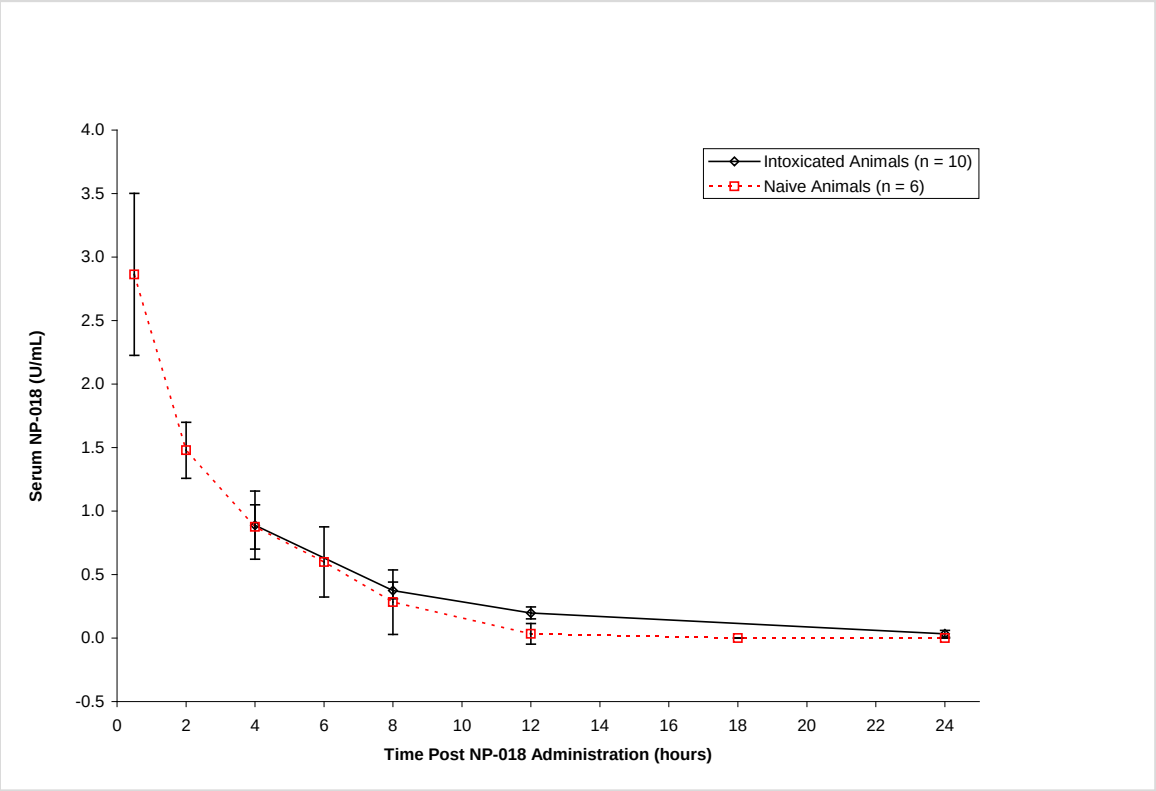
The various PK parameters in the Rhesus macaque were only determined for serotype A antitoxin therefore, non-compartmental PKs for H-BAT serotypes B through G have not been provided for the un-intoxicated or intoxicated Rhesus macaque model.

A comparison of the PK parameters between un-intoxicated (LBERI 056) and BoNT serotype A intoxicated (LBERI 061) Rhesus macaques can be found in Table 50 and Figure 15. The PK profile for both groups of animals appeared to be similar, indicating that the presence of BoNT at 4x $NHPLD_{50}$/kg had little effect on the various antitoxin PK parameters. This result is not unexpected given the huge excess of H-BAT administered compared to the amount BoNT injected (> 14,000 times).

Table 50: Pharmacokinetic Parameters (Mean and SD) for Antitoxin Serotype A in Rhesus Macaques Following IV Administration of 1.0x the Scaled Human Dose of H-BAT

Study Identifier	Treatment Group	$AUC_{0-\infty}$ (U*hr/mL)	C_{max} (U/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	CL (mL/hr/kg)	Vd (mL/kg)
LBERI 056	Un-intoxicated	9.7 (1.6)	2.9 (0.6)	0.5 (0)	3.0 (0.8)	13.1 (3.2)	57.2 (21.9)
LBERI 061	BoNT Serotype A Intoxicated	11.1 (3.5)	0.9 (0.3)	4 (o)	4.7 (1.2)	13.5 (2.9)	94.2 (31.9)

Figure 15: Serum Concentration of Botulism Antitoxin Serotype A in Un-intoxicated (LBERI 056) and Botulinum Neurotoxin Serotype A (LBERI 061) Intoxicated Rhesus Macaques



13.3 H-BAT Special Studies

13.3.1 Immunogenicity

All exogenous proteins, including H-BAT, have the potential to cause antibody formation. The development of ATA has the potential to impact the clinical safety (hypersensitivity reactions) and efficacy of protein therapeutics. Therefore, all subjects in clinical trials BT-001 and BT-002B were tested for immunogenicity against H-BAT at baseline (prior to dosing) and at the end of the studies using a validated immunogenicity assay. The immunogenicity testing was performed to detect anti-H-BAT antibodies in subjects dosed with either one or two vials of H-BAT. Samples were screened with a validated assay using the Biacore T100 instrument to determine reactivity. Reactive samples were tested to characterize the antibody profile (IgG, IgM, IgE and IgA). The assay did not produce quantitative levels of anti-H-BAT antibodies.

Of the 56 healthy subjects receiving H-BAT during clinical trials BT-001 and BT-002B, 17 subjects (30.4%) had anti-H-BAT antibodies at baseline before receiving study drug. The existence of anti-H-BAT antibodies in these subjects was likely due to previous environmental exposure to equine antigens. At the completion of the clinical trials following exposure to H-BAT, 28 (50%) subjects had anti-H-BAT antibodies. Therefore, 11 subjects (19.6%) developed anti-H-BAT antibodies (IgG, IgA, IgM) during the course of these clinical trials.

One subject from each clinical trial experienced a moderate allergic reaction during the administration of H-BAT. Both subjects were negative for anti-H-BAT antibodies at baseline and at the end of their respective studies. Therefore, the allergic reactions experienced by these subjects did not correspond to the presence of anti-H-BAT antibodies at baseline before H-BAT administration, nor did they did appear to develop anti-H-BAT antibodies in response to H-BAT administration.

The impact of anti-H-BAT antibodies in the efficacy of H-BAT could not be determined.

13.3.1.1 Study BT-001: Pharmacokinetics of a Heptavalent Equine-Derived Botulinum Antitoxin

In clinical trial BT-001, 21 healthy subjects (of 40, 53%) treated with either one or two vials of H-BAT tested positive for anti-H-BAT antibodies 28 days post-dosing (Table 51). Fourteen of these subjects were originally found to be reactive at baseline. Of the subjects testing positive, all developed IgG and IgA anti-H-BAT antibodies. No IgE anti-H-BAT antibodies were detected. There were no differences in the number of subjects testing positive post-dosing between subjects receiving one vial and two vials of H-BAT.

Table 51: Immunogenicity Testing Results for Clinical Trial BT-001

Time Point	Screening Assay		Confirmatory Assay				
	Negative	Reactive	Confirmed	IgG	IgM	IgE	IgA
Baseline	26 (65%)	14 (35%)	14 (100%)	14 (35%)	0	0	1 (3%)
Day 28	19 (47%)	21 (53%)	21 (100%)	21 (53%)	0	0	4 (10%)

IgA = Immune Globulin A; IgE =Immune Globulin E; IgG = Immune Globulin G; IgM = Immune Globulin M.

One subject from this clinical trial experienced a moderate allergic reaction during the administration of H-BAT. The subject was negative for anti-H-BAT antibodies at baseline and at the end of the study. Therefore, the allergic reaction experienced by this subject did not correspond to the presence of anti-H-BAT antibodies at baseline before H-BAT administration and the subject did not appear to develop anti-H-BAT antibodies in response to H-BAT administration.

13.3.1.2 Study BT-002 Stage A: Botulism Antitoxin Effects on Paralysis Induced by Type A and Type B Botulinum Toxins in the Extensor Digitorum Brevis Muscle

In clinical trial BT-002A, three subjects (of 5, 60%) receiving a single IV infusion of previously marketed Aventis Pasteur (Connaught) BAT AB product were found to be positive for anti-BAT AB antibodies post-dosing (Table 52). One subject in the control group (of 5, 20%) was originally found reactive at baseline. Of the four subjects with anti-BAT AB antibodies, all had IgG antibodies. None of the subjects had antibodies for IgA, IgE or IgM.

Table 52: Immunogenicity Testing Results for Clinical Trial BT-002 Stage A

Time Point	Screening Assay		Confirmatory Assay				
	Negative	Reactive	Confirmed	IgG	IgM	IgE	IgA
Baseline	9 (90%)	1 (10%)	1 (100%)	1 (10%)	0	0	0
Day 28	6 (60%)	4 (40%)	4 (100%)	4 (40%)	0	0	0

IgA = Immune Globulin A; IgE =Immune Globulin E; IgG = Immune Globulin G; IgM = Immune Globulin M.

13.3.1.3 Study BT-002 Stage B: Botulism Antitoxin Effects on Paralysis Induced by Type A and Type B Botulinum Toxins in the Extensor Digitorum Brevis Muscle

In clinical trial BT-002 Stage B, seven subjects (of 16, 44%) receiving a single IV infusion of H-BAT were found to be positive for anti-H-BAT antibodies post-dosing (Table 53). Three of these subjects were originally found reactive at baseline. Of the seven subjects, four subjects developed a primary immune response to H-BAT. All four subjects developed IgG antibodies. Two of the four subjects also developed antibodies specific for IgM. None of the subjects developed antibodies for IgA or IgE.

Table 53: Immunogenicity Testing Results for Clinical Trial BT-002 Stage B

Time Point	Screening Assay		Confirmatory Assay				
	Negative	Reactive	Confirmed	IgG	IgM	IgE	IgA
Baseline	13 (81%)	3 (19%)	3 (100%)	3 (19%)	0	0	1 (6%)
Day 28	9 (56%)	7 (44%)	7 (100%)	7 (44%)	2 (13%)	0	0

IgA = Immune Globulin A; IgE =Immune Globulin E; IgG = Immune Globulin G; IgM = Immune Globulin M.

One subject from this clinical trial experienced a moderate allergic reaction during the administration of H-BAT. The subject was negative for anti-H-BAT antibodies at baseline and at the end of the study. Therefore, the allergic reaction experienced by this subject did not correspond to the presence of anti-H-BAT antibodies at baseline before H-BAT administration and the subject did not appear to develop anti-H-BAT antibodies in response to H-BAT administration.

13.4 Animal Rule Essential Elements

Table 54: Summary of Animal Rule Essential Elements

Data Element	Animal		Human
	Guinea Pig	**Rhesus Macaque**	
A. Characteristics of the CBRN Agent that Influence the Disease or Condition			
1. Challenge agent	Serotype A – Hall Serotype B – Okra Serotype C – Stockholm Serotype D – 5995 (FDA) Serotype E – Alaska Serotype F – Langland Serotype G – G89	Serotype A – Hall	Serotype A – Hall Serotype B – Bean
2. Pathogenic determinants	BoNT targets the SNARE protein complex at the peripheral neuromuscular junction, blocking acetylcholine release	BoNT targets the SNARE protein complex at the peripheral neuromuscular junction, blocking acetylcholine release	BoNT targets the SNARE protein complex at the peripheral neuromuscular junction, blocking acetylcholine release
3. Route of exposure	Intramuscular	Intravenous	Foodborne Wound colonization Intestinal colonization Inhalation Iatrogenic
4. Quantification of exposure	Mouse bioassay	Mouse bioassay	Mouse bioassay
B. Host Susceptibility and Response to Etiologic Agent			
	Susceptible to all seven BoNT serotypes	Susceptible to all seven BoNT serotypes	Susceptible to all seven BoNT serotypes
C. Natural History of Disease: Pathophysiologic Comparability			
1. Time to onset of disease/ condition	Dependent on neurotoxin serotype, dose and route of exposure.	Dependent on neurotoxin serotype, dose and route of exposure.	Dependent on neurotoxin serotype, dose and route of exposure.
2. Time course of progression of disease/condition	Dependent on neurotoxin serotype, dose and route of exposure.	Dependent on neurotoxin serotype, dose and route of exposure.	Dependent on neurotoxin serotype, dose and route of exposure.
3. Manifestations (Signs and symptoms)	Lethargy Weak limbs Noticeable change in breathing Salivation Lacrimation Force abdominal Respiration Total paralysis	Ptosis Muscular weakness Respiratory distress Oral and nasal discharge	Fatigue Dysphagia Ptosis Dyspnea Arm weakness Leg weakness

Data Element	Animal		Human
	Guinea Pig	**Rhesus Macaque**	
D. Trigger for intervention			
	Right hind limb weakness Weak limbs Salivation Lacrimation Noticeable change in breathing	Ptosis Muscular weakness Respiratory distress	Suspected or confirmed botulism
E. Characterization of the Medical Intervention			
1. Product class	Equine-derived hyperimmune product		
2. Mechanism of action	Passive immunization		
3. *In vitro* activity	Not Applicable		
4. Activity in disease/condition of similar pathophysiology	H-BAT has not been tested in animal models of inhalation, wound or intestinal colonization botulism.		
5. PK in unaffected animals/humans	Varies with antitoxin serotype	Only antitoxin serotype A measured	Varies with antitoxin serotype measured
6. PK/PD in affected animals/humans	Not tested	Tested for BoNT serotype A	Tested for BoNT serotypes A and B
7. PK interactions with medical products likely to be used concomitantly	Not tested	Not tested	Not tested
8. Synergy or antagonism of medical products likely to be used in combination	Not tested	Not tested	Live attenuated virus vaccines Blood glucose monitoring systems involving glucose dehydrogenase pyrroloquinoline-quinone (GDH-PQQ)
F. Design considerations for animal efficacy studies			
1. Endpoints	Primary Survival Secondary Time to death Incidence of severe clinical signs Clinical course Recovery time Clinical severity score	Primary Survival Secondary Time to death Incidence of severe clinical signs Clinical course Recovery time Clinical severity score	Primary Duration of hospitalization Duration of ICU stay Duration on mechanical ventilation Secondary Survival

Data Element	Animal		Human
	Guinea Pig	**Rhesus Macaque**	
2. Timing of intervention	Four consecutive moderate or severe clinical signs of botulism including right hind limb weakness, weak limbs, salivation, lacrimation, and noticeable change in breathing rate or pattern	First moderate or severe clinical sign of botulism including ptosis, muscular weakness, and respiratory distress	H-BAT will be administered to patients with suspected or confirmed botulism
3. Route of administration	Intravenous	Intravenous	Intravenous
4. Dosing regimen	1x scaled human dose	1x scaled human dose	Label Claim for one vial of H-BAT Serotype A >4,500 U Serotype B >3,300 U Serotype C >3,000 U Serotype D >600 U Serotype E >5,100 U Serotype F >3,000 U Serotype G >600 U

G. Human Safety Information

H-BAT has been administered to 56 healthy subjects in two clinical trials (BT-001 and BT-002B). In both trials H-BAT was shown to be safe and well tolerated. One subject from each trial was withdrawn due to hypersensitivity-related AEs even though they were negative for skin sensitivity at screening and neither was positive for immunogenicity at the beginning or end of their respective trials.

H-BAT has also been administered to an additional 148 patients (as of December 31, 2011) under the CDC's expanded access program (BB-IND 6750). Eighteen patients experienced adverse reactions related to drug administration; two patients experienced SAEs, and one patient experienced serum sickness.

13.5 Survival Rates in Pivotal Guinea Pig Efficacy Study BBRC 1180 for Serotypes B through G

Figure 16: Survival Rate for H-BAT and Placebo Control Groups for Serotype B in Pivotal Guinea Pig Efficacy Study BBRC 1180

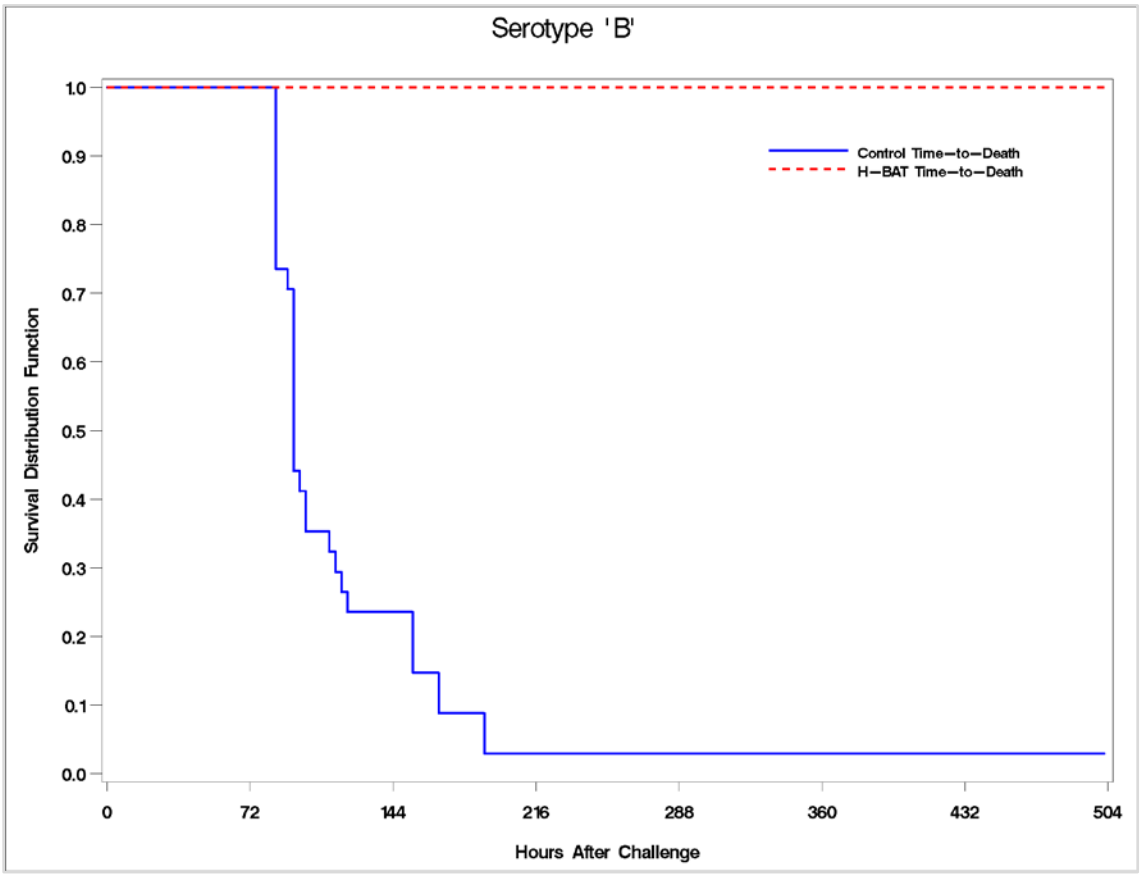


Figure 17: Survival Rate for H-BAT and Placebo Control Groups for Serotype C in Pivotal Guinea Pig Efficacy Study BBRC 1180

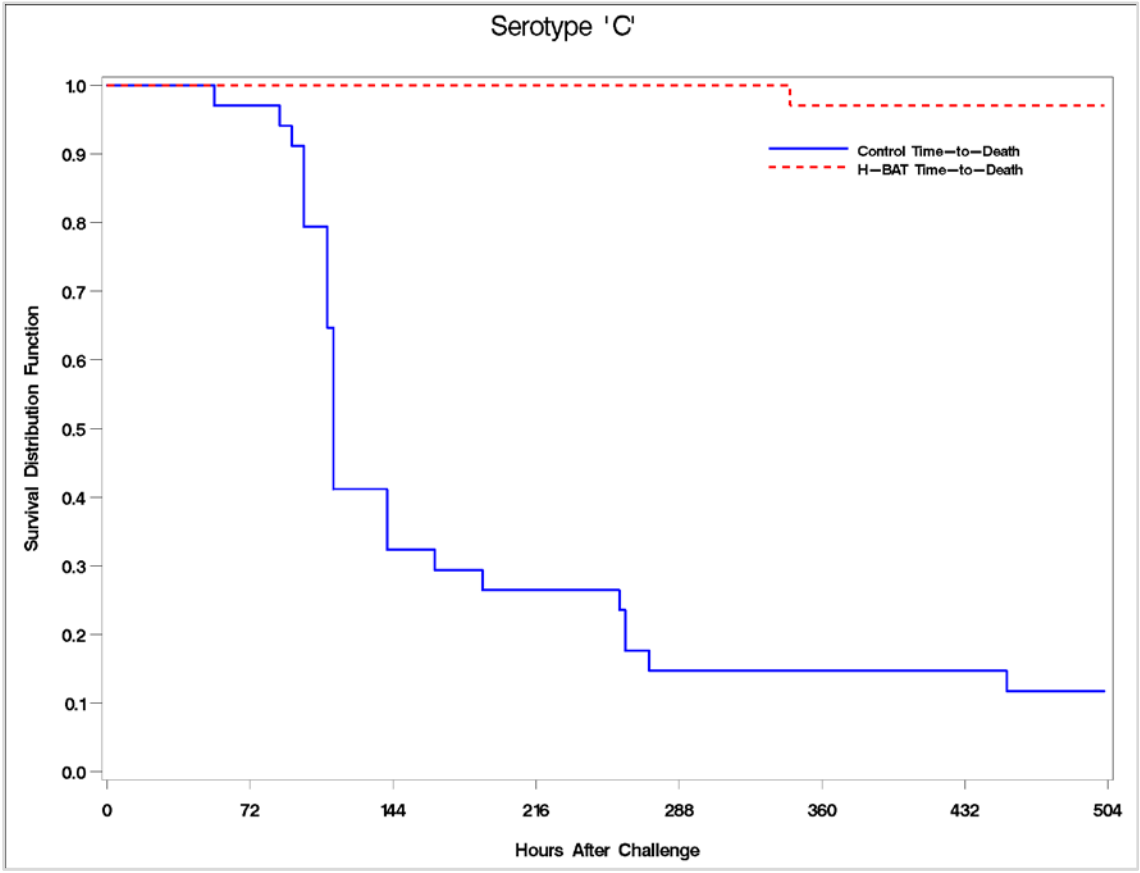


Figure 18: Survival Rate for H-BAT and Placebo Control Groups for Serotype D in Pivotal Guinea Pig Efficacy Study BBRC 1180

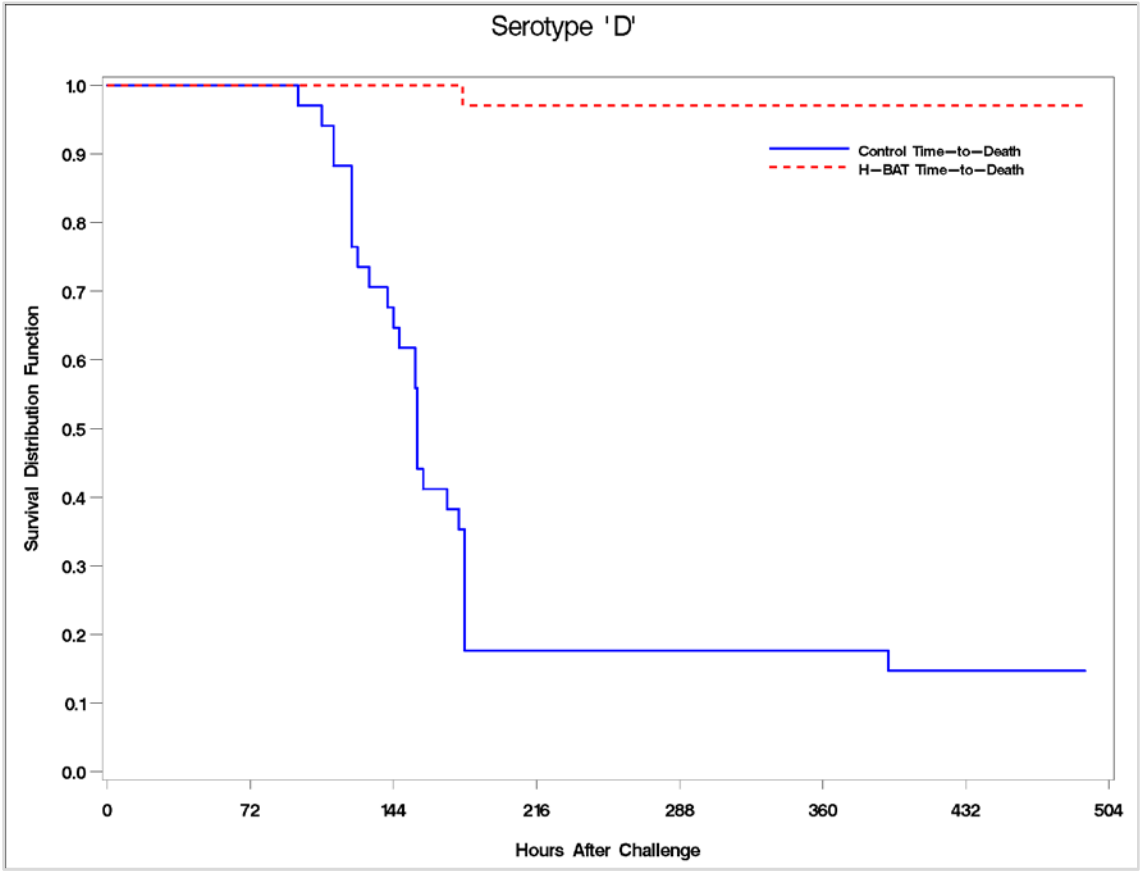


Figure 19: Survival Rate for H-BAT and Placebo Control Groups for Serotype E in Pivotal Guinea Pig Efficacy Study BBRC 1180

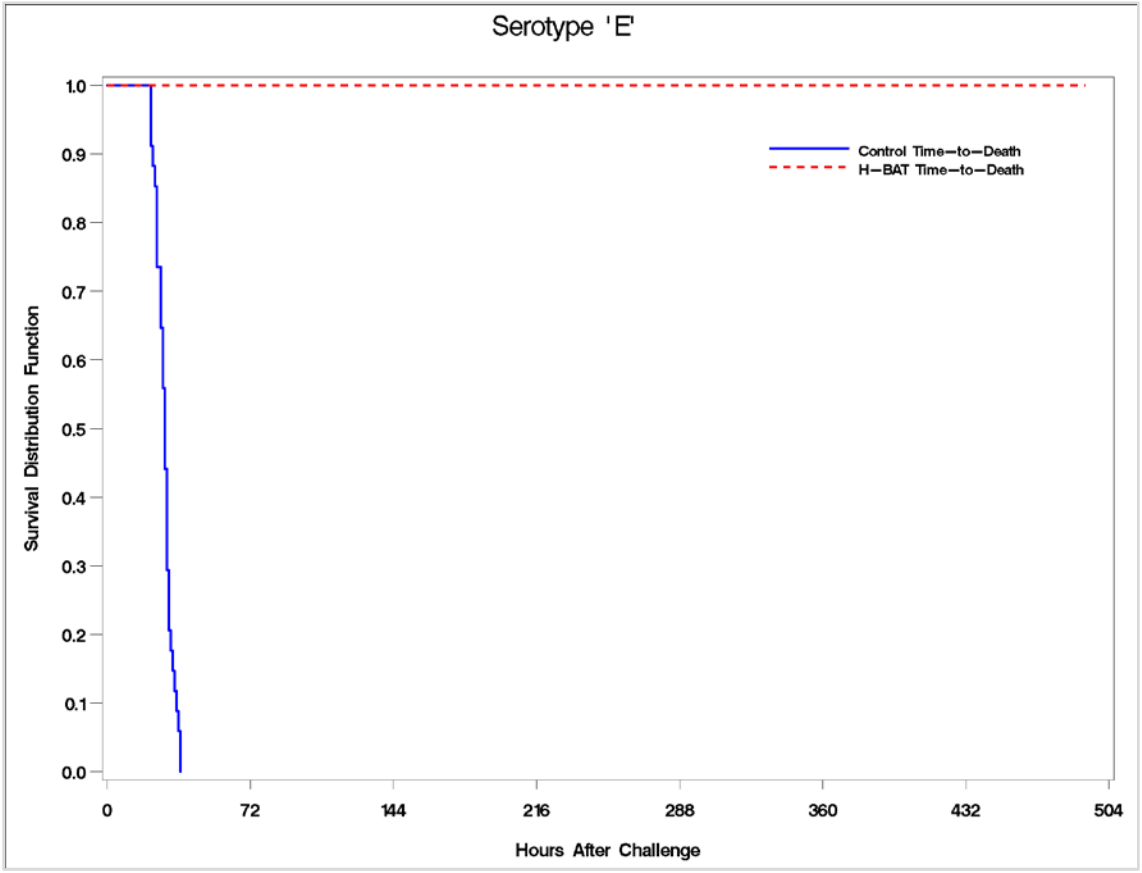


Figure 20: Survival Rate for H-BAT and Placebo Control Groups for Serotype F in Pivotal Guinea Pig Efficacy Study BBRC 1180

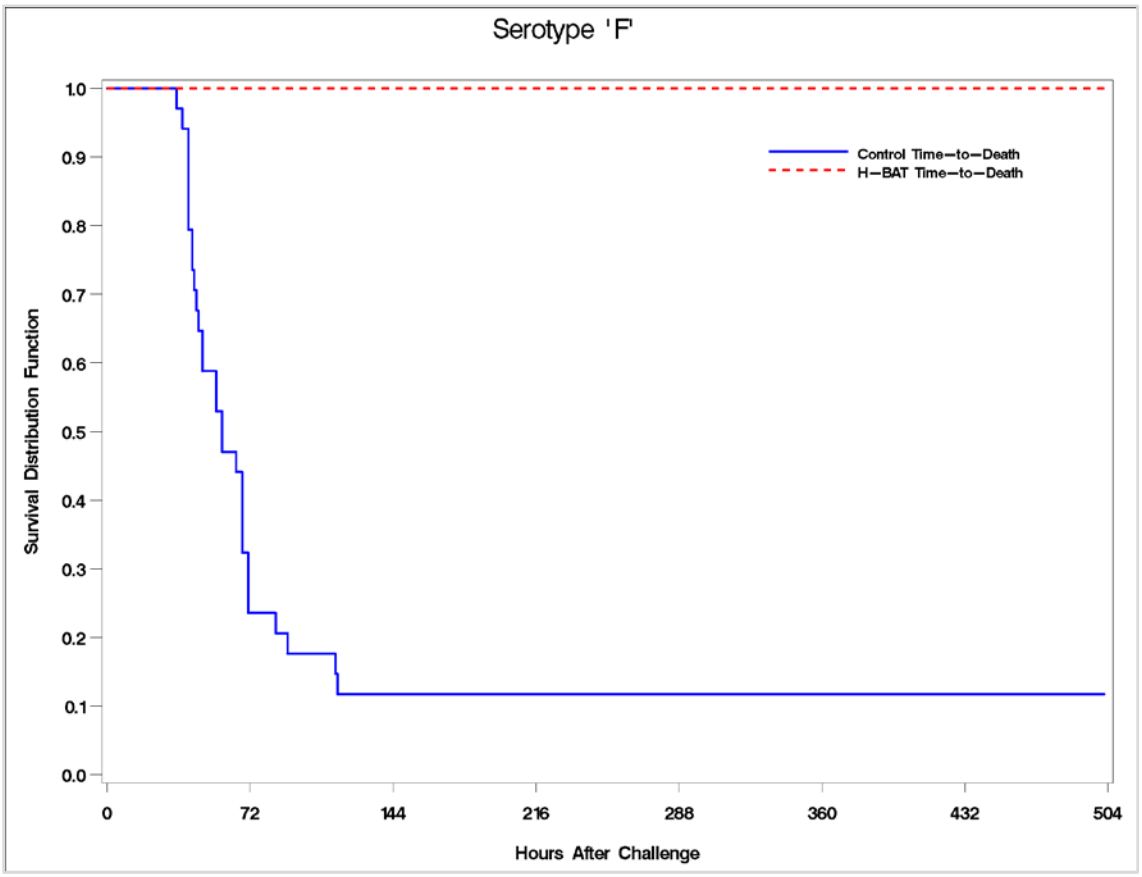
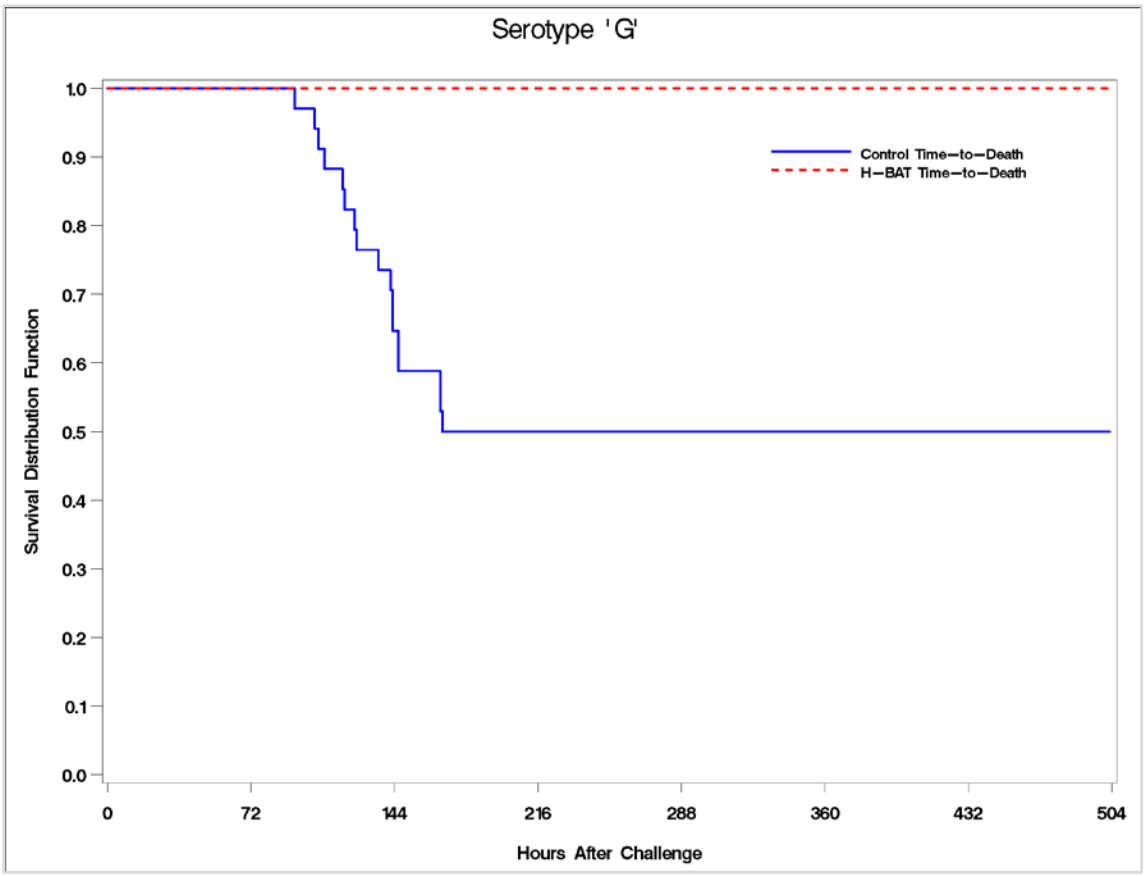


Figure 21: Survival Rate for H-BAT and Placebo Control Groups for Serotype G in Study BBRC 1180



13.6 Clinical Severity Scores in Guinea Pig Efficacy Study BRRC 1180 for Serotypes B through G

Figure 22: Clinical Severity Scores for H-BAT and Placebo Control Groups for Serotype B in Pivotal Guinea Pig Efficacy Study BBRC 1180

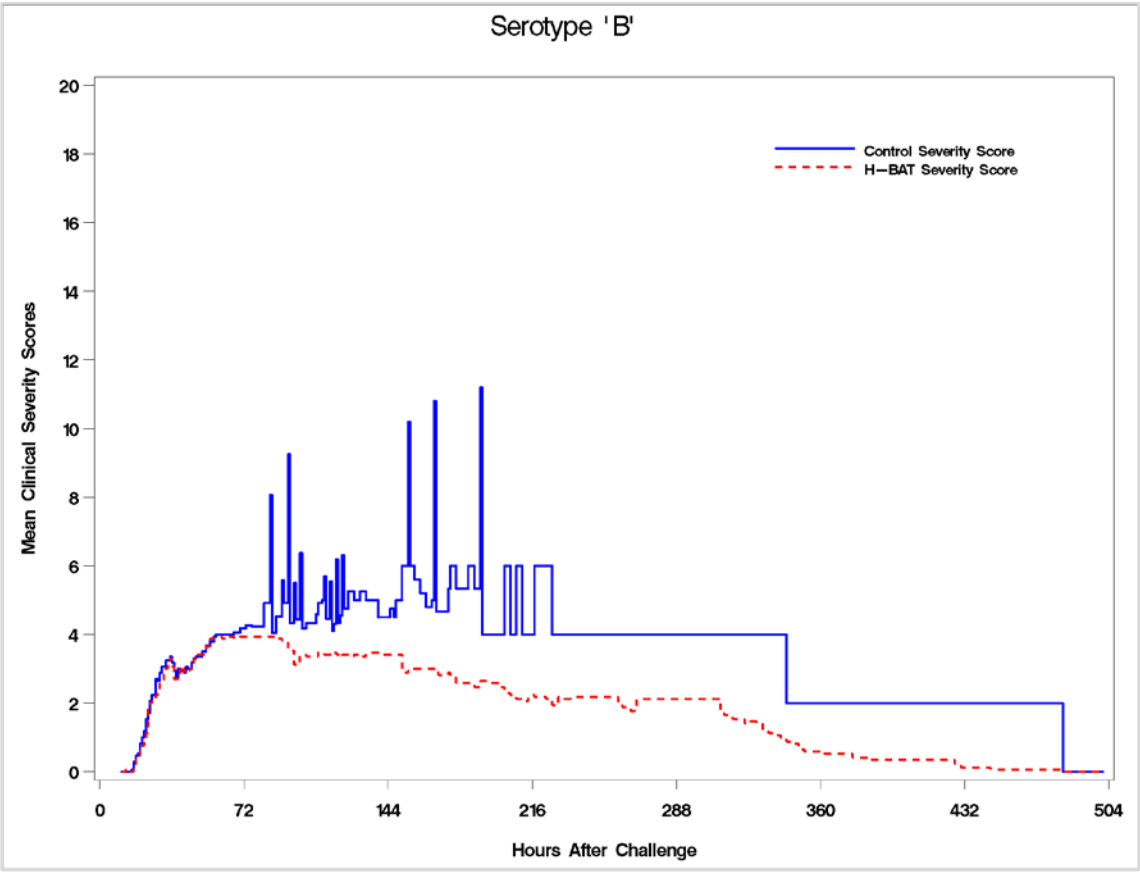


Figure 23: Clinical Severity Scores for H-BAT and Placebo Control Groups for Serotype C in Pivotal Guinea Pig Efficacy Study BBRC 1180

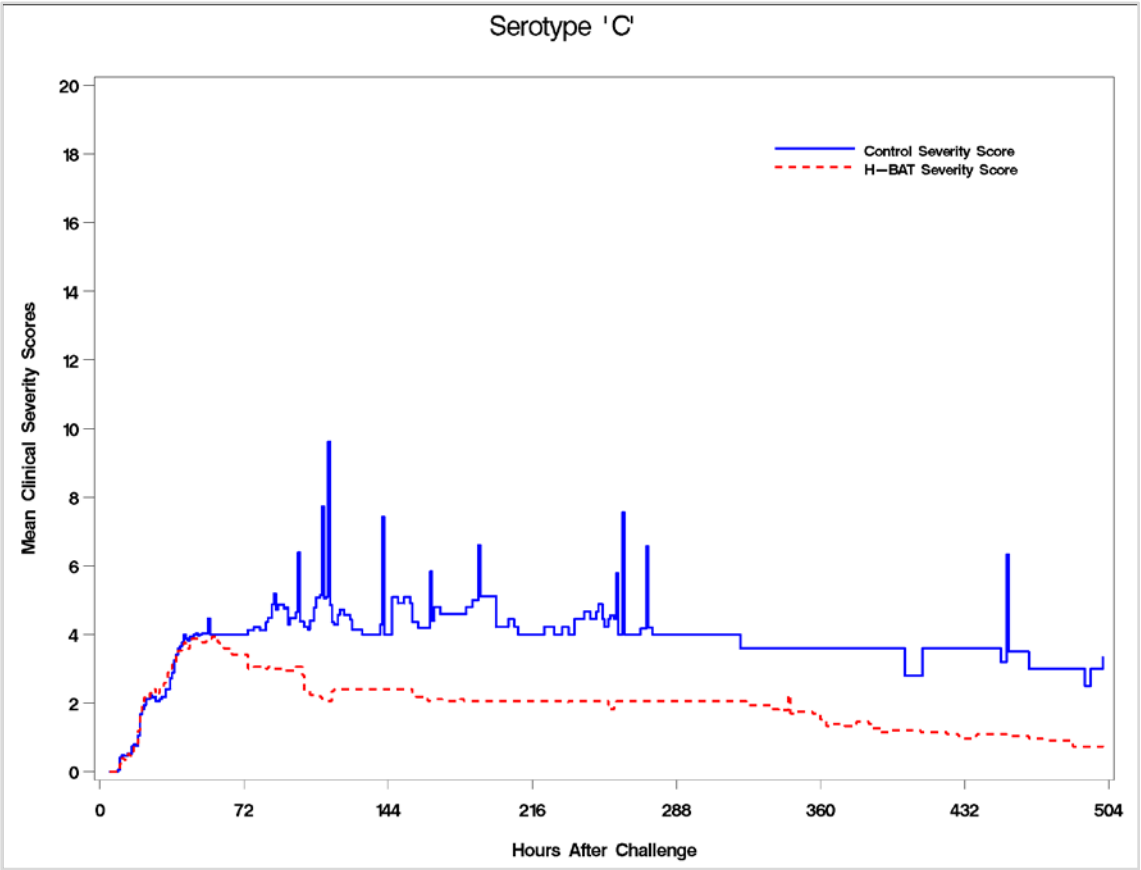


Figure 24: Clinical Severity Scores for H-BAT and Placebo Control Groups for Serotype D in Pivotal Guinea Pig Efficacy Study BBRC 1180

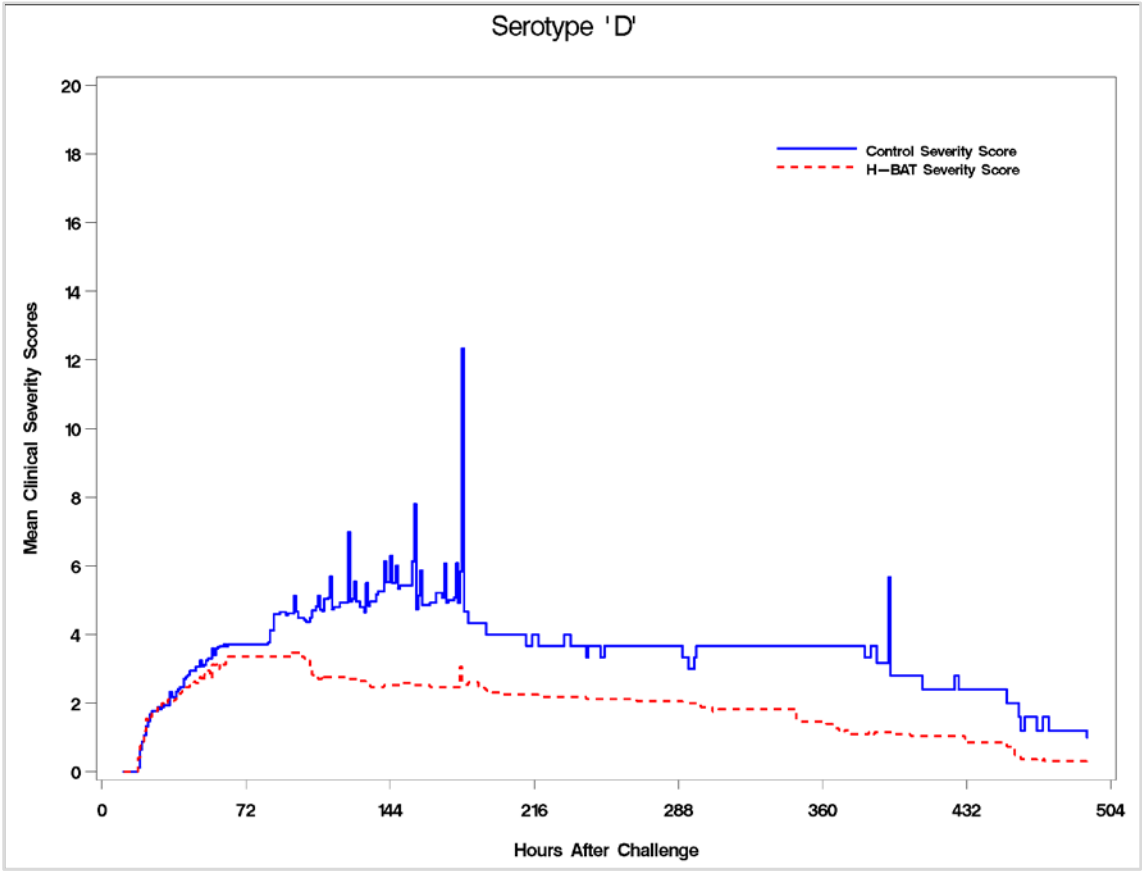


Figure 25: Clinical Severity Scores for H-BAT and Placebo Control Groups for Serotype E in Pivotal Guinea Pig Efficacy Study BBRC 1180

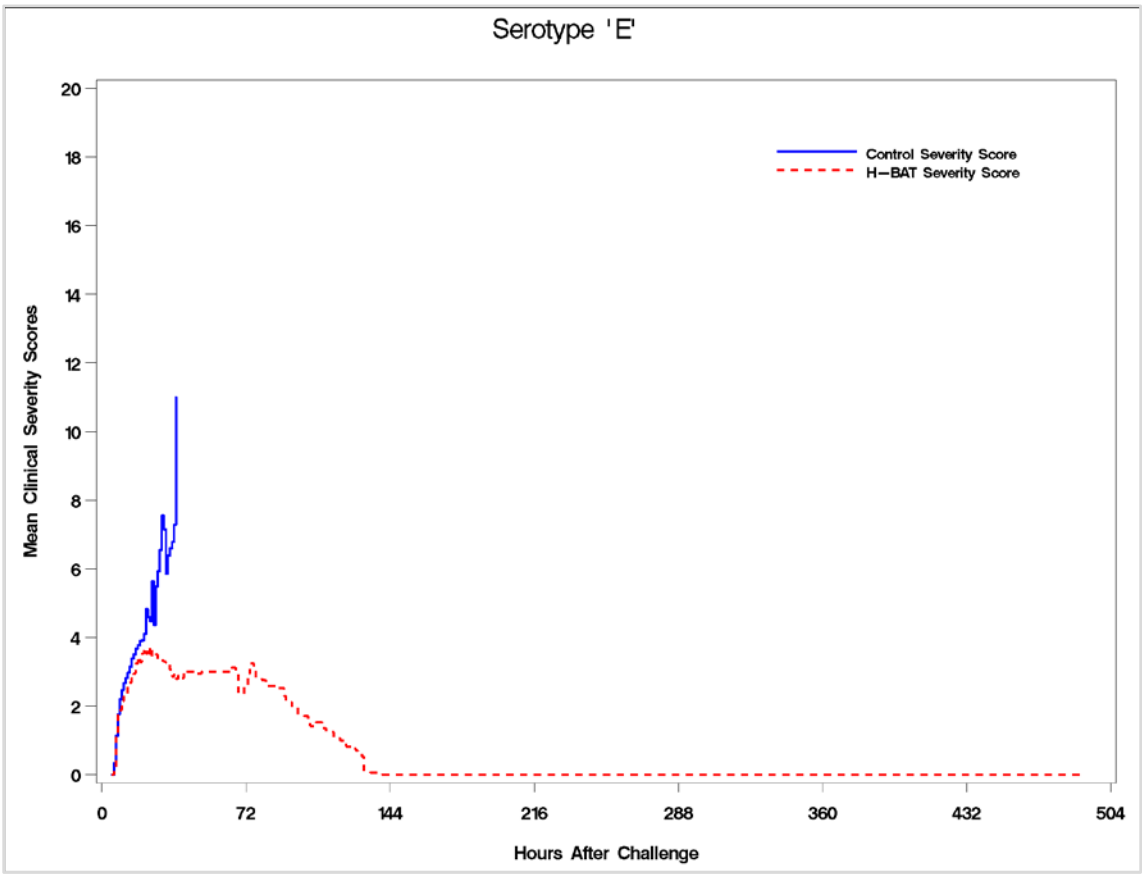


Figure 26: Clinical Severity Scores for H-BAT and Placebo Control Groups for Serotype F in Pivotal Guinea Pig Efficacy Study BBRC 1180

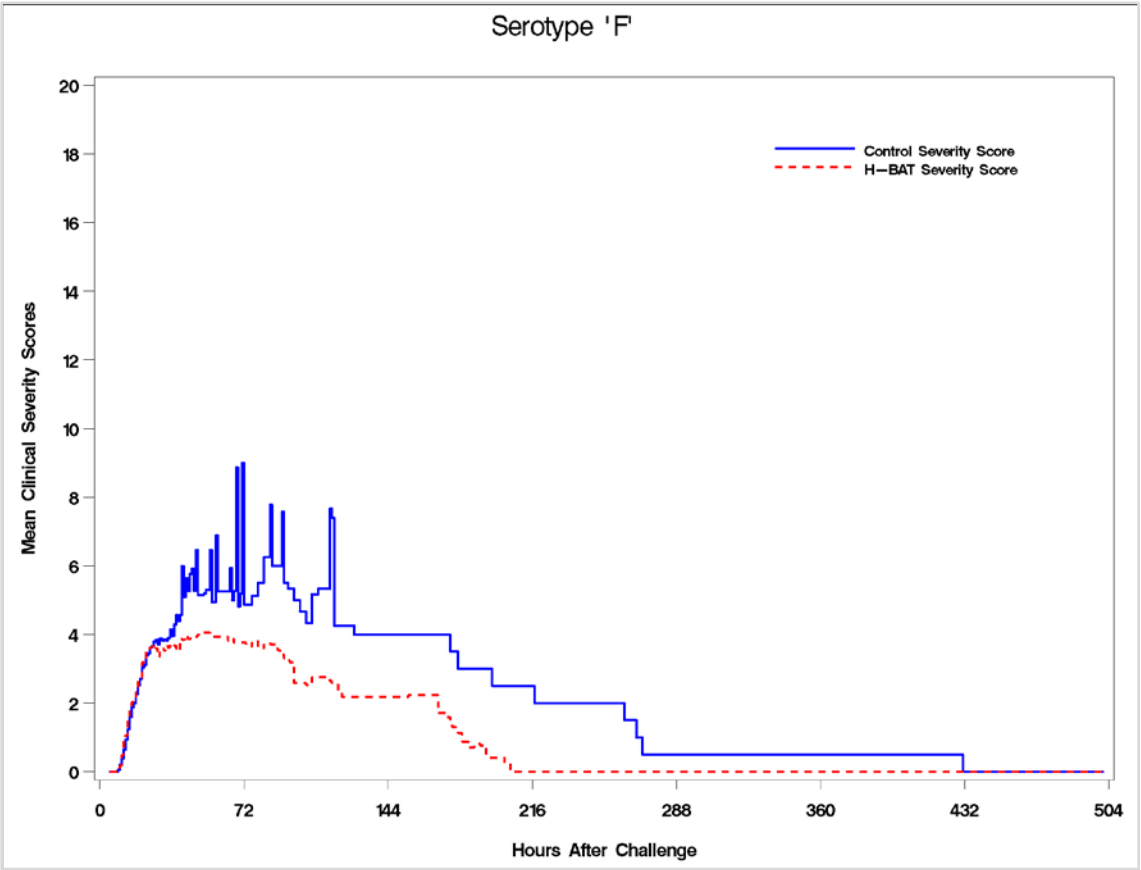
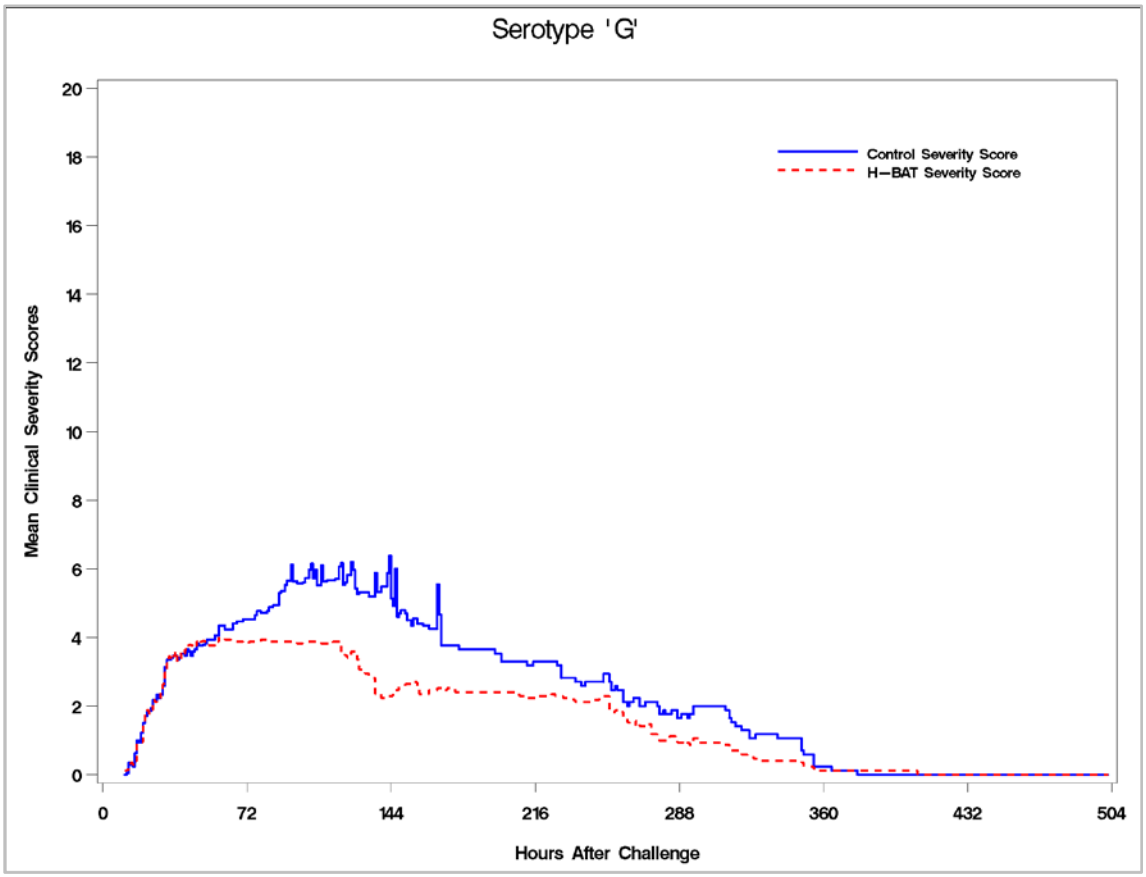



Figure 27: Clinical Severity Scores for H-BAT and Placebo Control Groups for Serotype G in Pivotal Guinea Pig Efficacy Study BBRC 1180