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

To: To File (BLA STN 125462/0)

From: Michael C. Kennedy, Ph.D., LPD/DH/OBRR, HFM-345

Through: Dorothy Scott, M.D., LPD/DH/ OBRR, HFM-345

CC: Nannette Cagungun, RPMB/DBA/OBRR, HFM-380

Applicant: Cangene Corporation

Product: Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) - (Equine)
Trade name: BAT

Subject: Final Review: NHP animal studies

Recommendation

This BLA is recommended for approval.

Executive Summary

Two Non-Human Primate (NHP) studies were conducted by Cangene in order to support the efficacy of NP-018 Botulinum Antitoxin Heptavalent Equine (BAT) for the treatment of intoxication by botulinum neurotoxin under the Animal Rule. These NHP studies were performed at Lovelace Biomedical and Environmental Research Institute in Albuquerque, New Mexico under Good Laboratory Practices (GLP) using Rhesus Macaques (*Macaca mulatta*). The earlier of the 2 studies was a pilot efficacy study followed by the pivotal efficacy study. These 2 studies shared a similar experimental design but had differences in the numbers of animals (18 vs. 60) used and the post-challenge study length (14 days vs. 21 days). Both studies utilized a 1.7x/kg Non-Human Primate lethal dose (NHPLD50) challenge of botulinum serotype A administered intravenously (IV) through a surgically implanted with an indwelling central venous catheter. Twenty-three hours following the toxin challenge the animals were monitored hourly for clinical signs of intoxication (ptosis, muscular weakness, and/or respiratory distress), and an assessment of food consumption. Immediately after the onset of clinical signs in each animal, NP-018 at 1x scaled human dose (0.26 mL/kg) or Botulinum Antitoxin Placebo (0.31 mL/kg, based on equivalent protein dose) was administered intravenously via the central venous catheter. Both studies resulted in ~48% survival in the BAT treatment groups compared to 0% survival in the placebo treated groups. Prior to conducting the 2 GLP efficacy studies in NHP, Cangene performed the following natural history, PK, PEP, and pilot NHP studies:

FY07- 027 GLP Lethal Dose and Clinical Course - Determine the lethal dose (NHPLD50) of botulinum serotype A in the Rhesus macaque - Animals administered a toxin dose equivalent to 0.625x, 1.0x, 1.5x,

and 4.0x/kg of the expected NHPLD50 as an IV injection and observed to determine survival and clinical progression. 16 animals, 8/sex, 4/toxin dose group

FY07- 056 GLP PK Study – Single IV Dose NP-018 to determine PK in 12 healthy non-intoxicated animals.

FY08-061 GLP Post-Exposure Prophylaxis (PEP) efficacy Study - Single IV Dose (0.1X or 1X scaled dose) NP-018 administered 4 hours after 4X NHPLD50/kg challenge of botulinum serotype A neurotoxin. All animals survived in both treatment groups and there was 100% mortality in the placebo control group. 30 animals, 10 per group (1x, 0.1x, placebo)

FY08-137 non-GLP Exploratory Therapeutic Efficacy - Single IV Dose (1X scaled dose) NP-018 administered after development of clinical signs of intoxication following a 4X NHPLD50/kg challenge of botulinum serotype A neurotoxin with some animals receiving full respiratory support. 4 animals

FY09-016 non-GLP Pilot Therapeutic Efficacy - Single IV Dose (1X scaled dose) NP-018 or placebo administered after development of clinical signs of intoxication following a 1.7X NHPLD50/kg challenge of botulinum serotype A neurotoxin. 14 animals, 10 in treatment group 5/sex, and 4 in placebo group 2/sex.

Background Summary

Cangene Corporation of Winipeg, Canada (Cangene) submitted a Biologics License Application (BLA) on September 20, 2012 for Botulism Antitoxin Heptavalent (A ,B, C, D, E, F, G) - (Equine), BAT. This application was submitted electronically and represents the first BLA submitted to CBER requesting approval under the "Animal Rule" 21 CFR 601 (subpart H). Cangene requested and received Orphan Drug Designation for BAT and priority review was granted to this BLA. Much of the development was funded by the U.S. government under the Biomedical Advanced Research and Development Authority (BARDA) and this product has previously been made available under a CDC held IND. The efficacy of BAT has been demonstrated in two animal models: the guinea pig intramuscular challenge model and the rhesus macaque intravenous challenge model (which is covered in this review). The application includes study reports for NHP botulism model development and 2 efficacy studies (1 pilot, 1 pivotal) using the intravenous botulinum neurotoxin challenge NHP model. Both efficacy studies administered a 1x scaled human dose of BAT (based on body weight) as a single intravenous infusion to animals challenged with a 1.7x/kg $NHPLD_{50}$ of botulinum neurotoxin serotype A that had developed clinical signs of botulism. BAT administered at the 1x scaled human dose statistically increased survival (~48% vs. 0%) and increased the survival time when compared to animals receiving placebo. The model development and efficacy data from this NHP efficacy model indicate that BAT is reasonably likely to provide clinical benefit in humans with botulism.

BAT is a clear to slightly opalescent, colorless to pale yellow sterile liquid containing enzymatically modified and purified equine-derived gamma immune globulin (IgG) antibodies to the seven known botulinum toxin types (A, B, C, D, E, F and G). The drug product is formulated with 10% maltose and 0.03% polysorbate 80. BAT is supplied in clear -(b)(4)- glass vials (20 or 50 mL) with ----(b)(4)---- ---------- rubber stoppers (20 mm), aluminum seals and plastic flip-top caps. Each vial contains approximately ---(b)(4)--- purified (Fab')2/Fab fraction per vial. Potency is expressed in units (U) based on the amount of toxin-specific neutralizing antibodies to each toxin serotype as determined by the Mouse Neutralization Assay (MNA). Filling is based on target potency per vial, expressed in U. Botulism Antitoxin Heptavalent contains no preservatives and is intended for single use by intravenous infusion. Prior to use, the product is diluted one in ten (1/10) with 0.9% Sodium Chloride Injection (USP).

Supplement Review Summary

Two Non-Human Primate (NHP) studies were conducted by Cangene in order to support the efficacy of BAT for the treatment of intoxication by botulinum neurotoxin under the Animal Rule. These NHP studies were performed at Lovelace Biomedical and Environmental Research Institute in Albuquerque, New Mexico under Good Laboratory Practices (GLP) using Rhesus Macaques (*Macaca mulatta*) and studies are summarized as follows:

FY09-114 Pilot Efficacy Study

Study Design: Eighteen (18) Rhesus macaques (9 per sex) were surgically implanted with an indwelling central venous catheter. Animals were randomly allocated two treatment groups; Group 1 animals (n = 10, 5/sex) for BAT treatment, and Group 2 animals (n = 8, 4/sex) for Placebo Control. One Group 2 female animal was removed from the study prior to Day 0 due to catheter damage. Therefore, 17 animals (10 in Group 1, and 7 in Group 2) completed the study. On Day 0, all animals received a dose equivalent to ~44 MIPLD50/kg (~1.7x $NHPLD_{50}$/kg) of Botulinum Neurotoxin Serotype A Complex as a single intravenous injection via a catheter placed in a saphenous vein. Starting about 23 hours after toxin administration, all animals were monitored approximately hourly for clinical signs of intoxication and assessment of food consumption. Immediately after the onset of clinical signs in each animal (ptosis and/or muscle weakness), BAT at 1x scaled human dose (0.26 mL/kg) or Botulinum Antitoxin Placebo (0.31 mL/kg, based on equivalent protein dose) was administered intravenously via the central venous catheter. Study personnel were blinded to which treatment group each animal belonged. Within 26 minutes of antitoxin or placebo administration, minimal support was initiated in all animals (Groups 1 and 2) as parenteral nutrition, administered through the central venous catheter via constant rate infusion by use of an ambulatory infusion pump. Dehydration was detected in four animals on Day 5, which was treated by administration of lactated Ringer's solution via the central venous catheter. Clinical signs in all animals in both groups were consistent with botulinum intoxication.

Results: The Kaplan-Meier median time to onset of initial clinical signs was 42.5 hours and 47 hours for Groups 1 and 2, respectively. Initial clinical signs included ptosis (n = 7), muscular weakness (n = 6), ptosis and muscular weakness (n = 3), and ptosis and respiratory distress (n = 1).
All (7/7) placebo-treated Group 2 animals were euthanized prior to study scheduled termination due to poor physical condition, with a median time to euthanasia of 65 hours.
Five out of ten (5/10) Group 1 animals recovered (i.e., without clinical signs excluding food consumption), and survived to the end of the study (Day 14). The remaining Group 1 animals (5/10) were euthanized due to poor physical condition, with a median time to death of 79 hours post-intoxication. The median time interval between the onset of clinical signs and euthanasia for Group 1 and Group 2 animals was 35 hours and 17 hours, respectively.

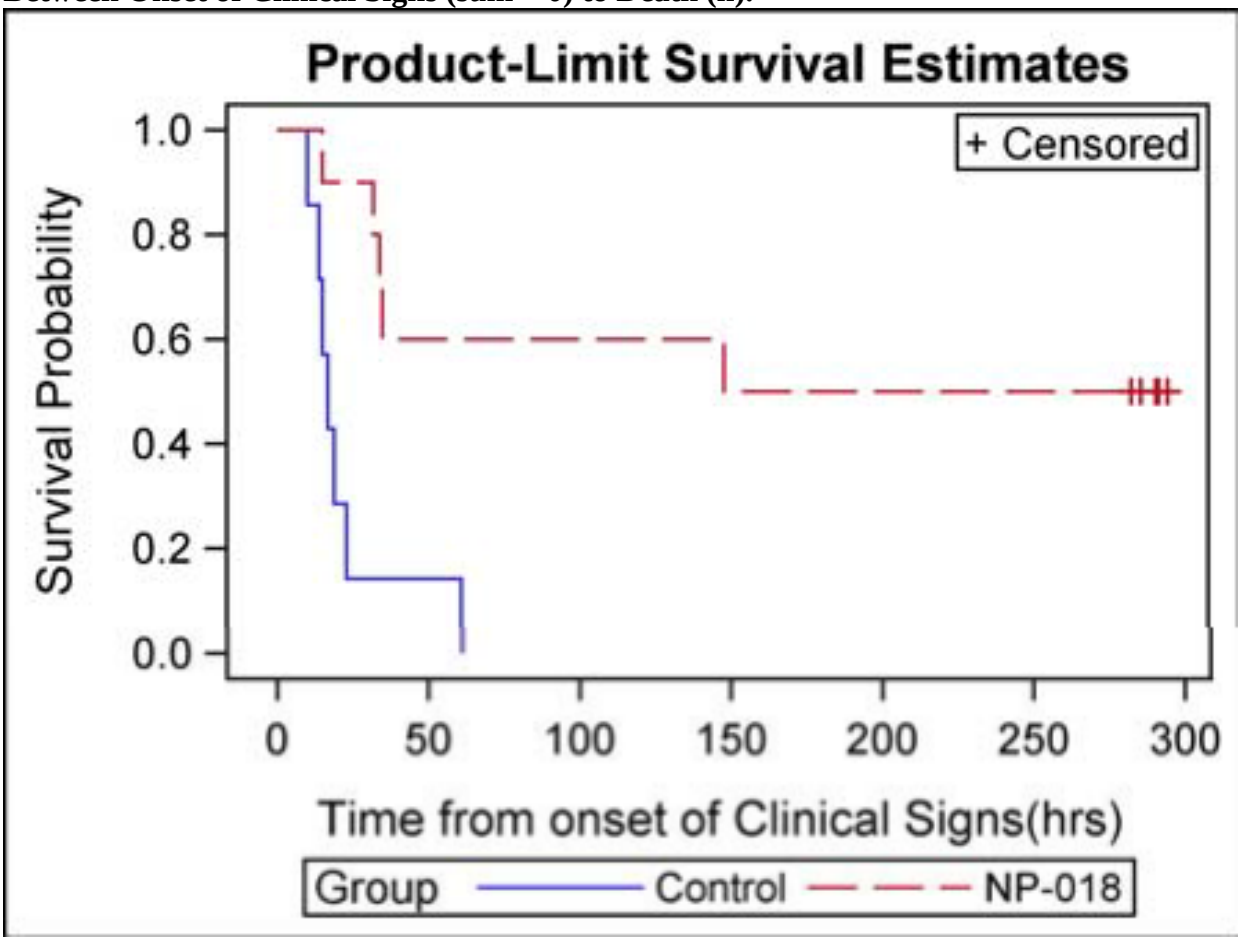
Table 1. Comparison of Mortality Rates in Rhesus Macaques Administered NP-018 Botulinum Antitoxin Heptavalent and Placebo

Study Group	Treatment	Mortality Rate (%)	P value

Group 1 NP-018 (n = 10)	~121 U/kg (or 0.26 mL/kg) NP-018 (1x scaled human dose)	50	0.044
Group 2 Placebo (n = 7)	0.31 mL/kg Botulinum Antitoxin Placebo	100	

a = Fisher's Exact Test

Figure 1. Kaplan-Meier Curves Associated with Duration of Clinical Signs Defined as the Interval Between Onset of Clinical Signs (sum > 0) to Death (h).



The analysis of these results show a statistically significant improvement in the survival of BAT treated animals.

Study Conduct: Study FY09-114 underwent 15 protocols changes during the study period and 16 known deviations occurred during the conduct of the study. Protocol changes were primarily directed at clarifying study procedures and the deviations which occurred were appropriately investigated and were generally minor such as missed observation times. Neither protocol changes or protocol deviations were deemed by this reviewer or by BIMO inspection personnel to have adversely impacted the quality of the study data or study results.

FY10-066 Pivotal Efficacy Study

Study Design: Sixty (63) rhesus macaques were surgically implanted with an indwelling central venous catheter; however, only 60 animals (29 males and 31 females) were used on study. Animals were first

randomly allocated to two treatment groups; Group 1 animals (n= 30, 15/sex) for Botulinum Antitoxin Heptavalent NP-018 treatment, and Group 2 animals (n = 30, 15/sex) for Placebo Control. Animals from each treatment group were then randomly allocated to cohorts. One Group 1 male animal was removed from the study prior to Day 0 because of health issues. This animal was replaced with a female because no other male animals were available. Therefore, 14 males and 16 females in Group 1 and 15 animals per sex in Group 2 completed the study. On Day 0, all animals received a dose equivalent to 1.7x $NHPLD_{50}$/kg (~44 $MIPLD_{50}$/kg) of Botulinum Neurotoxin Serotype A Complex as a single intravenous injection via a catheter placed in a saphenous vein. Starting 23 hr after intoxication, all animals were monitored hourly ($\pm$ 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), NP-018 Botulinum Antitoxin Heptavalent at 1x scaled human dose (0.26 mL/kg) or Botulinum Anitoxin Placebo (0.31 mL/kg, based on equivalent protein dose) was administered intravenously via the central venous catheter. Study personnel were blinded to which treatment group each animal belonged. Animals were observed for 21 days. Minimal supportive care (nutrition) was initiated in all animals (Groups 1 and 2) within 23 min of antitoxin or placebo administration and was either administered parenterally through the central venous catheter via constant rate infusion by use of an ambulatory pump, or via oral gavage of Liquid Rhesus monkey diet.

Results: None of the nonhuman primates challenged with botulinum neurotoxin serotype A complex and treated with Placebo survived to the end of the study, whereas, 14 of 30 (46.7%) nonhuman primates treated with BAT survived to the end of the study. Clinical signs in all animals in both groups were consistent with botulinum intoxication. Initial clinical signs included ptosis (n = 13), muscular weakness (n = 22), respiratory distress (n = 14), ptosis and muscular weakness (n = 1), ptosis and respiratory distress (n = 4), and muscular weakness and respiratory distress (n = 6). The duration of all clinical signs was longer for nonhuman primates treated with BAT, compared with animals treated with Placebo control. All (30/30) placebo-treated Group 2 animals were euthanized prior to study scheduled termination due to signs of botulism and poor physical condition, with a median time to death of 74.5 hr. However, fourteen of thirty (14/30) Group 1 animals recovered (i.e., without clinical signs excluding food consumption) after the onset of clinical signs, and survived to the end of the study (Day 21). The remaining Group 1 animals (16/30) died or were euthanized due to poor physical condition, with a median time to death of 189.5 hr post-intoxication.

Table 2. Analysis of Survival Rates at 21 Days Post-Challenge

Group	Survival Rate (No. of Survivors/No. in Group)	95% Confidence Interval	p-value
NP-018	0.47 (14/30)	(0.28, 0.66)	< 0.0001[a]
Placebo	0.00 (0/30)	(0.00, 0.12)	

[a]A statistically significant ($\alpha = 0.05$) difference was detected using Fisher's Exact test.

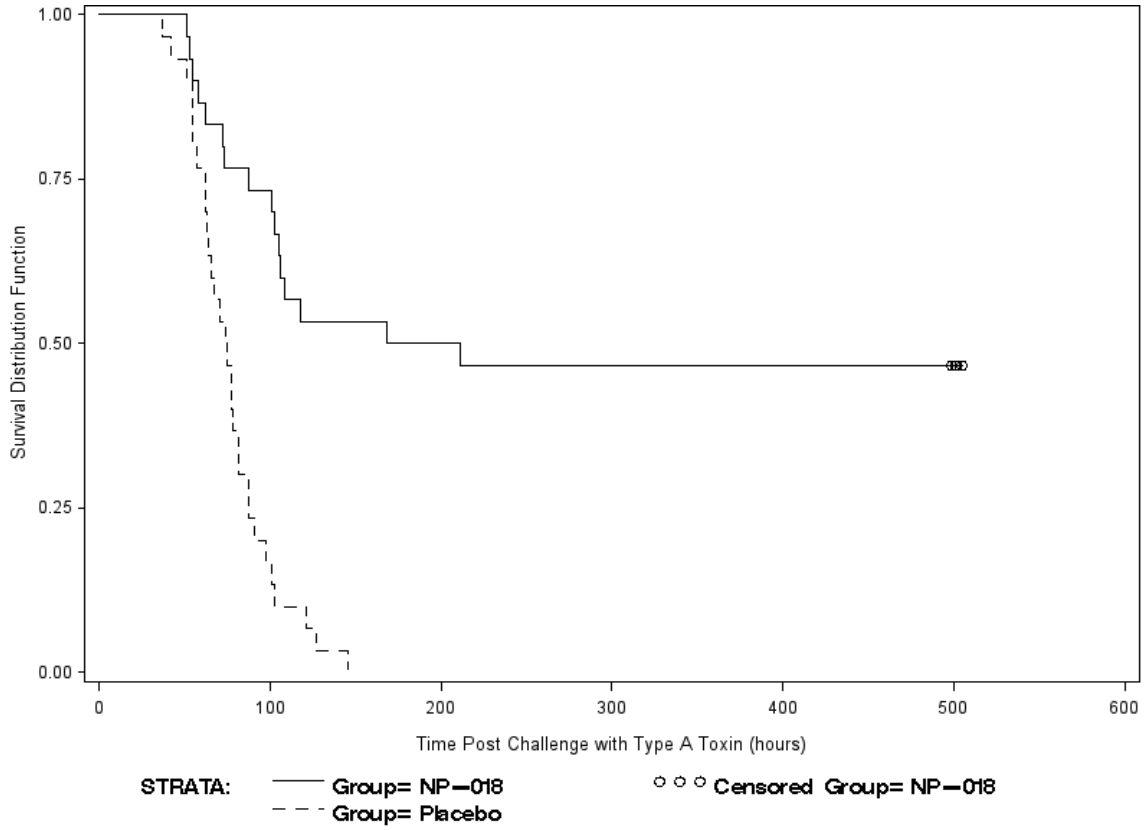
Table 3. Analysis of Time to Death

Group	Median time to death (hours)	95% Confidence Interval	p-value
NP-018	189.5	(102, –)[a]	< 0.0001[b]
Placebo	74.5	(63, 81)	

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

[b]A statistically significant ($\alpha = 0.05$) difference was detected using the log-rank test.

Figure 2. Kaplan-Meier Survival Curves for Time to Death



Analysis of this data supports that the treatment with BAT delays the progression of botulinum intoxication and the results in significant delays in time to death, when compared with the placebo treated group. These results also confirm a statistically significant improvement in the survival of BAT-treated animals compared to placebo treated animals.

Additional Supporting Studies:

FY09-16 non-GLP Pilot Therapeutic Efficacy

Study Design: Ten (10) Rhesus macaques (5 males and 5 females) underwent surgery for placement of a central venous Broviac catheter. After recovery from surgery, animals were randomly allocated to one of two treatment groups; Group 1 animals (n = 6, 3/sex) received BAT (NP-018) antitoxin treatment, and Group 2 (n = 4, 2/sex) received Placebo Control. All animals were administered a dose equivalent to 44 MIPLD50/kg (1.7x $NHPLD_{50}$/kg) of Botulinum Neurotoxin Complex Serotype A as a single intravenous injection via a catheter placed in the right saphenous vein. Beginning about 17 hours after intoxication, all animals were monitored approximately hourly for progression of clinical signs and assessment of food consumption. Immediately following each animal's onset of their first clinical sign, either NP-018 Botulinum Antitoxin Heptavalent 1x scaled human dose (0.16 mL/kg, for Group 1) or Botulinum Antitoxin Placebo (0.31 mL/kg, for Group 2) was intravenously administered via the Broviac catheter. Shortly after administration of antitoxin or placebo, minimal supportive care in the form of parenteral nutrition was administered to all animals through the Broviac catheter at a constant rate of infusion by use of an ambulatory infusion pump.

Results: The median time to onset of the initial clinical signs was 38 and 39 hours for Groups 1 and 2, respectively. Initial clinical signs (i.e., trigger for treatment) included ptosis (n = 5); muscular weakness (n

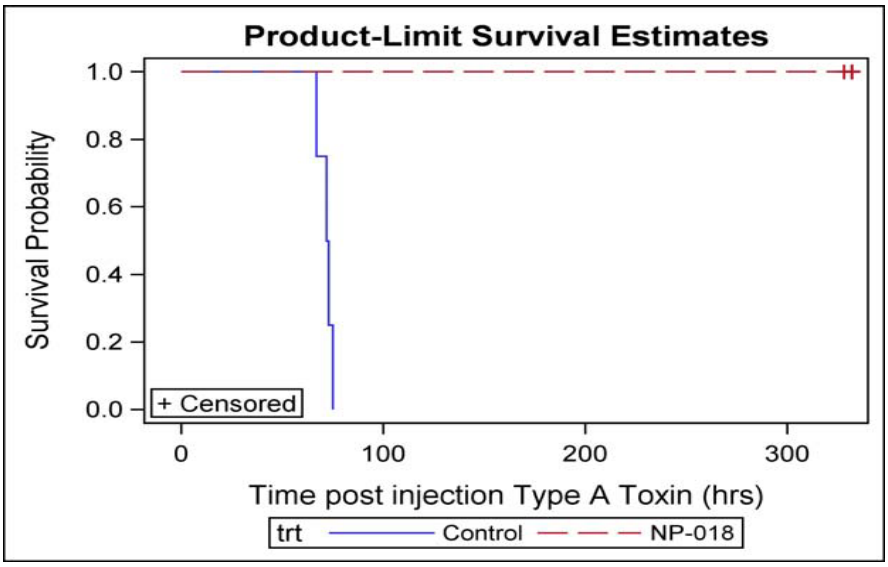
= 1); ptosis and muscular weakness (n = 2); respiratory distress (n = 1); and ptosis, muscular weakness, and respiratory distress (n = 1).
Initially, the progression of intoxication following toxin administration was comparable across both treatment groups, with all animals in both groups displaying moderate signs of intoxication. All six Group 1 animals administered BAT subsequently recovered (i.e., considered normal without clinical signs), and survived to the end of the study (Day 14). The clinical signs of intoxication in Group 2 animals continued to advance after placebo administration, until all animals were eventually euthanized due to poor physical condition (median time to death; 72.5 hours post-intoxication).
The median clinical course duration (i.e., time from onset of signs to death) for Group 2 animals was 34 hours.

Table 4 Comparison of Mortality Rates in Rhesus Macaques Administered BAT and Placebo

Study Group	Mortality Rate (%)	Exact 95% Confidence Interval	P value[a]
Group 1 NP-018 (n = 6)	0	(0, 0.46)	0.005
Group 2 Placebo (n = 4)	100	(0.40, 1.0)	

a = Fisher's Exact Test

Figure 3 Kaplan-Meier Survival Curves for Rhesus Macaques Administered NP-018 and Placebo



Gross pathologic examination of all major organs and histopathologic examination of the lungs were performed. No findings were considered test article-related.

FY08-061 GLP Post-Exposure Prophylaxis (PEP) efficacy Study

Study Design: Three groups of ten (10) rhesus monkeys (5 males and 5 females) received an intravenous injection of ~104 MIPLD50/kg (~4 NHPLD50) of Botulism toxin Type A. Two groups of 10 rhesus monkeys (5 males and 5 females) per group received BAT intravenously about 4 hours following the intravenous Botulism toxin Type A challenge. Treatment Group 1 received the projected scaled human dose or 0.16mL/kg (149 U/kg) of BAT intravenously and Treatment Group 2 received 1/10 of the

projected scaled human dose or 0.016mL/kg (14.9 U/kg). A third group of 10 rhesus monkeys (5 males and 5 females) received botulism antitoxin placebo.

Results: All animals in Treatment Groups 1 and 2 survived until scheduled sacrifice at study termination on days 14 or 15 post injection of toxin. All 10 animals in Treatment Group 3 pre-terminally died or were euthanized when exhibiting severe clinical signs of botulism toxin. The median time to death among Group 3 animals was 36.5 hours. This protection against lethality was statistically significant at $p<0.001$. One animal in group 2 had a single observation of oral discharge. Otherwise none of the animals in Treatment Groups 1 and 2 had any of the clinical signs of botulism intoxication of ptosis, muscular weakness, respiratory distress or oral or nasal discharge observed in botulism intoxication in Group 3 animals.

This protection from clinical signs was also statistically significant in both Treatment Groups 1 and 2 at $p<0.001$. Thus treatment with intravenous BAT at about 4 hours post injection of botulism neurotoxin complex Type A was highly effective at preventing the lethality from and the clinical signs of botulism.

The signs of botulism intoxication in Treatment Group 3 had an onset at about 27.5 hours (median time to ptosis=1) following toxin injection and progressed to death by about 36.5 hours (median time to death). Thus the time between the first observation of clinical signs and death was rapid (about 9 hours).

This data supports the conclusion that the treatment with intravenous BAT within 4 hours post injection of botulism neurotoxin complex Type A was highly effective at preventing the lethal effects and clinical signs of botulism intoxication.