DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Biostatistics and Epidemiology
Division of Biostatistics

STATISTICAL REVIEW AND EVALUATION
BLA (MID –CYCLE REVIEW)

BLA Supplement Number:	STN 125462/0
Product Name:	Botulism Antitoxin (Equine), Heptavalent (NP-18), ----------(b)(4)----------
Indication(s):	Treatment of Symptomatic Botulism Following Documented or Suspected Exposure to Botulinum Neurotoxin Serotype A, B, C, D, E, F, G
Applicant:	Cangene Corporation
Date(s):	Dec 07, 2012
Review Priority:	Fast Track /Orphan Drug Designation
Statistical Branch:	Therapeutics Evaluation Branch (TEB)
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1. EXECUTIVE SUMMARY

The sponsor submitted a biologic licensure application (eBAT NP-018) for Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)-(Equine) for the indication of treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin serotype A, B, C, D, E, F, G.

Clinical trials in a population exposed to botulinum neurotoxin (BoNT) are not feasible due to the small number of naturally occurring cases and the fact that it is unethical to deliberately expose healthy individuals to serious life-threatening pathogen, the efficacy of eBAT NP-018 is assessed in two animal models (guinea pig and non human primate) in accordance with the Animal Rule. The safety of eBAT NP-018 is assessed with two clinical trial studies that provided information on the pharmacokinetics (PK), pharmacodynamics (PD). A limited amount of efficacy and safety information was also gathered from patients treated with eBAT NP-018 under a CDC sponsored expanded access program (BB-IND 6705). The statistical assessment for this submission is still on-going and this memo is for the mid-cycle review commitment.

2. INTRODUCTION

2.1 Overview

Cangene Corporation is using the "Animal Rule" (Title 21 Code of Federal Regulations (CFR) 601 Subpart H) to seek licensure for its eBAT NP-018. The "Animal Rule" applies to new products that intend to reduce or prevent serious or life threatening conditions caused by exposure to lethal or permanently disabling toxic biological substances. Under this rule the Food and Drug Administration (FDA) may grant marketing approval based on adequate and well-controlled animal studies showing that the biological product is reasonably likely to produce clinical benefits in humans. Cangene Corporation originally submitted their intention to seek licensure under this pathway in 2004 (BB-IND 12052). FDA agreed that the efficacy demonstrated in two animal models (guinea pig and Rhesus macaque) would be adequate for consideration of licensure (FDA Pre-IND Meeting Minutes of August 26, 2004) under the "Animal Rule". Additionally, the clinical data collected by the Centers for Disease Control and Prevention (CDC) under their expanded access program (BB-IND 6750) is clinically relevant and supportive in nature to animal efficacy data for licensure under the "Animal Rule". The indication being sought for eBAT NP-018 is for the treatment of symptomatic botulism following documented or suspected exposure to BoNT serotypes A, B, C, D, E, F or G. This indication is supported by the data from two pivotal nonclinical studies; Pivotal Therapeutic Efficacy Study in the Guinea Pig (BBRC 1180-G005630) and Pivotal Therapeutic efficacy Study in the Rhesus Macaques (LBERI FY10-066) where eBAT NP-018 was administered intravenously to symptomatic animals thereby mimicking the intervention when administered to human patients with botulism. A post-exposure prophylaxis study evaluated the dose response of eBAT NP-018 in both guinea pig (BBRC 731-G005630) and non-human primate (NHP) models (LBERI FY08-061) where eBAT NP-018 was administered intravenously at a fixed time point to affected animals not yet showing signs of intoxication.

The rest of this review is organized as below: 1) brief description of the studies that provide the efficacy and safety information to support the licensure of eBAT-NP-018

(section 2.1.1 and 2.1.2), 2) the efficacy (3.1) and safety (3.2) in details, and 3) summary of the current review assessment.

2.1.1. Efficacy

2.1.1.1 Guinea Pig

The guinea pig was selected for use as a primary model for eBAT-NP-018 efficacy evaluation against all seven serotypes of BoNTs known to cause botulism in humans. Six efficacy studies were performed investigating the ability of eBAT NP-018 to improve survival of intoxicated animals when administered either at a range of dose levels in a post-exposure prophylactic study to asymptomatic animals, or at a scaled intended human dose as a therapeutic to symptomatic animals. Although each of the studies was designed to assess the efficacy of eBAT NP-018, study BBRC 1180-G005630 was the pivotal therapeutic efficacy study against all seven serotypes supporting licensure. Therefore this review memo focuses on results of study 1180.

2.1.1.2 Rhesus Macaque

Rhesus macaque was identified as the secondary model to demonstrate the product efficacy against BoNT serotype A only. Four efficacy studies were performed in Rhesus macaques, investigating the ability of eBAT NP-018 to improve survival of intoxicated animals when administered either as a post-exposure prophylactic to asymptomatic animals, or as a therapeutic to symptomatic animals. Although each of the four studies are designed to assess the efficacy of eBAT NP-018, only study LBERI FY 10-066 provided the pivotal therapeutic efficacy data against serotype A supporting licensure. Therefore, this review memo focuses on results of study LBERI FY 10-066.

2.1.1.3 Human

Since 2008, eBAT NP-018 has been available through a CDC sponsored expanded access program (BB IND 6750). This expanded access program was put in place to enable the use of NP-018 for the treatment of individuals with botulism as a result of naturally occurring outbreaks or in cases of isolated, unintentional incidents. The number of patients treated with eBAT NP-018 has increased since March of 2010 when eBAT NP-018 became the only botulism antitoxin (BAT) product available in the United States. This was a result of the expiration of the previously licensed Botulism Antitoxin Bivalent (Equine) Types A and B product and an investigational Botulism Antitoxin Monovalent (Equine) Type E product. Between January 15, 2008 and December 31, 2011, eBAT NP-018 was administered to one hundred and forty eight patients. Subsequently, the CDC provided Cangene Corporation with datasets developed from the case report forms from these patients. Using these case report datasets Cangene Corporation has generated a statistical analysis report which summarizes the data and provides results of an exploratory efficacy analysis. The datasets represent the most up to date information at the time of data transfer; however data collection and data cleaning activities are ongoing by the CDC. The sponsor anticipated that the complete database will be available after eBAT NP-018 licensure and that a final statistical analysis report will be generated. The results of this post-hoc analysis have been reviewed.

2.1.2 Safety

The clinical safety of eBAT NP-018 was obtained from two clinical trials (BT-001 and BT-002 Stage B), which included a total of fifty six healthy subjects administered either one or two vials of eBAT NP-018. A limited amount of safety information was also gathered from patients treated with eBAT NP-018 under a CDC sponsored expanded access program (BB-IND 6750).

2.2 Data Sources

All data sources are included in the sponsor's eCTD submission located in the FDA/CBER Electronic Room (EDR).

3. STATISTICAL EVALUATION

3.1 Evaluation of Efficacy

3.1.1 BBRC 1180-G005630

3.1.1.1 Study Design

Study Objectives:

The objective of the study was to evaluate the therapeutic efficacy of aBAT NP-018 when administered intravenous (IV) to guinea pigs at the onset of clinical signs (i.e., to symptomatic animals) following exposure to BoNT serotypes A to G. The study was conducted as a randomized, blinded and controlled GLP (Good Laboratory Practice) study. Secondary objectives included comparisons of (a) time to death, (b) incidence of clinical signs, (c) time to onset of clinical signs, and (d) resolution of clinical signs between treatment and placebo control groups.

Endpoints:

The primary study endpoint is the survival rate, which is defined as the proportion of animals in each group that survived to 21 days post-intoxication. The survival rate for each treatment group was calculated as

$$P = \frac{n}{N}$$

Where

n = the number of animals in the analysis set that survived to the scheduled phase termination on Day 21 post-intoxication for a given group.

N = the total number of animals in the analysis set for a given group.

In this study, death refers to all pre-terminal deaths, whether the animals were found dead or euthanized. Any animals meeting one of the following criterion were pre-terminally euthanized: (a) any animal has a 25.0% or greater weight loss (when compared to last preintoxication body weight) in conjunction with any concurrent severe sign of intoxication; (b) any animal has two consecutive observations of total paralysis; and (c) any animal that did not meet either of the first two criteria but was judged to be moribund. Only the study director (or, if study director was not available, the Battelle staff veterinarian in consultation with a lead technician as study director's designee) determined if an animal was moribund.

The secondary endpoints include: (a) time to death (number of hours from the time the challenge dose was administered until the time of death of animals in the analysis set), (b) incidence of clinical signs, (c) time to onset of clinical signs and (d) resolution of clinical signs between treatment and placebo control groups.

All endpoints were calculated separately for each serotype.

Sample Size:
The sample size was calculated based on the assumption that the placebo group has a survival rate of less than or equal to 65% and an expected survival rate in the NP-018-treated group of equal to or greater than 95%. A sample size of 34 in each group (17 males and 17 females) is needed to achieve greater than 80% power to detect the difference between the two treatment groups at the 0.05 significance level. The study actually contained 34 animals per group for each of the seven test groups.

Randomization Scheme:
1. Serotypes A, B, C, E, F and G
Each animal shipment of 44/sex (except for Serotype D) per serotype was randomized on Study Day -1 into two test groups of 17/sex/group and one spare group of 3/sex. For randomization, groups were filled with randomly selected animals that met the weight range on Day -1. If fewer than 37/sex met the weight range on Day -1, animals outside the weight range were randomized to study groups until a total of 37/sex were assigned. The random intoxication order for the 68 treatment and control animals were determined. The six spares were also assigned a random intoxication order, following the 68 treatment and control animals. A random replacement order was assigned to the extras.
On Study Day 0, animals that had Day -1 body weights <410.0 g or >490.0 g were reweighed. Starting with the study groups, any animals that did not meet the 400.0 to 500.0 g weight criteria were removed in the order they would have been intoxicated and replaced with the first available extra of the same sex that met the weight requirement. Each replacement animal assumed the random intoxication order previously assigned to the animal that was replaced. If there were insufficient extras available, spares were used to ensure that the treatment groups achieved 17/sex within the weight range whenever possible.
For serotype C, insufficient female extras or spares were available, and consequently 7 males were assigned as replacements in order to ensure that 34 treated/control and 6 spare animals within the weight range were available for intoxication. Animals with a last pre-intoxication weight outside 400.0 to 500.0 g on Day 0 were removed from the study.

2. Serotype D
Serotype D animals were randomized and replaced pre-intoxication by weight in a different manner than other serotypes. It is documented in the amendment that for Serotype D, the randomization on Study Day -1 was performed in steps:
(1) Animals that were within the 400.0 to 500.0 g weight range were randomized by gender and Study Day -1 weights to study groups;
(2) Animals under the weight range (weighing 350.0 to 400.0 g) were assigned to study groups in order of absolute deviation from the weight range until a total of 34/sex were

assigned. All remaining animals were assigned as extras. The random intoxication order for the 68 treatment and control animals were determined. On Study Day 0, Serotype D animals with body weights <410.0 g or >490.0 g on Study Day -1 were reweighed. Animals assigned to study groups that did not meet the 400.0 to 500.0 g weight criteria were replaced with an extra, using the following order of preference:
(a) an extra of the same sex in the weight range, if any were available; (b) an extra of the opposite sex in the weight range, if any were available; and (c) an extra of either sex under the weight range (350.0 to 400.0 g) in order of absolute deviation from the weight range. If no suitable replacement (based on the replacement preference order 1-3) was available, the original animal was used. Each replacement animal assumed the random intoxication order previously assigned to the animal that was replaced. Following all Study Day 0 pre-intoxication replacements, any extra or replaced animals within 350.0 to 525.0 g became spares and were intoxicated in cage order following all group-assigned animals. Animals with a last pre-intoxication weight below 350.0 g or above 525.0 g were not used in the study.

Blinding:
All study personnel involved in the observations or treatments were blinded to the study groups and treatment materials.

Population Proposed and Analyzed in the Protocols and BLA:
All animals that were intoxicated with BoNT and who survived to receive the NP-018 or control treatment material were included in the Intent-to-Treat (ITT) analysis set, in the treatment group to which they were assigned. The ITT analysis set was used to assess all study endpoints.
Animals that were intoxicated with BoNT, successfully received the assigned treatment and had scheduled clinical observations were included in the Per-Protocol (PP) analysis set, with the exception of animals that died or were removed from study due to reasons completely unrelated to toxin challenge or treatment related toxicity (based on pathologist and/or Study Director's decision). The PP analysis set was used to assess survival endpoints (survival rate and time-to-death) to evaluate the robustness of the conclusions based on the ITT population. Only Serotype C had a different PP analysis set vs. ITT analysis set, due to exclusion of animal ID #3711 from the PP analysis set.

Protocol Amendment and Deviation:
There are 12 amendments and 79 deviations listed in the final study report. This review only focuses on the ones that related to the study design and statistical analysis.
1. Amendment 10 expanded the animal weight range to 400 to 500 g for serotype D analysis. The impact of the amendment on the study is slight. In addition, statistical analysis included in the animal randomization report and supporting document for serotype D showed that there was no effect of weight on groups within each gender.

2. Deviation 11401, animal #286 and #294 were only weighted on Day -1, but were not weighted on Day 0. This deviation was acceptable since both of these animals were assigned to Extras on the Day -1 randomization and neither animal was used on the study.

3. Deviation 11388, animal #208 and #284's observation period on 8/10/2011 was incorrectly recorded and the errors were not caught by the verifier. Since both animals died 8/30/2011 and neither of these time points from animal #208 or #284 was included in the data package for statistical analysis.

3.1.1.2 Patient Disposition, Demographic and Baseline Characteristics

Seven separate 44/sex animal shipments were randomized pre-study to 14 groups, each group targeted to contain 17 animals /sex. Due to replacements, Serotype C and E had 18 males/16 females for the NP-018 treatment group; serotype D and F had 16 males/18 females for the NP-018 treatment group; and serotype C had 20 males/14 females for the placebo control group.

3.1.1.3 Statistical Methods

For the efficacy analysis of the primary endpoint, the null hypothesis was that there would be no improvement in survival between the NP-018 treated group and the placebo control group for each serotype. Survival rate at 21 days was calculated for each treatment and control group, along with an exact 95% confidence interval using Clopper-Pearson method. Tow-tailed Fisher's exact tests were used to determine if there was a statistically significant difference between the survival rates for the NP-018 treatment group and the placebo control group for each serotype. This analysis was performed on both ITT and PP analysis sets.

For the secondary endpoint, Kaplan-Meier curves along with log-rank tests were used to compare the time to death between the NP-018 treatment group and the placebo group for each serotype. The median time to death was determined along with a two-sided 95% confidence interval for each group using the product limit method. This analysis was performed on both ITT and PP analysis sets.

For the additional secondary endpoint, incidence of clinical signs in intoxicated guinea pigs, the statistical analysis method is the same as the method used for the primary endpoint (survival rate). The statistical analysis method for time to onset of clinical signs and time to solution of clinical signs was the same as the analysis method for time to death. In this review focus on the analysis of the primary endpoint: survival rate and the first secondary endpoint: time to death.

3.1.1.4 Results and Conclusions

Survival in the control-treated groups ranged from 0% to 15% except in the serotype G control group, which had 7/34 (50%) survival. NP-018 treatment provided significant protection against all botulinum neurotoxin serotypes (A, B, C, D, E, F and G). Two sided Fisher's exact tests showed that the survival rates were significantly higher for the NP-018 group than the placebo control group for all 7 serotypes at the 0.05 significance level. Table 1 presents the survival rates, 95 percent Clopper-Pearson confidence intervals and two-sided Fisher's exact test comparison between the NP-018 treated group and the control group for each serotype for the ITT analysis set.

Table 1. Survival Rates (95% Confidence Interval) and Two-Sided Fisher's Exact Test Comparisons between Treated and Control Groups, for the ITT Analysis Set.

Serotype	Group	Number Survived/Total Animals (Percent Survived) (95 Percent Confidence Interval) [a]	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%)	

* Comparison significant at the 0.05 level of significance.

[a] Clopper-Pearson confidence Interval

Time-to-death was censored at the end of the study for all surviving animals. The NP-018 treated groups had statistically significantly different (P<0.0001) time-to-death than the control groups for all seven serotypes. Table 2 presents the Kaplan-Meier median time-to-death and its 95 percent confidence interval, the p-values from log-rank test comparisons of the survival data between NP-018 treated and control animals for each serotype, for the ITT analysis set.

Table 2. Kaplan-Meier Median Time-to-Death, 95% Confidence Interval, and Log-Rank Test Comparisons between Treated and Control Groups, for the ITT Analysis Set.

Serotype	Group	Survival Time (hours) Kaplan-Meier Median (95% Confidence Interval)	Log-Rank Test Time-to-Death Comparison (P-Value)
A	A1 (Treated)	--(--)	<0.0001*
	A2 (Control)	99 (87, 113)	
B	B1 (Treated)	--(--)	<0.0001*
	B2 (Control)	94 (94, 112)	
C	C1 (Treated)	--(--)	<0.0001*
	C2 (Control)	114 (111, 141)	
D	D1 (Treated)	--(--)	<0.0001*
	D2 (Control)	156 (141, 180)	
E	E1 (Treated)	--(--)	<0.0001*
	E2 (Control)	29 (27, 30)	
F	F1 (Treated)	--(--)	<0.0001*
	F2 (Control)	58 (45, 68)	
G	G1 (Treated)	--(--)	<0.0001*
	G2 (Control)	168 (143, --)[d]	

-- The Kaplan-Meier median time-to-death could not be estimated since animal death was not observed for Serotypes A, B, E, F, and G and only one death was observed for Serotypes C and D.
* Comparison significant at the 0.05 level of significance.
[a] The upper bound of the 95 percent confidence interval could not be estimated due to the high incidence (50 percent) of censoring in the data.

Serotype C was the only serotype with different Intent-to-Treat (ITT) vs. PP analysis sets, because animal ID #3711 was euthanized for humane purposes following an injury (not related to BoNT intoxication), and therefore it was excluded from the PP analysis set.

For serotype C, the analysis result of the primary endpoint for the PP population is consistent with the analysis result for the ITT population.

The efficacy of Heptavalent Botulism Antitoxin (Equine) ABCDEFG (NP-018) was successfully demonstrated when administered therapeutically to guinea pigs intoxicated with 1.5x $GPIMLD_{50}$ of a single serotype (A, B, C, D, E, F or G) of Botulinu Neurotoxin Complex (BoNT). Treatment with NP-018 resulted in a statistically significant ($p<0.0001$) improvement in survival when compared to control animals for all serotypes.

3.1.2 LBERI FY10-066

3.1.2.1 Study Design

Study Objectives:

The objective of this study was to demonstrate the therapeutic efficacy of a single IV dose of Botulinum Antitoxin Heptavalent NP-018, when administered following the onset of clinical signs in reducing mortality among Rhesus macaques (*Macaca mulatta*) intoxicated with Botulinum Neurotoxin Serotype A Complex, with minimal supportive care provided.

Endpoints:

The primary endpoint is the survival rate at 21 days post-toxin exposure. The proportion of animals in each group survive to 21 days post-toxin exposure was calculated as

$$P = \frac{n}{N}$$

Where
n = the number of animals in the efficacy analysis set that survived to 21 post-intoxication for a given group.
N = the total number of animals in the efficacy analysis set for a given group.

Secondary endpoints include: (a) time to death, (b) time to onset of clinical signs, (c) duration of a clinical sign, (d) time from onset of clinical signs to recovery, (e) the proportion of animals recovering after the onset of clinical signs, (f) clinical severity scores, and (g) time to onset of the treatment.

Sample Size:
Sample size was determined based on the assumption that the placebo group has a survival rate of less than or equal to 10% and the NP-018 treatment group has an expected survival rate equal or greater than 45%. A total of 29 animals per group are needed to achieve 80% power to detect the assumed difference in the survival rates between treatment group and placebo group using the two-sided Fisher's exact test at the 5% significance level. In order to achieve gender balancing in each group, a total of 30 animals per group are proposed in the protocol. The study actually consisted of 60 animals with 14 males and 16 females in group 1 and 15 males and 15 females in group 2.

Randomization Scheme:
Nonhuman primates were allocated to study groups (and then to cohorts) by use of a stratified (body weight) randomization procedure, using a computerized data acquisition system (----------(b)(4)----------).

The original randomization procedure generated by --(b)(4)-- has been changed due to various reasons (See page 306, 308 & 309 of Appendix I of final report). This reviewer defers to the clinical reviewer to decide whether the randomization procedure is acceptable or not.

Blinding:
To control bias, randomization and loading of syringes with test and control article were performed by technicians not involved in dosing or post-dosing observations of nonhuman primates so that those observing animals were unaware of treatment group allocations.

Population Proposed and Analyzed in the Protocols and BLA:
The efficacy analysis set is defined to include all animals that have been successfully intoxicated with Botulinum Neurotoxin Serotype A Complex and survive to receive the assigned treatment. Both survival data and observational data based on clinical signs were evaluated on all 60 animals challenged with the toxin.

Protocol Amendment and Deviation:
There are several amendments and 45 deviations included in the report. This review focuses on the ones that related to the study design and statistical analysis.

1. Deviation 5. On the date specified, animal 08R0115 was euthanized. However, a necropsy was not performed due to technical error. This resulted in the loss of data from a single animal. None of the remaining 29 animals in the group showed significant

lesions or obvious signs of toxicity. This deviation is not going to affect the analysis of the primary endpoint.
2. Deviation 6. The toxin was flushed with 1-mL of 0.9% sterile saline (instead of 0.2% Gelatin Phosphate Buffer stated in the protocol) for animals 08R0120, 08R0143, 08R0147 and 08R0196. For those animals, the entire dose of toxin was delivered and no adverse events were noted other than expected toxin administration. All these animals are in the NP-018 treatment group and none of them survive to Day 21. Therefore, even if this deviation did not happen and all these animals survived to Day 21, it is only going to increase the survival rate of the NP-018 treatment group, which will not change the result that there is a significant difference in the survival rate for the two treatment groups.
3. Deviation 22, each treatment group was planned to consist of 30 animals (15/sex) in the protocol. However there is a gender imbalance due to incidents. The study was completed with 60 animals, 30 animals per group (group 1 included 14 males and 16 females; group 2 included 15 males and 15 females). A separate paragraph was added to the protocol to address the deviation from the planned sample size with respect to gender balance. An assessment of the impact to the study was also provided.
4. Deviation 28. After the animals were randomized as specified in the protocol, two pairs of animals: animal 06R018 and 08R0108, animal 08R0094 and 06R0094 were switched into the opposite treatment groups due to the similarity in identification. Since the swapped animals were all females and the study technicians were blinded to the treatment groups, the impact of this deviation is minor.

3.1.2.2 Patient Disposition, Demographic and Baseline Characteristics
Sixty nonhuman primates (30 animals/group) completed the study. Group 1 consisted of 14 males and 16 females and group 2 consisted of 15 males and 15 females.

3.1.2.3 Statistical Methods
Primary Analysis
The proportion of animals in the efficacy analysis set for each group that survived to 21 days post-toxin exposure was calculated along with two-sided 95% confidence intervals, using the exact binomial distribution. The proportion of animals that survived to 21 days post-toxin exposure in each group was compared using Fisher's exact test, with the probability of type I error set at alpha 0.05.

To conclude that the survival rate in NP-018-treated animals with minimal (nutritional) supportive care is greater that the survival rate in placebo-treated animals, the p-value of the two-sided Fisher's exact test must be less that 0.05 and the observed treatment difference must lie in the appropriate direction.

Secondary Analysis
This review focuses on the first two secondary endpoints: time to death and time-to-onset of clinical signs. The median time to death was calculated along with corresponding two-sided 95% confidence intervals for each treatment group, using the product-limit method, and Kaplan-Meier plots are provided. The survival curves were compared between the treatment and placebo groups using the log-rank test.

3.1.2.4 Results and Conclusions

None of the animals challenged with botulinum neurotoxin serotype A complex and treated with placebo survived to the end of the study; 14 of 30 (46.7%) animals treated with NP-018 survived to the end of the study. There is a statistically significant difference in the survival rate (0% vs. 46.7%), using a two-sided Fisher's Exact test ($p < 0.0001$). Table 3 lists the analysis results.

Table 3. Analysis of Survival Rates at 21 Days Post Challenge

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

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

The Kaplan-Meier median time to death and corresponding 95% confidence interval was calculated for each group (Table 4). Kaplan-Meier survival curves were produced and a statistically significant difference was found in the survival distribution for the two groups, using the log-rank test ($p < 0.0001$).

Table 4. Analysis of Survival Time (hours)

Group	Median Survival Time	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.

The Kaplan-Meier median times to onset of each clinical sign along with the corresponding 95% confidence intervals were calculated for each treatment group (Table 5). The time to onset of each clinical sign is defined as the first time-point where its score is greater than zero. The log-rank test was performed to compare the survival distributions between the two treatment groups for each clinical sign. There was no significant difference in the time to onset of all clinical signs except for nasal discharge.

Table 5. Analysis of Time-to-onset (hours) of Clinical Signs

Clinical sign	NP-018 Median (95% CI)	Placebo Median (95% CI)	p-value
Ptosis	62 (55, 66)	64 (55, 67)	0.2939
Muscular weakness	60.5 (53, 63)	59 (52, 63)	0.1294
Respiratory distress	59.5 (55, 63)	58 (53, 63)	0.3466
Oral discharge	61.5 (58, 65)	56 (53, 60)	0.0718
Nasal discharge	107 (90, –)[a]	84 (67, 110)	0.0481[b]

Clinical sign	NP-018 Median (95% CI)	Placebo Median (95% CI)	p-value
Food intake	24 (23, 28)	23.5 (23, 30)	0.4791

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

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

3.1.3 BB-IND 6750

3.1.3.1 Study Design

Study Objectives:

The objective of this statistical analysis is to determine if the data collected through the CDC expanded access program demonstrate the effectiveness of Cangene Corporation's eBAT NP-018 product in treating patients with suspected or confirmed botulism.

Endpoints:

The primary efficacy endpoint is the duration of hospitalization.

Randomization Scheme and Blinding:

N/A

Datasets and Population Analyzed in the BLA:

In total, data from 148 patients treated with eBAT NP-018 was submitted to Cangene Corporation by the CDC. All 148 patients who received at least one dose of eBAT NP 018 are included in the safety analysis population, regardless of whether the final diagnosis was botulism, Guillain-Barré syndrome, myasthenia gravis, tick paralysis or other. The efficacy analysis population includes patients with a discharge diagnosis of suspected or confirmed botulism, or where the final diagnosis was unknown. The inclusion of patients for which the discharge diagnosis is unknown is based on the principle of intent-to-treat. A total of 109 patients are included in the efficacy analysis population including 97 patients with a final diagnosis of botulism per the treating physician or based on confirmation from the CDC; 10 patients with the final diagnosis unknown; and 2 patients with a diagnosis of suspected botulism captured under "other".

A subset of the efficacy analysis population includes only those patients with a CDC confirmed diagnosis of botulism and/or serotype identification (n=51).

Missing data were treated as missing and no imputation methods were used.

3.1.3.2 Patient Disposition, Demographic and Baseline Characteristics

As of December 31, 2011, 148 patients with suspected or confirmed botulism have received eBAT NP-018 under the CDC's expanded access program (BB-IND 6750 [1 patient was treated under emergency IND: BB-IND 13615]). These patients consisted of 105 males (70.9%) and 43 females (29.1%) ranging in age from 10 days to 88 years at the time of eBAT NP-018 treatment. The majority of patients were Caucasian (41.2%) or of unknown race (41.9%). A summary of patient demographics is provided in Table 6.

Table 6. Summary of Patient Demographics

Parameter		
Age (Years)	Mean	46.1
	SD	17.63
	Median	46.5
	Range	10 days - 88 years
Age Group	<18	7 (4.7%)
	18-39	49 (33.1%)
	40-64	72 (48.6%)
	65-75	11 (7.4%)
	>75	9 (6.1%)
Sex	Female	43 (29.1%)
	Male	105 (70.9%)
Ethnicity	Hispanic/Latino	54 (36.5%)
	Non-Hispanic/Non-Latino	45 (30.4%)
	Unknown	49 (33.1%)
Race	African-American/Black	3 (2.0%)
	Alaska Native	8 (5.4%)
	American Indian, Alaska Native	2 (1.4%)
	Asian	6 (4.1%)
	White	61 (41.2%)
	Other	6 (4.1%)
	Unknown	62 (41.9%)

3.1.3.3 Statistical Methods

A log linear model was fitted to the data. The sponsor called the analysis a logistic regression model in the report. Since the primary endpoint was the duration of hospitalization in days and log transformation was applied to the analysis, the model should be called a log linear model. Only patients who were confirmed to have foodborne or wound forms of botulism were included in the log linear model analysis since the sample sizes in the rest of the other categories is small. Botulinum neurotoxin serotype was not included in the model as a factor due to small sample sizes for all serotypes other than A. After a backward selection procedure with an alpha level of 0.10, three factors were included in the model: (a) botulism route of exposure (food-borne or wound), (b) intubation (1 = required or 2 = not required) and (c) time from onset of symptoms to treatment with eBAT NP-018.

In order to evaluate the effectiveness of eBAT NP-018 in the treatment of botulism, patients were stratified on the basis of the time from onset of symptoms to the first administration of eBAT NP-018. It is anticipated that delayed treatment with eBAT NP-018 will have limited efficacy based on the mechanism of action, thus patients in the delayed treatment group will act as a comparator group for the evaluation of efficacy. A

stratification cutoff of 2 days from the onset of symptoms to first administration of eBAT NP-018 was selected because circulating toxins are anticipated to still be present 2 days from the onset of symptoms. Two days is also a sufficient period of time to allow a diagnosis of suspected botulism to be made by the attending physician and for eBAT NP-018 to be shipped by the CDC. In order to evaluate the impact of defining early administration as being within 2 days from the onset of symptoms, the model was also fitted using 3 days from symptom onset as the definition of early administration.

Two separate log linear models were fitted to the data, one for each of the two efficacy analysis populations (n = 40 for confirmed cases and n= 72 for all suspected cases). The statistical modeling was performed using the generalized linear model (GLM) procedure in SAS version 9.2.

In addition to the log linear modeling performed on the duration of hospitalization data, a simple two sample t-test was performed on the log-transformed duration of hospitalization for patients treated early (0 – 2.0 days) or late (>2.0 days) from the time of onset of symptoms. Patients who were confirmed to have iatrogenic, infant or other forms of botulism were also included in the analysis. Subset analysis on patients with confirmed case was also conducted.

Summary statistics were also generated to describe the patient population treated with eBAT NP-018. Statistics include the mean, median, standard deviation (SD), minimum and maximum for all continuous variables, and include the count and percentage for all categorical variables.

3.1.3.4 Results and Conclusions

For both analyses on the population with confirmed botulism and population with suspected botulism, the duration of hospitalization of patients was statistically significant different between the early treatment group and the late treatment group at 0.10 significance level (P=0.0611). Route of exposure and intubation were also statistically significant in the model (P=0.0705 and P<0.001 respectively). The result of the two sample t-test was consistent with the log linear model analysis result (P=0.0640).

For the subgroup analysis of population with confirmed botulism, similar results were obtained. Intubation and early/late treatment were both statistically significant at 0.10 significance level (p<0.001 and p=0.0158 respectively). However, route of exposure was not significant any more in the model (p=0.5713). The two sample t-test also showed significant difference between the early treatment group and the late treatment group (p=0.0208) at 0.10 significance level.

Since these efficacy analyses were performed *post-hoc*, the choice of statistical methods and the cutoff point for the time from onset of symptoms to first treatment with eBAT NP-018 were examined to determine if there was any impact on the overall efficacy results. The positive relationship between time to treatment and duration of hospitalization was also demonstrated when the cutoff point was 3 days instead of 2 days

for the time from onset of symptoms to first treatment. The results were consistent with the results from the analysis with the 2 days cutoff point.

The sponsor considered the results reported in study BB-IND 6750 as preliminary and are intended to be verified using additional data collected through the CDC expanded access program which is currently ongoing. The sponsor anticipated that an additional 50 or more patients may be available prior to the licensure of eBAT NP-018 based on current project timelines and previous enrolment rates.

3.2 Evaluation of Safety

3.2.1. Study BT-001: Pharmacokinetics of a heptavalent equine-derived botulinum antitoxin (NP-018)

Clinical trial BT-001 was a single center, double blind, randomized, parallel arm study to assess the safety and PK of eBAT NP-018 following IV administration of either a single dose (one vial; n = 20) or double dose (two vials; n = 20) of eBAT NP-018 to normal healthy subjects. The study was conducted in a single Phase I clinic at -----(b)(4)----- ----------. The primary objective of the study was to evaluate the safety of eBAT NP-018 based upon clinical observations, adverse events (AE) and laboratory assessments. The subjects were followed for twenty eight days. The secondary objective was to assess the PK of the seven botulism antitoxin serotypes contained in eBAT NP-018 following IV administration.

No formal statistical analysis but only summary statistics was provided for the safety assessments conducted during clinical trial BT-001. There were no substantial safety issues and no serious adverse events occurred during this clinical trial. One subject was withdrawn due to AEs resulting from a moderate allergic reaction. The most frequently reported adverse events were mild and moderate headache (possibly or probably treatment related) and mild somnolence (unlikely treatment related). The remaining adverse events occurred in less than 10% of subjects. The adverse events were reported as mild or moderate in severity and most were resolved without concomitant therapy. Drug-related AEs included headache, dysphagia, flatulence, nausea, throat irritation, feeling cold, pain, pyrexia, swelling, pharyngolaryngeal pain, hyperhydrosis, pruritus, pruritus generalized, skin disorder and urticaria.

3.2.2. Study BT-002 Stage B: Botulism antitoxin effects on paralysis induced by type A and type B botulinum toxins in the extensor digitorum brevis muscle

Clinical trial BT-002 Stage B was a single center, double blind, randomized, parallel arm study to assess the safety and PD of eBAT NP-018 (n = 16) or placebo (0.9% saline; n = 10) following IV administration to normal healthy subjects. The primary objective of the study was to evaluate the effect of eBAT NP- 018 in preventing paralysis of the extensor digitorum brevis (EDB) muscle following the administration of botulinum neurotoxin serotypes A (Botox®) or B (Myobloc®) in the human body prior to uptake by the cholinergic nerve endings. Since the principle effect of exposure to botulism in humans is muscle paralysis, inhibition of muscle paralysis induced by BoNT serotype A (Botox) or BoNT serotype B (Myobloc), was used as a surrogate endpoint to demonstrate the effectiveness of eBAT NP-018 in humans. The secondary objective was to evaluate the

safety of eBAT NP-018 in normal healthy subjects. The subjects were followed for a total of twenty eight days.

Safety evaluations included monitoring AEs, laboratory results, physical examinations, vital signs and electrocardiograms (ECG). No formal statistical analysis but only summary statistics was provided for the safety assessments conducted during clinical trial BT-002 Stage B.

There were no notable differences in the number of adverse events or laboratory abnormalities between the eBAT NP-018 and placebo treated groups. Some laboratory findings were found to be out-of-range (OOR) but were judged as not being clinically significant by the Principal Investigator (PI). However, one subject developed adverse events related to eBAT NP-018 administration including urticaria, elevated body temperature and chest discomfort within five minutes of the start of the eBAT NP-018 infusion. Elevated fibrinogen levels post-dosing were also detected in this subject.

3.2.3. BB-IND 6750: Use of NP-018 heptavalent equine-based botulinum antitoxin (HBAT) after exposure to *Clostridium botulinum* toxin or other closely-related botulinum toxin-producing *Clostridia* species due to a naturally-occurring outbreak or isolated, unintentional incident.

Refer to section 3.1.3 for details in study design of BB-IND 6750. No formal statistical analysis was conducted for safety evaluation, but only summary statistics was provided. Adverse reaction information was available from one hundred and forty six of the one hundred and forty eight patients treated with eBAT NP-018. It was well tolerated by one hundred twenty eight patients (87.7%) of the patients, with eighteen patients experiencing adverse reactions related to eBAT NP-018 administration. No patients experienced anaphylaxis however; one patient did experience mild serum sickness. A second patient experienced hemodynamic instability including two episodes of severe bradycardia with one episode progressing to cardiac arrest. A third patient experience rebound toxicity. Six deaths (4.1%) were also reported to the CDC however, all were determined by the CDC's botulism subject matter expert (SME), based on information provided by the treating physician/hospital, to be unrelated to eBAT NP-018 treatment.

4. SUMMARY AND CONCLUSIONS

There are three efficacy and safety studies reviewed in the memo. From statistical point of view, this review did not find any major discrepancy (only minor typographical errors) or any major problems regarding the efficacy and safety analysis of the studies currently. Our review is still ongoing and this statistical review memo serves as a mid-cycle assessment for the biological license application under STN 125462/0.