Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research

To:	File for STN 125462
From:	Robert W. Fisher, DH, OBRR, HFM-345
Through:	Michael Kennedy, Team Leader LPD, DH, OBRR, HFM-345
CC:	Nannette Cagungun, RPM, RPMB, DBA, OBRR, HFM-380
Applicant:	Cangene Corporation
Product:	Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-(Equine); BAT
Subject:	Final review, STN 125462/0 guinea pig studies

Recommendation:

Approval.

Executive Summary:

Cangene Corporation (Cangene) has submitted a Biologics License Application for Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-Equine with an indication for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin serotypes A, B, C, D, E, F, or G in adults and pediatric patients. The efficacy of BAT has been demonstrated in two animal models: the guinea pig intramuscular challenge model (reviewed in this memorandum) and the rhesus macaque intravenous challenge model. The application includes study reports for guinea pig botulism model development and a pivotal efficacy study using the guinea pig intramuscular botulinum neurotoxin intoxication model. Cangene's model development data was found to be consistent with the 'Essential Data Elements of an Animal Model' described in the 2009 "Draft Guidance for Industry: Animal Models- Essential Elements to Address Efficacy Under the Animal Rule". The pivotal efficacy study administered a 1x scaled human dose of BAT (based on body weight) as a single intravenous infusion to guinea pigs challenged with a 1.5x lethal dose 50% quantity of botulinum neurotoxin serotype A, B, C, D, E, F, or G and that were exhibiting clinical signs of botulism. BAT administered at the 1x scaled human dose statistically increased survival and decreased the incidence of severe clinical signs compared to placebo in guinea pigs challenged with any of the seven botulinum neurotoxin serotypes. The model development and efficacy data from the guinea pig model indicate that BAT is reasonably likely to provide clinical benefit in humans with botulism.

Background:

1. STN 125462/0 is an eCTD format original Biologics License Application (BLA) for Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-Equine (BAT) submitted by Cangene Corporation.
    a. This submission was received at DCC on 20 September 2012 and a chair assigned on 21 September 2012.
    b. Fast Track designation for BAT development was granted on 18 January 2007.
    c. Orphan drug status was granted for 'treatment of botulism' on 29 June 2011.
    d. The BLA was filed on 12 November 2013.
    e. Priority review was requested in the BLA and was granted due to the unmet medical need for a product to treat botulism.
    f. This submission was the subject of a Blood Products Advisory Committee meeting in February 2013.
    g. The Action Due Date is 22 March 2013.
2. BAT is a polyclonal antibody preparation manufactured from the plasma of horses immunized with botulinum neurotoxin serotypes A, B, C, D, E, F, G or with toxoids prepared from BoNT serotypes.
    a. It contains both $F(ab')_2$ and $F(ab')_2$-related immune globulin fragments that bind to circulating BoNT and prevent internalization of the toxin and the resulting flaccid paralysis.
    b. Potency of the product is demonstrated via use of a mouse neutralization assay specific for each BoNT serotype.
3. Botulism is a rare paralytic illness triggered by intoxication with neurotoxins produced by *Clostridium botulinum* and related species such as *C. butyricum* and *C. baratii*. Naturally occurring botulism is rare, however the extreme potency of the botulinum neurotoxins (BoNT) in conjunction with the ubiquitous nature of the *Clostridium* species and relative ease of producing purified toxin has justified the inclusion of BoNT on the CDC category A list with regards to use as a potential biowarfare agent. Naturally occurring botulism usually involves contaminated food or colonization of wounds or the gastrointestinal tract. Bioterrorism scenarios usually involve ingestion of deliberately contaminated food; however lethal BoNT aerosol exposures have been demonstrated in animal models.
    a. Food-borne botulism is caused when food is contaminated with spores of *Clostridium* spp. and subsequently stored under anaerobic conditions. This usually involves inadequately cooked home-prepared canned foodstuffs. In 2010 only 8% of U.S. botulism cases were attributable to food-borne routes of exposure.
    b. Wound botulism is caused by colonization of deep wounds with spores of *Clostridium* spp. The anaerobic conditions allow germination of the spores and production of BoNT by vegetative bacteria. 15% of botulism cases in the U.S. occurring in 2010 were wound botulism.
    c. Intestinal colonization may occur when infants (typically under < 6 months) ingest *Clostridium* spp. spores. It is suspected that in these cases the normal intestinal flora has not developed to the degree necessary for resistance to *Clostridium* overgrowth. Adult intestinal colonization has also been described, but occurs at a much lower incidence and is typically associated with gastrointestinal tract abnormalities or antibiotic use that has disrupted the normal adult intestinal flora. The majority of botulism cases in the U.S. are attributable to infant botulism (76% in 2010).
    d. Inhalational botulism in humans has only been documented in one laboratory-associated exposure. Lyophilized, aerosolized BoNT was explored as an offensive biological warfare agent in World War II and numerous animal model experiments have demonstrated that a lethal dose may be delivered in this manner, albeit inefficiently compared to other routes.
    e. Iatrogenic exposures to BoNT have occurred due to overdoses of toxins as therapeutic agents. BoNT serotypes A and B (BoNT/A and BoNT/B) are licensed in the U.S. for

cosmetic use and for treating cervical dystonia, respectively. One recent case involved use of a non-licensed BoNT/A preparation and resulted in several lengthy hospitalizations.

4. Botulism toxins are zinc metalloproteases that can be categorized based on seven antigenically different serotypes (and are designated accordingly with letters A-G). They are produced as a single 150 kDa polypeptide, which undergoes proteolytic cleavage into a disulfide linked light chain (Lc) and heavy chain (Hc). The 50 kDa light chain contains the metalloprotease activity, while the 100 kDa heavy chain incorporates receptor binding and translocation domains.
    a. The neurotoxins are released from the bacteria as part of a multimeric protein complex containing hemagglutinin and other components. The accessory proteins do not contribute directly to BoNT toxicity, although they may stabilize the active toxin in the presence of low pH environments or proteases found in the stomach.
    b. The pathophysiology of BoNT is reasonably well understood. The BoNT heavy chain binds gangliosides or phosphatidylethanolamine on neurons and then interfaces with a protein receptor that triggers endocytosis. The heavy chain then undergoes a conformational shift due to the low endosomal pH and forms a transmembrane channel that allows the light chain to enter the cytosol. The fundamental mechanism of action is BoNT light chain mediated cleavage of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins in neurons. Loss of SNARE proteins prevents acetylcholine-containing vesicles from fusing with the plasma membrane at neuromuscular junctions thereby preventing neurotransmitter release and triggering a flaccid paralysis.
    c. While all BoNT serotypes are exceedingly potent neurotoxins, toxicity can vary widely between the serotypes. Humans are susceptible to all seven serotypes, although most naturally occurring cases of botulism are due to serotypes A, B, and E. Toxins of different serotypes exhibit specificity for different SNARE substrates and also demonstrate different kinetics in terms of recovery. BoNT-mediated SNARE cleavage can persist from day to months in an intoxicated neuron (Keller, Neale, Oyler, & Adler, 1999).
5. The pharmacokinetics of BoNT are not well understood and have been studied primarily with serotypes A and B in the intravenous rat model. Most of the toxin remains unbound in the circulation and is eliminated or metabolized with a half life of 200-400 minutes(Al-Saleem et al., 2008; Ravichandran et al., 2006). BoNT/A, B, and C have been shown to cross intestinal epithelial cells via transcytosis (Maksymowych & Simpson, 1998), suggesting a likely mechanism for intoxication via the oral route. The matrix in which the toxin is suspended can influence bioavailability (Cheng & Henderson, 2011), complicating experiments utilizing this exposure route.
6. Cangene submitted final study reports for 11 guinea pig studies. This total includes the initial dose ranging study (621-G005630), a confirmatory dose ranging/natural history study (670-G005630), a pilot oral challenge study (975-G005630), a PK study in unintoxicated guinea pigs (684-G005630), a post exposure prophylaxis study (731-G005630), a therapeutic efficacy pilot study using a 4x Guinea Pig Intramuscular Lethal Dose 50% ($GPIMLD_{50}$) toxin challenge (843-G005630), an additional dose ranging/natural history study (964-G005630), several pilot therapeutic efficacy pilot studies at a 1.5x $GPIMLD_{50}$ toxin challenge dose (993-G005630, 1005-G005630, 1124-G005630), and the pivotal efficacy study (1180-G005630).
    a. All studies involving BAT utilized the same lot of material (lot 2060401Y).
    b. BAT was delivered as a single dose infusion via a jugular vein catheter surgically implanted by the animal vendor.
7. Study 621-G005630 was the initial BoNT dose ranging study performed in guinea pigs.
    a. This was a Good Laboratories Practice (GLP; per 21 CFR Part 58) study designed to determine the $GPIMLD_{50}$ of BoNT serotypes A, B, C, D, E, F, and G. The study was initiated 10 November 2006 with the in-life portion completed by 19 January 2007, and was performed at Battelle Biomedical Research Center, West Jefferson, OH (BBRC). The

BoNT test material was obtained from the ----------(b)(4)---------- ---------- (serotypes A-E) or directly from -----(b)(4)----- (serotypes F and G). Partially purified BoNT Serotypes A-F were received as ----------(b)(4)----------. BoNT Serotype G was received as a solution in ----(b)(4)--- from the manufacturer. The toxins were diluted to form a working stock, aliquoted, and assigned a BBRC batch number. The original lot number, potency, and assigned batch numbers are indicated in Table 1.

i. The toxins were identified based on reactions with serotype specific antisera in the mouse neutralization assay. The potency of each lot of toxin was determined using the mouse potency assay, and purity assessed with ----(b)(4)----.

ii. A single lot of each BoNT serotype was used for this study (and for all subsequent studies as well).

iii. The challenge material was tested for potency using the mouse potency assay. In general there was good agreement with the historical mouse potency data for the toxin challenge material. Results ranged from 64 percent to 97 percent of the historical values, with coefficient of variations ranging from 3 percent to 40 percent. The highest variability was observed in the serotype F results. See Table 2.

b. The study system used male --(b)(4)-- guinea pigs (*Cavia porcellus*) from -----(b)(4)--- ---------- in the 400-500 gram range. See Table 3 for the challenge groups and doses used.

i. For BoNT serotypes A-E $GPIMLD_{50}$ values identified in BBRC task 97-51 (performed under contract to the Department of Defense's Joint Program Office for Biological Defense) were verified by intoxicating groups of 5 guinea pigs with 0.2-5x $GPIMLD_{50}$ delivered via intramuscular injection of 0.1 mL of the desired toxin concentration. Additional BoNT serotype E animals were injected with 0.52, 0.60, or 0.69x $GPIMLD_{50}$ due to the steep dose response observed with this serotype (per protocol amendment #5).

ii. For BoNT serotypes F and G a two stage study design was used, since no guinea pig data was available for these toxin serotypes. Stage one used 2 guinea pigs per group and toxin doses of 10-1000 $MIPLD_{50}$ to estimate a preliminary dose response curve. Stage 2 used five groups of 5 guinea pigs and dose ranges of 0.25-4x $GPIMLD_{50}$ for BoNT serotype F and 0.45-2.2x $GPIMLD_{50}$ for BoNT serotype G.

1. BoNT serotypes E and G were activated by proteolysis with trypsin before use.

iii. The study continued for 14 days post-intoxication. Weights were measured on the day prior to intoxication and twice weekly thereafter. Animals were observed twice daily during normal working hours for mild, moderate, and severe clinical signs.

1. Mild signs included ruffled fur and hind limb (local) paralysis.
2. Moderate signs included salivation, lacrimation, weak limbs, noticeable change in breathing rate or pattern, and droopy eyelids.
3. Severe signs included forced abdominal respirations and total paralysis.

iv. Euthanasia criteria was predefined and was applied in any animal exhibiting 20% or greater weight loss in conjunction with severe signs of intoxication, or any animal judged moribund by the life sciences technician, veterinarian, or study director.

c. A probit dose response model was used to analyze the lethality proportion as a function of the logarithm of toxin dose. See Figure 2 and Table 4. Other study endpoints included time to onset of the first clinical sign and time to death.

i. The $GPIMLD_{50}$ values calculated from study 621-G005630 were not significantly different than historical values for BoNT serotypes A and B. The values established for serotypes C, D, and E were lower by 60-70%. See Table 5.

ii. The dose response curves were extremely steep, and as expected the difference between the LD50 values and the LD99 values across all serotypes was slight. See Table 6.

iii. Observed clinical signs were serotype and dose dependent, as was the mean time to the onset of clinical signs (Figure 3) calculated using a regression model. Interestingly the time to onset began to plateau for all serotypes around the 3x$GPIMLD_{50}$ value.

1. BoNT serotype A intoxicated animals exhibited lethargy, hind limb paralysis, ruffled fur, weak limbs, change in breathing rate or pattern, lacrimation, salivation, ptosis, soft stool, prostration, forced abdominal respirations, and total paralysis. Severe clinical signs (paralysis, forced abdominal respirations) were only observed in groups 6 and 7 (BoNT/A doses >2.2x the historical $GPIMLD_{50}$)
2. BoNT serotype B intoxicated animals exhibited clinical signs including lethargy, hind limb paralysis, ruffled fur, weak limbs, lacrimation, salivation, ptosis, diarrhea, changes in breathing rate or pattern, forced abdominal respirations, and total paralysis. Severe clinical signs were only observed in the 5x $GPIMLD_{50}$ group.
3. BoNT serotype C intoxicated animals exhibited lethargy, hind limb paralysis, ruffled fur, weak limbs, noticeable changes in breathing rate or pattern, lacrimation, salivation, forced abdominal respirations, and total paralysis. Severe clinical signs were observed in animals receiving 1.3x $GPIMLD_{50}$ or higher doses of BoNT/C.
4. BoNT serotype D intoxicated animals exhibited clinical signs including lethargy, hind limb paralysis, ruffled fur, weak limbs, lacrimation, salivation, changes in breathing rate or pattern, forced abdominal respirations, prostration, and total paralysis. While the study report indicated that two animals in the 0.8x $GPIMLD_{50}$ group were noted with severe clinical signs, the euthanasia trigger for animals in the higher dose groups was not specified.
5. BoNT serotype E intoxicated animals exhibited clinical signs including lethargy, hind limb paralysis, weak limbs, noticeable changes in breathing rate or pattern, prostration, and total paralysis. Severe clinical signs were observed in animals receiving 0.8x, 1.0x, or 1.3x $GPIMLD_{50}$ doses. Animals receiving 2.2x or 5x $GPIMLD_{50}$ died without manifesting severe clinical signs. Nine guinea pigs died with no prior abnormal clinical signs.
6. BoNT serotype F intoxicated animals exhibited clinical signs including lethargy, noticeable changes in breathing rate or pattern, ruffled fur, hind limb paralysis, salivation, weak limbs, lacrimation, forced abdominal respirations, droopy eyelids, and audible gasping. Severe clinical signs were observed in animals receiving 1.0x $GPIMLD_{50}$; animals receiving higher doses died without manifesting severe clinical signs. Seven guinea pigs died with no prior abnormal clinical signs.
7. BoNT serotype G intoxicated animals exhibited clinical signs including lethargy, hind limb paralysis, ruffled fur, salivation, weak limbs, lacrimation, noticeable change in breathing rate or pattern, droopy eyelids, and forced respiratory respirations. Severe clinical signs were observed in animals receiving 1.0x $GPIMLD_{50}$; animals receiving higher doses died without manifesting severe clinical signs. Two guinea pigs died with no prior abnormal clinical signs.

d. In conclusion study 621-G005630 was adequately conducted and provided reasonable dose response estimates for each serotype of BoNT when administered via intramuscular injection in guinea pigs.

8. Study 670-G005630 was a GLP follow-up study to 621-G005630 with the objective to determine the clinical course of intoxication for all seven BoNT serotypes following an intramuscular challenge with 4x $GPIMLD_{50}$.
    a. Groups of ten guinea pigs (five male, five female) were injected into the musculature of the right hind limb with 4x $GPIMLD_{50}$ of BoNT serotype A, B, C, D, E, F, or G in a volume of 0.1mL.
    b. Animals were monitored hourly (serotypes A, B, C, D, F, G) or every 30 minutes (serotype E) through study day 3, and twice daily thereafter.
    c. All animals administered BoNT (except one) exhibited clinical signs consistent with botulism and were either found dead or euthanized according to the predetermined euthanasia criteria. Weakness in the injection site limb, lethargy, and breathing changes were noticed in the majority of animals (Table 7). The single animal that died without showing clinical signs had received BoNT serotype G and died approximately 33 hours later.
    d. The mean time to onset of clinical signs and the mean time to death for each serotype is provided in Table 8.
        i. Note that lethargy precedes the onset of hind limb weakness, and that death typically occurs < 24 hours after the onset of hind limb weakness (except for BoNT serotype C).
        ii. This indicates a short treatment window opportunity for use of BAT in the guinea pig model at this challenge dose (4x $GPIMLD_{50}$).
9. Study 731-G005630 was a GLP study to evaluate the post-exposure prophylaxis utility of BAT in the 4x $GPIMLD_{50}$ guinea pig challenge model.
    a. A post-exposure prophylaxis indication is not being considered for BAT. The utility of this study was to help provide data for the sensitivity analysis performed to evaluate human dosing. Please refer to the final clinical pharmacology review memorandum for details.
    b. Guinea pigs (~20/serotype) were challenged with 4x $GPIMLD_{50}$ of each BoNT serotype, and then administered 0.008x, 0.04x, 0.2x, or 1x scaled human doses of BAT or a dose of placebo as a single intravenous infusion either 12 hours post challenge (serotypes A, B, C, D, F, G) or 6 hours post challenge (serotype E). BAT was administered prior to the onset of clinical signs.
    c. Guinea pigs receiving placebo exhibited 100% mortality for all BoNT serotypes.
    d. Guinea pigs receiving BAT demonstrated an increased mean time to death regardless of the BoNT challenge serotype.
    e. A statistically significant increase in survival compared to placebo was observed at all BAT dose levels for serotypes A, B, C, F, and G. A statistically significant increase in survival was also noted at BAT doses $\geq$ 0.2x for serotype D, and $\geq$ 0.04x for serotype E. See Table 9.
10. Study 843-G005630 was a GLP, blinded, placebo-controlled study to demonstrate the therapeutic efficacy of BAT administered after the first onset of clinical signs of botulism.
    a. Groups of guinea pigs (~33/serotype) were challenged with 4x $GPIMLD_{50}$ of BoNT serotypes A, C, D, or F delivered as an intramuscular injection into the right hind limb. At the first onset of clinical signs of botulism, a single intravenous dose of BAT (1x scaled human dose) was administered.
    b. No survival benefit was observed in the BAT treated guinea pigs compared to control. There was however a statistically significant increase in the mean time to death in BAT treated animals challenged with BoNT serotypes A, C, and D. See Table 10.

11. Based in part on the results of study 843-G005630, Study 964-G005630 was performed to 1) re-evaluate the time between onset of clinical signs and death for BoNT serotypes A and E, and 2) compare clinical observations taken by two independent teams of technicians.
    a. Three doses of each serotype were evaluated (1.5x, 2.0x, and 4.0x) with ten guinea pigs per dose.
    b. The presence of clinical signs was monitored by two independent teams of observers.
    c. Mortality data are summarized in Figure 4 and a comparison of data taken from the two observer teams is presented in Table 11.
        i. Data from the two teams was comparable.
    d. Based on the increased duration between onset of clinical signs and death at the 1.5x $GPIMLD_{50}$ BoNT challenge dose it was determined that this dose would provide an adequate treatment window in which to demonstrate BAT efficacy.
12. Studies 993-G005630 and 1005-G005630 were non-GLP, blinded, placebo-controlled pilot studies performed to evaluate the therapeutic efficacy of BAT administered after the onset of clinical signs of botulism.
    a. Study 993-G005630 utilized BoNT serotypes A and E as the challenge agent
    b. Study 1005-G005630 utilized BoNT serotypes B, C, D, F, and G as challenge agents.
    c. Guinea pigs (22/group/serotype) received a 1.5x $GPIMLD_{50}$ BoNT challenge delivered in the right hind limb.
    d. Upon the first observation of a clinical sign of botulism, animals were treated with a 1x scaled human dose of BAT.
    e. BAT administration resulted in a statistically significant increase in survival compared to placebo for all BoNT serotypes (see Table 12 and Table 13).
    f. BAT administration also decreased the frequency of severe clinical signs in symptomatic guinea pigs.
13. Study 1124-G005630 was a non-GLP, blinded, placebo-controlled pilot study performed in guinea pigs to demonstrate the therapeutic efficacy of BAT administered after the onset of clinical signs of botulism. The study was performed as a follow-up to studies 993-G005630 and 1005-G005630.
    a. In contrast to previous studies, treatment was delayed until the fourth consecutive observation of clinical signs of botulism. This change in the treatment trigger was the result of discussions between Cangene and the Agency. The Agency had expressed concerns that guinea pigs treated at the first observation of clinical signs may represent an overly optimistic treatment scenario and that right hind limb weakness may be confounded due to local paralytic effects.
    b. Guinea pigs (22/group/serotype) received a 1.5x $GPIMLD_{50}$ challenge with BoNT serotype A, B, C, D, E, F, or G delivered into the musculature of the right hind limb.
    c. Upon the fourth consecutive observation of a clinical sign of botulism, animals were treated with a 1x scaled human dose of BAT.
        i. In the majority of animals, the treatment trigger was the fourth consecutive observation of right hind limb weakness. The time to treatment trigger for each serotype is provided in Table 14.
    d. BAT administration resulted in a statistically significant increase in survival compared to placebo for all BoNT serotypes (see Table 15).
14. Study 1180-G005630 was a blinded, placebo-controlled study performed in guinea pigs to demonstrate the therapeutic efficacy of BAT administered after the onset of clinical signs of botulism.
    a. This pivotal efficacy study was performed under GLP at BBRC.
    b. The primary objective of the study was to determine any statistically significant improvement in survival between BAT and placebo control groups for all seven serotypes in the guinea pig IM model.

i. The primary endpoint was survival at 21 days post-challenge.

ii. Secondary endpoints included time to death, incidence of clinical signs, time to onset of clinical signs, and resolution of clinical signs between treatment and control groups.

c. Specific pathogen free---(b)(4)-- guinea pigs (*Cavia porcellus*) between 350 and 525 grams were randomized to 14 test groups (34 animals per group; 17 males and 17 females) and exposed to 1.5x $GPIMLD_{50}$ units of BoNT serotypes A-G delivered as a single intramuscular injection into the right hind limb. The guinea pigs were purchased from ------(b)(4)----- and were received from the vendor with a surgically implanted jugular vein catheter.

i. While each shipment of 88 animals (44 per sex) was randomized into three groups (test article, placebo, or spare) with 17/sex/group in the treatment groups and 3/sex in the spare group, animals that did not meet the weight criteria were removed from the study and replacements (from the spare group) assigned. In some phases of the study, there were insufficient spares and animals of the opposite sex were used to ensure that a minimum of 34 animals were used on study.

d. The treatment trigger was 4 consecutive signs of any moderate or severe clinical sign. Animals were individually treated based on the presence of the treatment trigger.

i. Upon observation of the 4^{th} occurrence, animals were administered a 1x humanized dose of test article or equivalent volume of placebo via the implanted jugular catheter.

ii. Moderate signs were defined as salivation, lacrimation, right hind limb weakness, weak limbs, and change in breathing sounds or patterns.

iii. Severe signs were defined as forced abdominal respirations or total paralysis.

e. The test article was BAT lot 2060401Y. The control article was BAT-placebo, lot 10703480.

i. Proposed adult human dosing of BAT is 1 vial per patient.

ii. Dosing of the test article was based on animal body weight and was calculated to yield a human equivalent dose of 0.16 mL BAT/kg. This was adjusted with normal saline to a treatment dose volume of 2 mL/kg with normal saline. The final solution for infusion was a 12.5 dilution of the stock BAT and contained 4.48 mg/mL protein.

iii. The control article was diluted with normal saline to yield an equivalent protein concentration.

f. Two analysis sets were considered: the intent to treat (ITT) set and the per protocol (PP) set. The ITT analysis set included all animals that were intoxicated with BoNT and survived to receive the test or control articles. The PP analysis set included all animals that had been intoxicated with BoNT, successfully treated with test or control article, and had the scheduled clinical observations. The PP set excluded animals that died or were removed from the study for reasons unrelated to BoNT challenge or treatment related toxicity per the pathologist and/or the Study Director.

i. The study conclusions were based on the ITT analysis set. The ITT and PP analysis sets were identical for all serotypes except C; this group contained an animal in the placebo group (#3711) that was euthanized due to a pelvic injury unrelated to BoNT/C intoxication.

g. Euthanasia criteria were predefined and included 25% or greater weight loss in conjunction with any concurrent severe signs of intoxication, two consecutive observations of paralysis, or a determination that the animal was moribund.

i. The majority of animals that were euthanized met the paralysis criteria.

h. Blinding

i. The study director, qualified lead technicians, and all personnel involved in BoNT administration, clinical observations, and/or treatments were blinded to the randomization and group designations. The blinding included the sponsor's (Cangene's) representative, although members of BBRC's Quality Assurance Unit and Micro technicians preparing test/control articles were unblinded at the beginning of each challenge cohort. Blinding was not applied to the specific serotype used for intoxication.

i. Pathology

i. Only animals that died between study days 7 and 21 without showing moderate or severe signs of intoxication in the previous 24 hour period were required to be necropsied, although necropsy could be performed after study day 3 at the study director's discretion.

ii. Three animals were necropsied (animals 3764, 3806, and 3868). Cause of death was uncertain, but two animals (3806 and 3868) that had been challenged with BoNT serotype D had abundant pleural effusion and pulmonary edema.

j. Results

i. The time to treatment trigger varied based on serotype and was typically shorter than those observed in study 1124. See Table 16. Most animals were treated based on the right hind limb weakness observation.

ii. The study met the primary endpoint and demonstrated statistically significant survival advantage in the animals treated with BAT. See Table 17.

1. Survival in untreated guinea pigs was low (<85%) for all serotypes except serotype G.
2. Treatment with BAT did not immediately arrest clinical signs. Nearly all treated animals 233/238) developed additional clinical signs after receiving BAT, regardless of the BoNT serotype used for intoxication.
3. Animals in the BAT treatment arm had a lower mean clinical score, and a decreased incidence of severe clinical signs of botulism. See Figure 5.

k. Protocol Amendments and Deviations

i. There were twelve protocol amendments to the final study protocol. It was difficult to ascertain how the amendments related to the timing of the in-life portion of the study.

1. Cangene was asked to provide a timeline of the in-life portion of the study annotated to indicate what protocol amendments were made in relation to the actual performance of the study.
2. Three amendments were made prior to the in-life portion of the study, and nine made during the in-life portion. Most amendments were made to clarify procedures or correct documentation. Those that had a potential to impact the study performance were appropriate (e.g. in response to the problem with the original serotype F animals).

ii. Study deviations were provided for study 1180-G005630. Most of the deviations were minor and not uncommon for a study of this type (documentation errors, slightly late or early observations, a deceased animal was not refrigerated prior to necropsy, etc.).

1. The most significant deviation with regard to potential study outcome was the receipt of a cohort of guinea pigs for the serotype F challenge that exhibited mortality prior to toxin challenge (deviation DR-11410). Details were provided in the investigation report for this deviation (IR-497).
2. The affected group of 44 guinea pigs was received at Battelle from ---(b)(4)---------- on 18 August 2011, and 5 had died by the planned challenge day on

23 August 2011. All 5 animals were necropsied and the Battelle staff veterinarian found evidence of improper jugular vein catheter placement and/or thoracic injury.

a. All five animals had the catheters implanted at -----(b)(4)----- by a single staff member (identified as staff member "A").

3. Battelle stopped study activities on this set of animals mid-day 23 August 2011 prior to toxin challenge and notified Cangene.
4. An additional 5 animals died or were euthanized due to poor condition between 23 August 2011 and 29 August 2011, when the surviving animals were euthanized. Four of these animals had catheters implanted by staff member "A", and necropsy documented improper catheter placement and/or thoracic trauma.
5. These results were provided to -----(b)(4)-----, who removed staff member "A" from surgical procedures pending full re-training and re-approval. The vendor also initiated pre-order verification of surgeons assigned to animals for study 1180. Surgeons were required to perform the catheter implant on a minimum of 2 guinea pigs, which were euthanized to verify correct placement and procedure.
6. Surgeon verification was provided to Battelle along with a full replacement set of guinea pigs.

l. Review comments for study 1180-G005630

i. The primary issue identified during the review was a cluster of animals in the serotype B and C challenge groups that did not appear to meet the euthanasia criteria. A review of the clinical observation sheet and weigh monitoring sheets could not determine an obvious reason for the euthanasia of animals 614, 616, 623, 3707, 3727, 3738, 3749, 3759, 3763, and 3775. The only commonality seems to be that these animals were observed and euthanized during the morning shift.

1. Animals 614, 616, and 623 were in the placebo group for the serotype B challenge.
   a. Animal 614 was euthanized at 1016 on day 7 post challenge; the observations taken the hour prior (at 0905) indicated lacrimation, weak limbs, and a change in breathing patterns.
   b. Animal 616 was euthanized at 1012 on day 8 post challenge; the observations taken the hour prior (at 0930) indicated lacrimation, weak limbs, change in breathing patterns, and "other" with the latter remarked upon as "no stool".
   c. Animal 623 was euthanized at 1011 on day 8 post challenge; the observations taken the hour prior (at 0930) indicated salivation, weak limbs, change in breathing patterns, and "other" with the latter remarked upon as "no stool".
2. Animals 3707, 3727, 3738, 3749, 3759, 3763, and 3775 were in the placebo group for the serotype C challenge.
   a. Animal 3707 was euthanized at 0957 on day 11 post challenge. The observation made at 0922 indicated weak limbs and a change in breathing patterns.
   b. Animal 3727 was euthanized at 0950 on day 7 post challenge. The observation made at 0923 indicated salivation, weak limbs, and a change in breathing patterns.
   c. Animal 3738 was euthanized at 1005 on day 6 post challenge. The observation made at 0916 indicated lacrimation, weak limbs, and a

change in breathing patterns.

d. Animal 3749 was euthanized at 0951 on day 8 post challenge. The observation made at 0925 indicated weak limbs, a change in breathing patterns, and "other", with the latter remarked upon as "mucosal stool".

e. Animal 3759 was euthanized at 1005 on day 6 post challenge. The observation made at 0916 indicated lacrimation, weak limbs, and a change in breathing patterns.

f. Animal 3763 was euthanized at 0956 on day 11 post challenge. The observation made at 0906 indicated weak limbs and a change in breathing patterns.

g. Animal 3775 was euthanized at 1007 on day 6 post challenge. The observation made at 0934 indicated weak limbs and a change in breathing patterns.

3. Cangene indicated that these animals were judged to be moribund by the study director and that euthanasia was therefore warranted. Approval by the study director was noted in the raw data.

m. A Bioresearch Monitoring GLP inspection was requested and performed for study 1180-G005630. No issues impacting data quality were found, an FDA Form 483 was not issued to BBRC, and the inspection was classified as NAI.

15. Essential Data Elements of an Animal Model[1]

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

i. Challenge Agent

1. The partially purified BoNT complexes used for animal studies submitted in support of this application were originally purchased from ------(b)(4)----------. The serotype A, B, E, F, and G challenge materials may be considered reasonably similar to the etiologic agents responsible for human botulism and were purified from the appropriate *C. botulinum* cultures; certificates of analysis were provided for each toxin serotype. Serotypes C and D have not been associated with human disease but are responsible for botulism in animals (birds, cattle, and horses). A single purchased lot of each toxin serotype was aliquoted into a working stock, and the stocks for BoNT/ A-E have been in use at BBRC since 1997. BoNT/F was purchased in 2001 and used in experiments since December 2003. BoNT/G was purchased in 2005. BBRC routinely monitors the potency of these toxins via the mouse bioassay and extends the assigned expiry date as appropriate. The potency of toxin dilutions used in the pivotal animal studies was verified via mouse bioassay.

ii. Pathogenic Determinants

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

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

with fatal outcomes attributed to respiratory paralysis (or complications thereof).

iii. Route of exposure

1. Humans can be exposed to BoNT through a variety of different exposure routes, with inhalation and ingestion the most likely avenues for a bioterrorism event. Parenteral, inhalation, and oral routes of BoNT exposure have been studied in guinea pigs and nonhuman primates. In general, the parenteral routes appear to be the most efficient means of intoxication, followed by inhalation and oral exposure. A 1998 study performed by BBRC for the U.S. Department of Defense exposed guinea pigs to BoNT serotypes A-E delivered via intramuscular or intraperitoneal injection. The resulting LD_{50} estimates were comparable, and were lower than LD_{50} estimates previously reported for inhalational exposure in a guinea pig model. Less data is available for the nonhuman primate, especially with BoNT serotypes other than BoNT/A; however like the guinea pig model, parenteral routes of exposure appear to be similar while the oral and inhalational routes require higher toxin levels to achieve similar mortalities (Herrero, Ecklung, Streett, Ford, & King, 1967; Sanford et al., 2010; Scott & Suzuki, 1988).
2. An important factor to consider in terms of animal model development is the reproducibility of dosage. Aerosol and oral deliveries are associated with high variability, and for oral exposure the matrix in which the toxin is suspended can affect bioavailability (Cheng & Henderson, 2011). The toxin form also influences oral toxicity, with accessory proteins playing an important role in protecting the protein from proteolysis during the transition through the gastrointestinal tract (Chen, Kuziemko, & Stevens, 1998; Cheng et al., 2008; Ohishi, Sugii, & Sakaguchi, 1977). At the suggestion of the Agency, Cangene did sponsor a study to examine the utility of an oral intoxication (study 975-G005630) and established a guinea pig oral lethal dose 50% level of 388 $MIPLD_{50}$, although the time to onset of clinical signs was more variable in comparison to the IM model. Robustness of the animal model depends on reproducibility of challenge agent quantities, and since parenteral routes of intoxication provide similar results in terms of clinical progression and mortality, an IV or IM route was preferred for the efficacy model. These issues were discussed with Cangene at the IND stage and the Agency agreed that parenteral intoxication would be acceptable in order to decrease experimental variability, especially given the uncertainty around the various human exposure scenarios.
3. Limited data is available on the pharmacokinetics of BoNT. Bioavailability and metabolism have been examined for BoNT/A in the mouse and rat (Ravichandran et al., 2006) and BoNT/B in the rat (Al-Saleem et al., 2008). These studies suggest that the toxin is not subject to significant biotransformation and is held in the plasma compartment in an unbound state. Once consideration in terms of using the parenteral routes of exposure chosen for the BAT development program is that the disease course is shortened due to the bypass of absorption mechanisms.

iv. Quantification of exposure

1. While in vitro assays for quantifying BoNT are in development and have shown some promise in terms of sensitivity and turn around time, the most widely accepted assay is still the mouse potency bioassay. Serially diluted

samples are injected into mice and a probit analysis is performed to calculate the $MIPLD_{50}$ value. This assay provides a direct readout of the biological potency of the toxin; unfortunately the assay requires large numbers of mice and 4-6 days to complete.

2. The mouse bioassay was used to monitor potency of the BoNT serotype batches used in the BAT development program, and was also used to verify the potency of dilutions used in the various animal studies. Circulating BoNT levels in intoxicated animals was not monitored. To establish the quantity of each BoNT serotype required for lethality in the guinea pig following intramuscular injection, study 621-G005630 was performed at BBRC. Dilutions of BoNT serotypes A-E were prepared and injected into the muscles of the right hind leg of guinea pigs. The animals were monitored twice a day for 14 days for clinical signs and mortality. Probit dose response models applied to the mortality data were used to determine the guinea pig intramuscular lethal dose 50% ($GPIMLD_{50}$) value for each toxin serotype. The data was also compared to historical data from a previous BBRC study in guinea pigs using BoNT serotypes A-E as the challenge agent. The comparisons are provided in Table 5. The newly established $GPIMLD_{50}$ values and 95% confidence intervals for BoNT/F and BoNT/G were 25 (20.8-30.0) and 53.2 (48.7-58.0) $MIPLD_{50}$, respectively.
3. Cangene originally chose a 4x $GPIMLD_{50}$ challenge dose for their efficacy model. However, study 843-G005630 demonstrated that initiation of BAT treatment at the onset of clinical signs did not provide a survival benefit, although the median time to death was increased for serotypes A, C, and D Since the time to death was 16-38 hours after the onset of symptoms, it was speculated that disease progression was too rapid to allow for an accurate test of BAT efficacy.
4. In order to address these concerns, Cangene sponsored studies 964-G005630, 993-G005630, and 1005-G005630 in order to establish the clinical course of a lower (1.5 x $GPIMLD_{50}$) toxin challenge dose and test efficacy in the revised model. Study 964-G005630 did demonstrate that lowering the toxin dose could extend the clinical course of disease for serotypes A and E (see Figure 4).
5. Although BAT was effective at increasing survival in studies 993-G005630 and 1005-G005630 an unexpectedly large proportion of control animals survived. It was determined that the precision of the original $GPIMLD_{50}$ calculations was insufficient due to a small sample size. The toxin doses were reexamined by combining control animal data from studies 621-G005630, 964-G005630, 993-G005630, 1005-G005630 (and for serotype F, study 1124-G005630); it was determined that the original $GPIMLD_{50}$ values for serotypes B, C, D, E, F, and G had been overestimated (Table 18). The toxin dose was increased accordingly for the pivotal study 1180-G005630.

v. Host Susceptibility and Response to Etiologic Agent

1. Naturally occurring botulism has been documented in numerous animal species including wild ducks, chickens, cattle, and horses (Aiello, 2010). Toxin serotypes C and D are usually associated with animal botulism, although serotype B is responsible for shaker foal syndrome (analogous to infant botulism in humans) in the eastern United States. In general, most species are susceptible at some level of toxin exposure although rats are

relatively resistant to BoNT/B intoxication due to sequence variation in the BoNT Lc cleavage target in VAMP proteins (Patarnello, Bargelloni, Rossetto, Schiavo, & Montecucco, 1993), and carrion eaters are also relatively resistant (Gupta, 2012). Experimental botulism has been demonstrated in mice, rats, guinea pigs, rabbits, dogs, and nonhuman primates. Cangene utilized two of these species for its animal model development program, the guinea pig and the nonhuman primate.

2. Guinea pig
    a. The guinea pig has been utilized as a botulism model for many years, primarily for vaccine research in the biodefense field (Cardella, Jemski, Tonik, & Fiock, 1963). Cangene justified the use of the guinea pig based on its sensitivity to all seven BoNT serotypes, similarities in the clinical course of disease compared to other species, a similar pathophysiology compared to human botulism, and the reproducibility of the model. The guinea pig also provides a small animal model that allows for studies to be adequately powered through the use of sufficient animals; an important consideration when all seven BoNT serotypes are being tested in the model system. Limitations of the guinea pig system include a truncated clinical progression; the time between intoxication and the development of clinical signs was typically <2 days (Table 19), in contrast to humans that may develop symptoms from 12 hours to 8 days after ingesting BoNT contaminated food (Arnon et al., 2001) or 72 hours after inhaling aerosolized toxin (Holzer, 1962). The ability to deliver supportive care in guinea pigs is also quite limited.

vi. Natural History of Disease

1. Time to Onset of Disease/Condition
    a. The time to onset in human botulism following exposure to BoNT is highly variable and depends on serotype, dose, and route of exposure. Botulism caused by intestinal or wound colonization typically exhibits a time to onset greater than disease triggered by ingestion or inhalation. Interestingly, in the 2004 iatrogenic exposure case, patients who received intramuscular injections of up to $8x10^6$ $MIPLD_{50}$ of BoNT/A in the facial region did not present with clinical botulism until 48-72 hours after the injections (Chertow et al., 2006).
    b. As is the case with human botulism, the time to onset of clinical signs for botulism in guinea pigs experimentally intoxicated with BoNT is also dependent on serotype, dose, and route of exposure. Study 621-G005630 established a dose response relationship between BoNT serotypes A-G and onset of clinical signs with the exception of high doses where animals died rapidly and animal observation frequency was not high enough to capture the disease progression. The data from this study was used to perform a regression analysis and predict mean time to onset of clinical signs for multiples of the $GPIMLD_{50}$ as shown in Figure 3 below. Time to onset of clinical signs was also monitored during the model development studies and the pivotal efficacy study. Considerable deviation from the predicted values was observed at the 4x $GPIMLD_{50}$ dose for serotypes A, B, C, and E. For studies where the 1.5x $GPIMLD_{50}$

challenge dose was utilized, the onset of signs was considerably more rapid than predicted, especially for the pivotal study 1180 (see Table 19). The variability may be due to the inherent variability in the mouse potency assay used to quantify the challenge dose of each serotype or due to small dosing errors amplified by the steep dose response curve of the neurotoxins.

2. Time Course of Progression of Disease/Condition and Manifestations (Signs and Symptoms)
    a. Humans with botulism may present with mild signs or symptoms such as ptosis, speech difficulties, or diplopia or rapid progress to severe symptoms including full respiratory paralysis. The disease progression involves cranial nerve dysfunction, followed by a descending flaccid paralysis along with autonomic instability. Patients usually present with vision or swallowing difficulties and in the absence of treatment progress to a loss of control of facial and neck muscles, generalized weakness, and finally to dysphagia, loss of pharyngeal reflex, and respiratory paralysis (Arnon et al., 2001). Depending on the dose of toxin and the route of exposure, disease progression may be rapid; a patient who was intoxicated with BoNT/F progressed to respiratory failure within 24 hours of the onset of symptoms with an estimated circulating toxin level of ~ 1 $MIPLD_{50}$/mL plasma (Sobel et al., 2009).
    b. Like the human, disease progression in the guinea pig is a function of BoNT serotype, dose, and route of exposure and follows a characteristic, reproducible pattern (within a specific serotype). Animals usually display mild signs such as lethargy or ruffled fur, then progress to moderate signs including limb weakness, changes in breathing rate and/or pattern, salivation, or lacrimation. Severe signs of intoxication present last and include total paralysis or forced abdominal respirations. The most consistent clinical signs across all BoNT serotypes were right hind limb weakness and changes in breathing pattern or sounds. Guinea pigs challenged with BoNT/E were interesting in that lacrimation was observed at a higher frequency than with the other serotypes.
    c. Disease progression in nonhuman primates challenged with BoNT/A was different compared to guinea pigs, but did follow the general pattern of a descending flaccid paralysis. The first clinical sign observed was typically ptosis, sometimes in combination with muscle weakness and/or respiratory distress. A comparison of the disease manifestations between humans, guinea pigs, and rhesus macaques is presented in Table 20.

vii. Trigger for intervention

1. Diagnosis of botulism in humans can be difficult due to the nonspecific initial symptoms (see Table 20). In the animal models this is further complicated by the difficulties in recognizing clinical signs such as dry mouth, dysphagia, diplopia, and dilated/fixed pupils. Since Cangene is seeking a treatment indication, it was necessary to identify clinical signs in intoxicated animals that could be reproducibly recognized and serve as a consistent trigger for intervention. Bioassays would not suffice for this purpose due to the rapid disease course and low levels of circulating toxin

in the animals.

2. Based on the model development studies (621-G005630, 670-G005630), pilot post-exposure prophylaxis study 731-G005630, and pilot efficacy studies (993-G005630, 1005-G005630, and 1124-G005630) it was determined that observation of moderate or severe signs of intoxication would be an appropriate treatment trigger in the pivotal efficacy study. In order to ensure reproducibility and minimize the risk that animals might be dosed prematurely, the fourth consecutive observation of any moderate or severe sign of intoxication (salivation, lacrimation, right hind limb weakness, weak limbs, change in breathing sounds or patterns, forced abdominal respirations, or total paralysis) was set as the treatment trigger for the pivotal study 1180-G005630.
3. Personnel involved in clinical observation of intoxicated guinea pigs were trained and qualified in this procedure. One performance qualification plan (QD-294) was executed to train and qualify the study personnel at BBRC and involved a written exam, hands-on-training, and a series of clinical observation training sessions on intoxicated animals. Pivotal observations were made by pairs of qualified personnel and verified by the lead technician or Study Director.

viii. Characterization of Medical Intervention

1. Product Class
    a. BAT is a polyclonal hyperimmune product manufactured from the plasma of horses immunized with BoNT or BoNT toxoids. The equine IgG has been digested with pepsin to yield $F(ab')_2$ fragments in order to decrease immunogenicity arising from the equine Fc portion of the molecule. The product contains $F(ab')_2$ fragments specific for each of the seven BoNT serotypes. The labeled potency (per vial, regardless of fill volume) is at least 4,500 units for serotype A, 3,300 units for serotype B, 3,000 units for serotype C, 600 units for serotype D, 5,100 units for serotype E, 3,000 units for serotype F, and 600 units for serotype G. Each unit is defined as the amount of antitoxin that can neutralize 10,000 $MIPLD_{50}$ of serotypes A, B, C, D, F, and G, and 1,000 $MIPLD_{50}$ of serotype E.
    b. BAT is not a novel product; equine botulism antitoxins have been previously licensed in the United States and an equine $F(ab')_2$ antitoxin for use in scorpion envenomation was licensed in 2011.
2. Mechanism of Action
    a. Cangene has not studied the *in vitro* activity of BAT. The product potency is measured, however, using a bioassay whereby dilutions of product are mixed with each serotype of BoNT prior to injection into mice and neutralization capacity determined by probit analysis of the resulting survival curves. Neutralization in this assay is a result of the sequestration of toxin by the polyclonal antibody fragments, thus precluding BoNT binding to neurons and resulting toxicity. In vivo, the protective effect of antibodies directed against BoNT involves both steric hindrance between the antibody-bound toxin and its receptors as well as immune-mediated clearance of the antibody-toxin complexes(Cheng, Stanker, Henderson, Lou, & Marks, 2009). These immune complexes are likely removed via hepatic clearance mechanisms (Al-Saleem et al., 2008;

Ravichandran et al., 2006). Since the mechanism of action depends on clearance of circulating toxin prior to entry into the neuron, BAT will not reverse clinical symptoms or signs once they develop and should be administered as soon as possible after the onset of neurological symptoms

3. Pharmacokinetics
    a. Briefly, pharmacokinetic studies were performed in humans, guinea pigs, and rhesus macaques. The details of these studies are described in the pharmacokinetic review memo.
4. Synergy or antagonism of medical products likely to be used in combination
    a. Antihistamines and/or corticosteroids are frequently dosed concomitantly with non-human plasma derivative products in order to reduce the risk of allergic reactions, and NSAIDS are often used to manage minor adverse reactions. Non-aminoglycoside antibiotics may be used to manage secondary infections acquired during the lengthy hospitalization stays associated with severe botulism.
    b. No combination treatments were studied in the BAT animal model development program, and pretreatments with antihistamines/corticosteroids were not utilized in the human safety trials. No information was available on the use of pretreatments in CDC's Expanded Access Treatment Program.

ix. Design considerations for Animal Efficacy Studies

1. Endpoints
    a. The primary endpoint for both guinea pig and rhesus macaque pivotal animal efficacy studies was survival at 21 days post-intoxication. Predetermined euthanasia criteria were utilized to provide a humane and consistent survival endpoint.
2. Timing of intervention
    a. For the pivotal therapeutic studies, animals were dosed with placebo or BAT upon clinical observations defined as the treatment trigger. Each animal was evaluated individually and treated separately upon exhibiting the treatment trigger.
3. Route of Administration
    a. The route of administration of BAT in the animal studies is identical to that used in humans, specifically via intravenous infusion.
    b. Dosing Regimen
        i. Both guinea pigs and rhesus macaques received a 1x scaled human dose (based on weight) delivered as a single intravenous infusion. For a description of the dose scaling, please refer to the pharmacokinetic review memo.

16. On 12 February 2013, the 106th meeting of the Blood Products Advisory Committee considered the topic of Cangene's BLA for BAT.
    a. FDA sought input from the committee on whether the animal efficacy studies and human safety studies supported licensure.
    b. Five questions and an issue for discussion were posed to the committee. Three of these related directly to the animal model studies and are listed below, along with the committee vote on the question.

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

evidence that the product is reasonably likely to provide clinical benefit for the treatment of humans with symptomatic botulism?

The Committee agreed unanimously. Vote: 14 yes; 0 no

2. **Do the results from safety studies in healthy human volunteers, efficacy studies in animal models, and clinical data from CDC's use of BAT under IND (Expanded Access protocol) support an acceptable risk to benefit profile for use of BAT?**

   The Committee agreed unanimously. Vote 14 yes, 0 no

   Committee members did express concern regarding the severe adverse events of asystole, reported in the ten year old boy who received BAT. The Committee recommended Cangene conduct thorough postmarket safety studies, especially in children.

3. **Do the animal studies and simulation modeling adequately support the proposed dosing in humans**
   a. **for adults?**

      Vote: 14 yes, 0 no

      b. for children (no data) ?

      Vote: 12 yes, 1 no, 1 abstain

   The Committee member who abstained from voting noted that it was not possible to form an opinion without data.

   Other Committee members commented that in the absence of such data, intense monitoring and robust data collection is necessary to help determine appropriate dosing in children.

17. In conclusion, data from the guinea pig animal challenge model was found to be adequate to support licensure of BAT under the 21 CFR 601 Subpart H "Animal Rule" regulations. Efficacy of BAT in the BoNT challenged guinea pigs provides evidence that the product is reasonably likely to have a clinical benefit in humans. Approval of this BLA is recommended.

Tables and Figures

Figure 1. Timeline of guinea pig studies in support of STN 125462/0

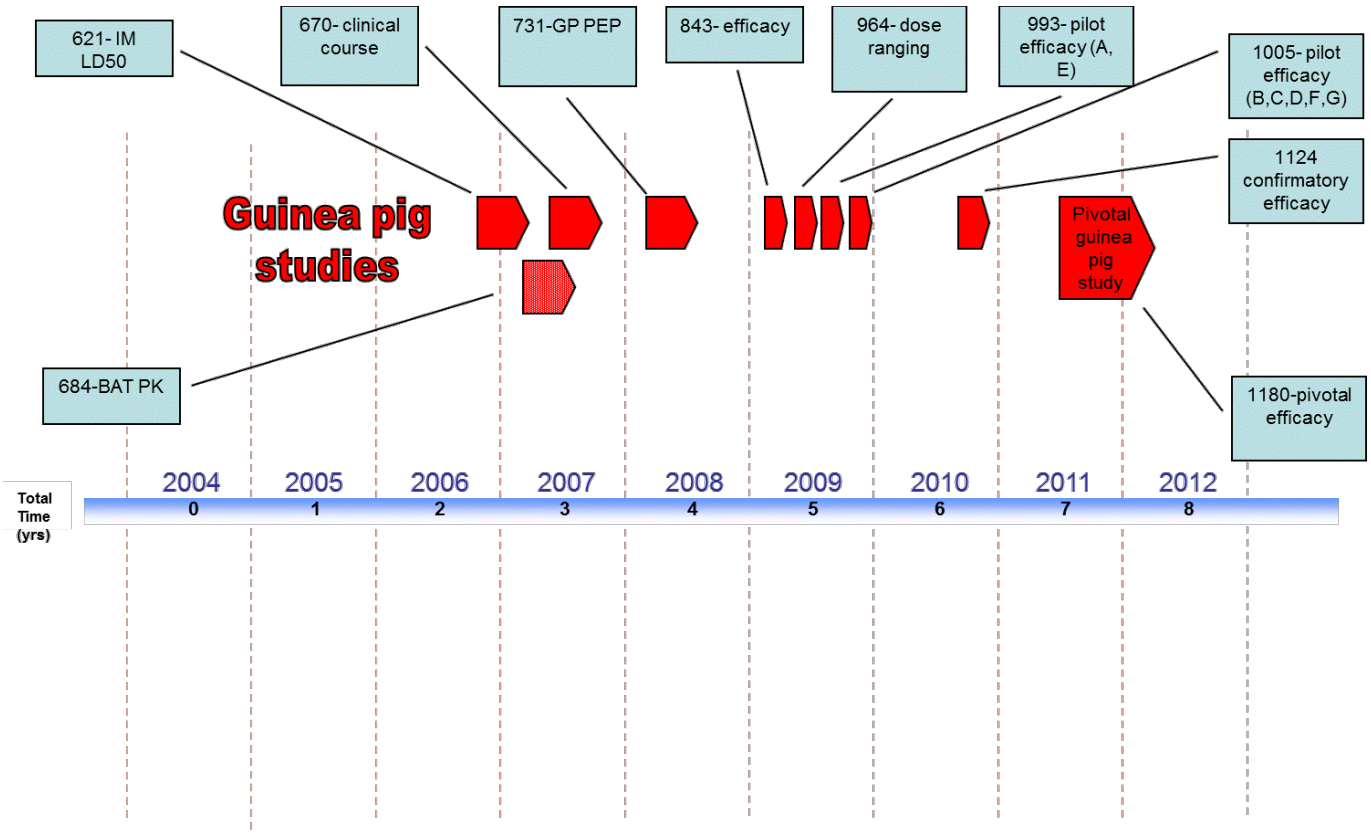

Table 1. BoNT lots used in dose ranging study 621-G005630.

Serotype	Lot number	Diluted working concentration batch number	Potency ($MIPLD_{50}$/mL)
A	A011995	2a	172,559
B	B011995	2a	928,682
C	C012495	2a	12,009
D	D022505	2a	196,640
E	E011295	2a	333,122
F	F033001-01	2a	90,318
G	G092905-01	4a	399,056

Table 2. Mouse potency assay results ($MIPLD_{50}$/mL) for Study 621-G005630 challenge material (BoNT) and comparison against historical potency for each serotype.

	Serotype A	Serotype B	Serotype C	Serotype D	Serotype E	Serotype F	Serotype G
Historical Potency ($MIPLD_{50}$) units/mL*	**200,000**	**978,000**	**12,500**	**202,000**	**316,000**	**66,400**	**329,000**
Assay Replicate 1	192,251	946,218	11,504	184,061	441,789	66,667	320,419
Assay Replicate 2	105,861	848,205	12,016	200,000	211,561	70,268	333,333
Assay Replicate 3	184,061	1,000,000	12,016	208,061	339,568	79,610	471,236
Assay Replicate 4	208,061	920,305	12,500	194,436	339,568	144,725	471,236
Average potency result of 4 Assay Replicates	**172,559**	**928,682**	**12,009**	**196,640**	**333,122**	**90,318**	**399,056**
Standard Deviation	45,567	63,088	407	10,080	94,285	36,680	83,513

Table 3. Challenge groups and doses, study 621-G005630.

		Serotype A	Serotype B	Serotype C	Serotype D	Serotype E	Serotype E (additional groups)
Dose Group[1]	**Factor of Historical $GPIMLD_{50}$**	**Historical $GPIMLD_{50}$ 4.3**	**Historical $GPIMLD_{50}$ 6.9**	**Historical $GPIMLD_{50}$ 3.1**	**Historical $GPIMLD_{50}$ 8.7**	**Historical $GPIMLD_{50}$ 101.9**	**Historical $GPIMLD_{50}$ 101.9**
1	x0.2	0.9	1.4	0.6	1.7	20.3	N/A
2	x0.45	1.9	3.1	1.4	3.9	45.9	N/A
3	x0.8	3.4	5.5	2.5	7.0	81.5	N/A
4	**x1.0**	**4.3**	**6.9**	**3.1**	**8.7**	**101.9**	N/A
5	x1.3	5.6	9.0	4.0	11.3	132.5	N/A
6	x2.2	9.5	15.2	6.8	19.1	224.2	N/A
7	x5	21.5.	34.5	15.5	43.5	509.5	N/A
8	x0.52	N/A	N/A	N/A	N/A	N/A	53.1
9	x0.60	N/A	N/A	N/A	N/A	N/A	61.3
10	x0.69	N/A	N/A	N/A	N/A	N/A	70.7

Groups in bold font were challenged at x1the historical $GPIMLD_{50}$.

Figure 2. Dose response curves for guinea pigs challenged with various doses of all seven BoNT serotypes.

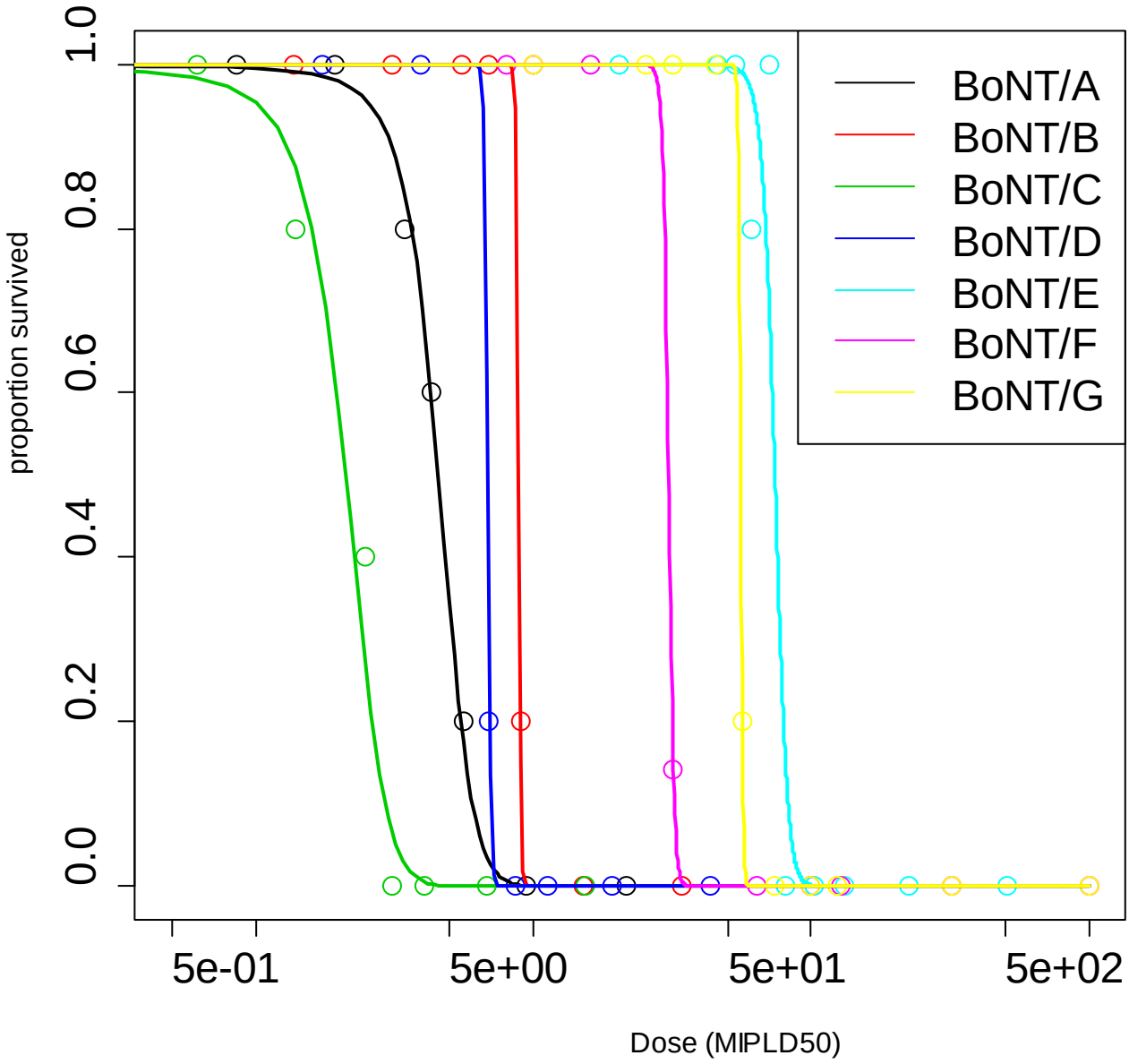

Table 4. Estimated $GPIMLD_{50}$ values from study 621-G005630.

Serotype	Study No.	Total Animals Dead/ Total Tested	Probit Slope Estimate	50% Lethal Dose Percentile	
				$GPIMLD_{50}$	95% Confidence Interval
A	621-G005630	17/35	8.4 *	4.5	(3.3, 6.7)
B	621-G005630	14/35	(Non-est.) #	8.5 #	(7.4, 9.8) #
C	621-G005630	24/35	7.1 *	2.0	(1.2, 2.6)
D	621-G005630	24/35	(Non-est.) #	5.7 #	(4.9, 6.5) #
E	621-G005630	26/50	20.9 *	73.2	(66.4, 86.4)
F	621-G005630	22/35	(Non-est.) #	25.0 #	(20.8, 30.0) #
G	621-G005630	20/35	(Non-est.) #	53.2 #	(48.7, 58.2) #

* The probit slope was statistically significant at the 0.05 level.

\# The probit slope was infinitely steep and could not be accurately estimated (Non-est.); therefore, the Spearman-Karber method was used to calculate the $GPIMLD_{50}$ and the 95 percent confidence intervals.

Table 5. Comparison of $GPIMLD_{50}$ values observed in study 621-G005630 to historical controls (BBRC Task 97-51)

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

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

Table 6. Guinea pig lethal dose 50%, 90%, and 99% values (expressed as MIPLD$_{50}$ units) calculated by CBER based on the study 621-G005630 dataset.

	LD50	LD90	LD99
Serotype A	4.6	6.0	7.7
Serotype B	8.8	9.0	9.2
Serotype C	2.1	2.9	3.8
Serotype D	6.8	7.0	7.2
Serotype E	74.2	82.8	92.1
Serotype F	30.7	32.3	34.0
Serotype G	55.9	57.0	58.3

Figure 3. Mean times to onset of clinical signs at multiples of the GPIMLD$_{50}$ for each serotype as predicted by the regression model, guinea pig study 612-G005630.

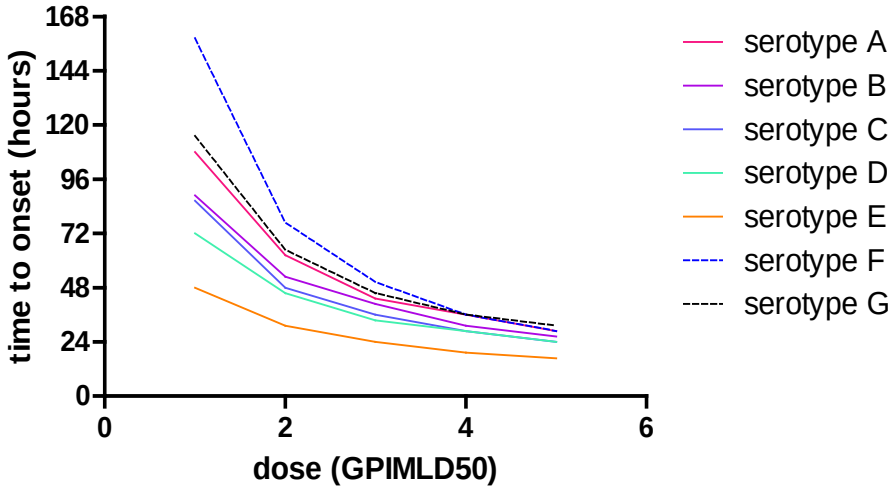

Table 7. Frequency of clinical signs in BoNT challenged guinea pigs, study 670-G005630.

Clinical Sign	Number of Animals that Showed Clinical Sign in Each Study Group of 10, by BoNT Serotype							
	A	B	C	D	E	F	G	Control
Lethargy	10	10	10	10	10	10	9	1
Hind limb Local Paralysis/Weak Limbs	10	10	10	10	10	10	9	0
Salivation	8	6	8	4	0	1	9	0
Lacrimation	4	4	8	1	0	0	4	0
Noticeable Change in Breathing Pattern or Rate	10	10	10	9	9	9	6	0
Forced Abdominal Respirations	10	7	10	7	8	8	8	0
Cannot Rise	8	9	10	8	6	1	3	0
Total Paralysis	4	3	2	2	0	1	0	0
Any Clinical Sign	10	10	10	10	10	10	9	1
Lethargy Plus Hind Limb Local Paralysis/Weak Limbs	10	10	10	10	10	10	9	0

Table 8. Time to onset of clinically relevant early signs of BoNT intoxication in guinea pigs, study 670-G005630.

BoNT Serotype	Lethargy	Hind Limb Local Paralysis/Weak Limbs	Mean (Range) Time to Death, in Hours
A	28 (25, 39)	32 (26, 46)	55 (36, 73)
B	33 (25, 51)	39 (26, 47)	58 (46, 76)
C	30 (25, 45)	33 (26, 42)	74 (55, 84)
D	27 (25, 31)	33 (26, 46)	48 (33, 62)
E	12 (10, 15)	13 (11, 14)	17 (12, 20)
F	26 (23, 31)	27 (23, 31)	32 (26, 45)
G	36 (26, 43)	39 (31, 41)	52 (34, 71)
Control	27 (--)	Not applicable	Not applicable

(--) **Range** could not be calculated since only one animal showed the clinical sign.

Table 9. Mortality, mean time to death, and mean time to onset of clinical signs, post-exposure prophylaxis study 731-G005630.

BoNT Serotype	Group	Treatment Dose Level*	Mortality	Kaplan-Meier Mean Time to Death (Range) in Hours	Kaplan-Meier Mean Time to Onset of Any Moderate Clinical Sign (Range), in Hours
A	1	1×	0/20***	--	64 (63, 64)
A	2	0.2×	1/20***	372 (372, 372)	91 (60, 95)
A	3	0.04×	0/20***	--	112 (49,123)
A	4	0.008×	9/20***	294 (159, 323)	108 (49, 153)
A	5	Control**	20/20	83 (65, 157)	53 (41, 95)
B	1	1×	1/20***	171 (171, 171)	122 (53, 144)
B	2	0.2×	0/20***	--	118 (79,122)
B	3	0.04×	3/20***	296 (195, 304)	244 (52, 292)
B	4	0.008×	0/20***	--	86 (29, 125)
B	5	Control**	20/20	86 (70, 147)	52 (42, 57)
C	1	1×	0/20***	--	99 (99,99)
C	2	0.2×	2/20***	413 (347, 417)	99 (99,99)
C	3	0.04×	2/20***	373 (321, 376)	120 (73, 125)
C	4	0.008×	6/20***	239 (158, 253)	75 (55,89)
C	5	Control**	20/20	85 (60, 117)	45 (25,55)
D	1	1×	0/20***	--	--
D	2	0.2×	0/20***	--	107 (75, 111)
D	3	0.04×	18/20	124 (84, 156)	67 (46, 91)
D	4	0.008×	20/20	64 (45, 81)	41 (32, 54)
D	5	Control**	20/20	51 (41, 74)	34 (26, 41)
E	1	1×	0/19***	--	42 (14, 114)
E	2	0.2×	1/20***	150 (150, 150)	45 (14, 112)
E	3	0.04×	2/20***	92 (79, 92)	17 (14, 23)
E	4	0.008×	19/19	31 (19, 52)	16 (13, 20)
E	5	Control**	20/20	21 (15, 30)	14 (11, 18)
F	1	1×	0/20***	--	--
F	2	0.2×	0/20***	--	95 (38, 98)
F	3	0.04×	2/20***	335 (160, 344)	70 (34, 75)
F	4	0.008×	3/20***	173 (68, 180)	55 (32, 87)
F	5	Control**	20/20	52 (35, 122)	34 (27, 52)
G	1	1×	1/20***	75 (75, 75)	68 (68, 68)
G	2	0.2×	0/20***	--	--
G	3	0.04×	0/20***	--	123 (51, 136)
G	4	0.008×	3/20***	129 (113, 131)	73 (50, 114)
G	5	Control**	20/20	57 (41, 73)	40 (35, 49)

-- Either the clinical sign was not observed, or the Kaplan Meier estimates could not be calculated due to censoring.

* Compared to proposed human clinical NP-018 dose (milliliter per kilogram basis). Assuming one vial of NP-018 (containing 11.17 mL) is equal to one human dose (of 70 kg), the dose volume per kg is 0.16 mL/kg (11.17 mL/70 kg).

** Normal Equine Immune Globulin

*** $p < 0.05$ via Fisher's Exact Test, compared to the same Serotype Controls (group 5)

Table 10. Mortality, mean time to onset of clinical signs, and mean time to death, study 843-G005630.

BoNT Serotype	Group	Treatment Dose Level	Mortality (Percent)	Kaplan-Meier Mean (Range) Time (Hours) to Onset of Clinical Signs and Time to Death		
				Any Moderate Clinical Sign***	Any Severe Clinical Sign	Time to Death
A	1	1.0x NP-018*	33/33 (100)	35 (30, 44)	64 (34, 113)[a]	68 (42, 131)[a]
	2	Placebo Control**	33/33 (100)	35 (29, 43)	49 (34, 62)	54 (40, 65)
C	1	1.0x NP-018*	30/35 (86)	34 (20, 45)	97 (56, 125)[a]	105 (63, 131)[a]
	2	Placebo Control**	34/34 (100)	35 (22, 48)	68 (49, 110)	73 (59, 112)
D	1	1.0x NP-018*	31/31 (100)	29 (22, 32)	85 (44, 110)[a]	86 (38, 131)[a]
	2	Placebo Control**	32/32 (100)	28 (21, 43)	63 (41, 73)	60 (47, 80)
F	1	1.0x NP-018*	29/31 (94)	30 (23, 42)	46 (24, 94)	42 (28, 61)
	2	Placebo Control**	32/32 (100)	31 (23, 42)	56 (24, 121)	47 (25, 129)

* Compared to proposed human clinical NP-018 dose (mL/kg basis)
** Normal Equine Immune Globulin
*** Corresponds to mean treatment time
[a] Statistically significant difference between the NP-018 Treated and Placebo control animals

Figure 4. Time to death for 1.5x, 2.0x, and 4.0x GPIMLD$_{50}$ for IM challenge with BoNT serotypes A and E, study 964-G005630

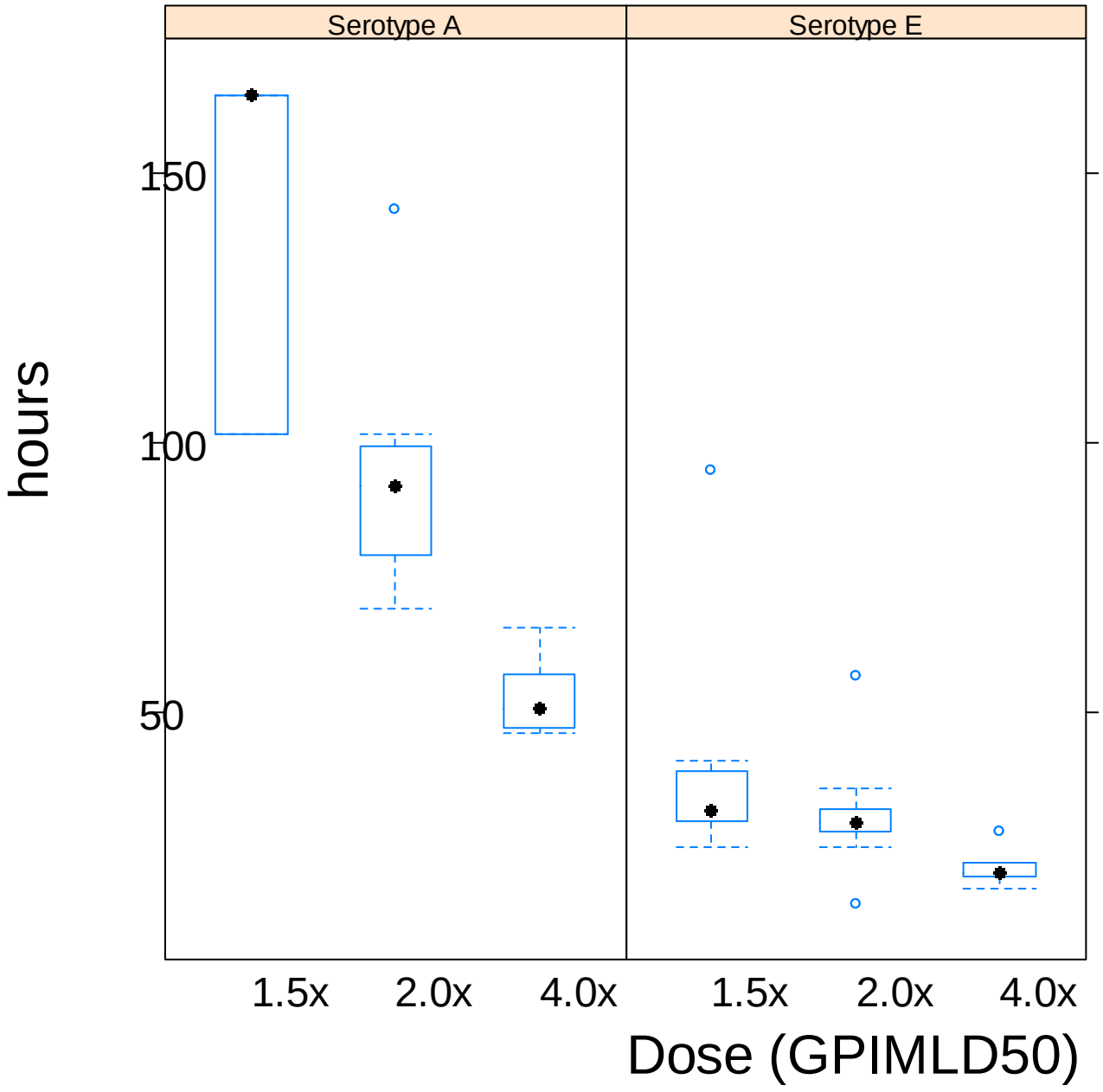

Table 11. Summary table comparing results from the two observer teams, study 964-G005630.

Technician Team	Serotype	Group	Toxin Dose in GPIMLD$_{50}$ (MIPLD$_{50}$)	Time to Onset of Moderate Clinical Signs (Hours)	Duration of Clinical Signs (Hours)	Time to Death (Hours)
1	A	1	1.5x (6.75)	37 (32, 42)	121 (72, 129)	165 (102, 165)
		2	2.0x (9.0)	27 (27, 31)	63 (52, 71)	92 (79, 99)
		3	4.0x (18.0)	24 (21, 25)	26 (25, 34)	51 (47, 57)
	E	4	1.5x (109.8)	14 (10, 17)	21 (12, 24)	32 (30, 39)
		5	2.0x (146.4)	16 (12, 18)	14 (11, 19)	30 (28, 32)
		6	4.0x (292.8)	13 (12, 13)	8 (7, 9)	20 (20, 22)
	Control	7	0 (0)	--	--	--
2	A	1	1.5x (6.75)	37 (32, 42)	121 (72, 129)	165 (102, 165)
		2	2.0x (9.0)	27 (27, 31)	62 (52, 71)	92 (79, 99)
		3	4.0x (18.0)	24 (21, 25)	26 (25, 34)	51 (47, 57)
	E	4	1.5x (109.8)	15 (10, 17)	20 (12, 24)	32 (30, 39)
		5	2.0x (146.4)	16 (12, 18)	14 (11, 19)	30 (28, 32)
		6	4.0x (292.8)	13 (12, 13)	8 (7, 9)	20 (20, 22)
	Control	7	0 (0)	--	--	--

-- Kaplan-Meier estimates could not be calculated due to censoring

Table 12. Summary of mortality, mean time to death, and number of animals with at least one severe clinical sign, study 993-G005630.

BoNT Serotype	Group	Treatment Dose Level	Mortality (percent)	Kaplan-Meier Mean Time to Death (Range) in Hours	Number of Animals with at least one Severe Clinical Sign
A	1	1.0x NP-018*	0/22[a] (0)	--	0
	2	Placebo Control**	20/22 (91)	184 (127, 283)	10
E	3	1.0x NP-018*	2/22[a] (9)	41 (35, 41)	6
	4	Placebo Control**	16/22 (73)	56 (26, 86)	16

* Compared to proposed human clinical NP-018 dose (mL/kg basis)

** Normal Equine Immune Globulin

[a] Comparison between treated and control groups significant at the 0.05 level of significance.

-- Animal death was not observed. Time to death was censored at 336 hours (14 days).

Table 13. Summary of mortality, mean time to death, and number of animals with at least one severe clinical sign, study 1005-G005630.

BoNT Serotype	Group	Treatment Dose Level	Mortality (percent)	Kaplan-Meier Mean Time to Death (Range) in Hours	Number of Animals with at least one Severe Clinical Sign
B	1B	1.0x NP-018*	0/22 [a] (0)	--	0
	2B	Placebo Control **	9/21 (43)	189 (142, 206)	7
C	1C	1.0x NP-018*	0/22 [a] (0)	--	1
	2C	Placebo Control **	9/20 (45)	200 (139, 211)	8
D	1D	1.0x NP-018*	0/20 [a] (0)	--	0
	2D	Placebo Control **	20/22 (91)	153 (115, 235)	19
F	1F	1.0x NP-018*	0/20 [a] (0)	--	0
	2F	Placebo Control **	7/22 (32)	144 (73, 160)	14
G	1G	1.0x NP-018*	0/22 [a] (0)	--	0
	2G	Placebo Control **	19/22 (86)	135 (67, 208)	18

* Compared to proposed human clinical NP-018 dose (mL/kg basis)
** Normal Equine Immune Globulin
[a] Comparison between treated and control groups significant at the 0.05 level of significance.
-- Animal death was not observed. Time to death was censored at 336 hours (14 days).

Table 14. Time to trigger, study 1124-G005630.

Serotype	Group	Time-to-Trigger (Hours)						
		N	Mean	Standard Deviation	Standard Error	Median	Min	Max
A	1 (Treated)	24	24	2	0	23	20	27
	2 (Control)	24	23	2	0	23	20	27
B	3 (Treated)	24	30	2	0	31	26	34
	4 (Control)	24	29	3	1	29	25	34
C	5 (Treated)	24	23	3	1	23	17	29
	6 (Control)	24	22	2	1	22	18	25
D	7 (Treated)	23	31	3	1	32	23	36
	8 (Control)	19	31	3	1	32	23	35
E	9 (Treated)	23	10	1	0	10	8	13
	10 (Control)	24	10	2	0	10	7	12
F	11 (Treated)	24	22	4	1	23	14	27
	12 (Control)	23	22	3	1	22	16	27
G	13 (Treated)	24	29	5	1	29	19	35
	14 (Control)	24	29	4	1	29	21	37

-- = Not applicable.

Table 15. Survival comparisons, study 1124-G005630.

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

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

Table 16. Time to treatment trigger, study 1180-G005630.

Serotype	Group	Time-to-Trigger in Hours						
		N	Mean	Standard Deviation	Standard Error	Median	Min	Max
A	A1 (Treated)	34	17	2	0	17	15	23
	A2 (Control)	34	18	3	0	17	16	29
B	B1 (Treated)	34	25	3	0	26	20	29
	B2 (Control)	34	24	3	0	25	19	29
C	C1 (Treated)	34	20	4	1	22	12	26
	C2 (Control)	34	20	4	1	22	12	26
D	D1 (Treated)	34	25	4	1	24	22	37
	D2 (Control)	34	25	5	1	24	22	37
E	E1 (Treated)	34	9	2	0	9	7	16
	E2 (Control)	34	9	1	0	8	8	10
F	F1 (Treated)	34	15	2	0	15	11	20
	F2 (Control)	34	15	2	0	15	10	20
G	G1 (Treated)	34	22	3	1	23	15	28
	G2 (Control)	34	22	3	1	22	16	29

Table 17. Intent to treat group survival comparisons, study 1180-G005630.

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

Figure 5. Clinical scores for BoNT challenge groups, study 1180-G005630. The dotted line indicates the mean treatment trigger time.

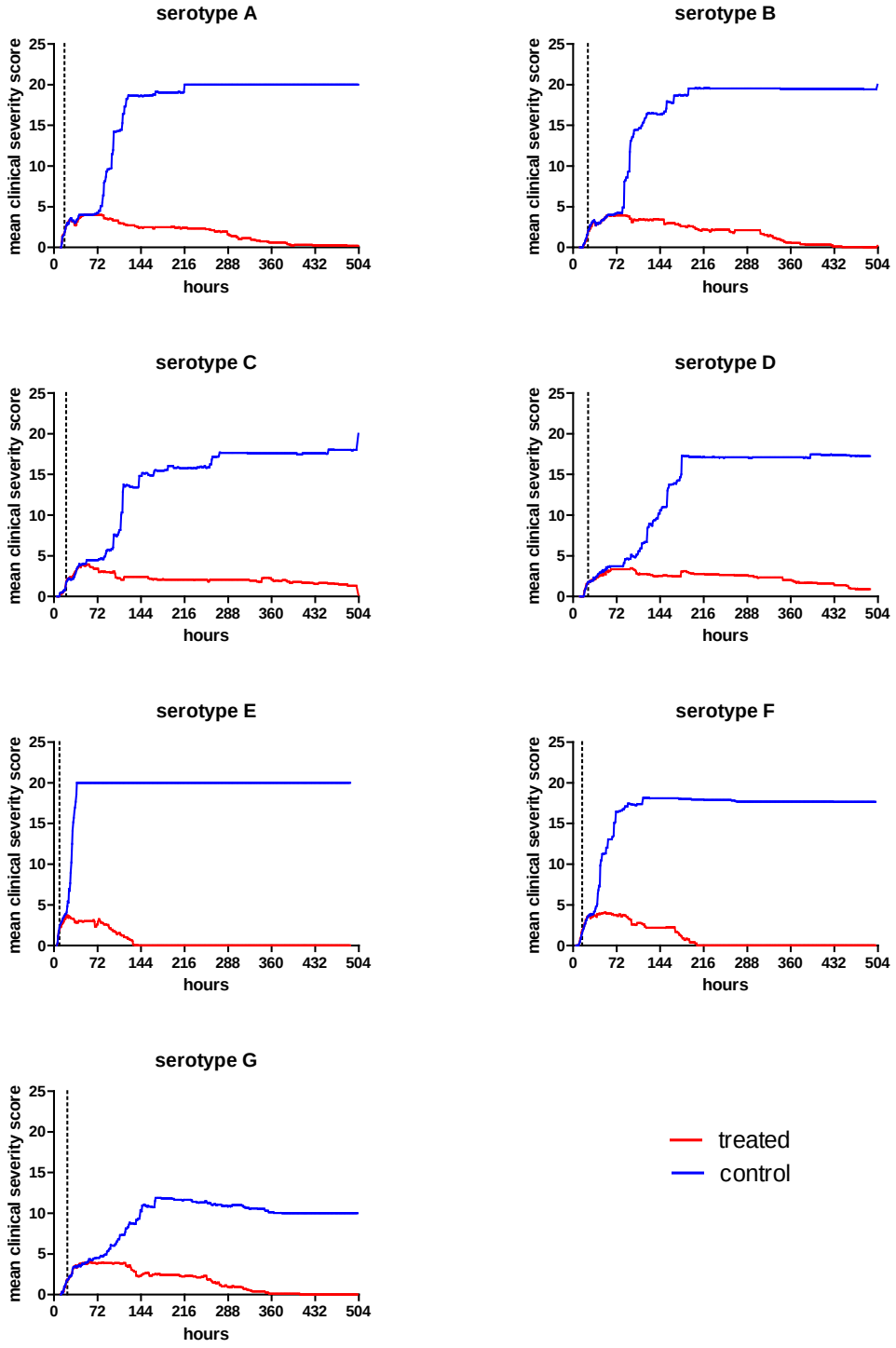

Table 18. Summary of toxin dose recalculation

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

Table 19. Summary of median time (hours) and 95% confidence interval to onset of the first clinical sign across control groups for guinea pig IM challenge studies performed for STN 125462. *Times for study 621 (shaded) are estimates of the mean time to onset based on the regression model from that study.

Study	Challenge dose ($GPIMLD_{50}$)	Serotype						
		A	B	C	D	E	F	G
621	4x*	34 (30-38)	31 (26-36)	28 (23-33)	28 (25-30)	19 (18-21)	35 (28-42)	36 (33-38)
670	4x	28 (25-39) N=10	33 (25-47) N=10	30 (25-42) N=10	27 (25-31) N=10	12 (10-14) N=10	26 (23-31) N=10	36 (26-40) N=9
731	4x	46 (43-52) N=20	51 (46-53) N=20	44 (43-47) N=20	32 (31-35) N=20	13 (13-14) N=20	33 (31-36) N=20	38 (38-42) N=20
843	4x	35	-	34	28	30	-	-
964	4x	24	-	-	-	13	-	-
621	1.5x*	81 (75-87)	68 (61-75)	64 (57-71)	57 (53-60)	38 (37-40)	109 (100-119)	85 (82-89)
993	1.5x	33 (31-35) N=22	-	-	-	17 (15-18) N=22	-	-
964	1.5x	37 (32-42) N=5	-	-	-	15 (10-17) N=5	-	-
1005	1.5x	-	36 (32-43) N=21	30 (29-39) N=20	26 (24-32) N=22	-	24 (21-25) N=22	23 (21-24) N=22
1124	1.5x	20 (18-21) N=24	25 (24-27) N=24	19 (17-21) N=24	28 (27-29) N=19	8 (7-10) N=24	20 (18-22) N=23	26 (21-28) N=24
1180	1.5x	14 (14-15) N=34	21 (20-23) N=34	19 (13-20) N=34	21 (19-23) N=34	7 (7-8) N=34	14 (12-15) N=34	17 (16-19) N=34

Table 20. Interspecies comparison of the frequency of signs and symptoms following exposure to BoNT/A

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

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

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