

CLINICAL PHARMACOLOGY BLA REVIEW

Division of Hematology
Office of Blood Review & Research

BLA 125462

Product: Heptavalent equine-derived botulinum antitoxin, Types A, B, C, D, E, F and G (NP-018)

Sponsor: Cangene Corporation

Indication: For the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin (BoNT) serotypes A, B, C, D, E, F or G.

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EXECUTIVE SUMMARY

Botulism is a rare and sometimes fatal paralytic illness that occurs when neuromuscular transmission is interrupted by botulinum neurotoxins (BoNTs) produced by *Clostridium botulinum* and other related clostridial species. Botulinum neurotoxins are some of the most potent neurotoxins and exist in seven antigenically distinct serotypes, designated by the letters A through G. Humans are susceptible to all seven BoNT serotypes.

Botulism Antitoxin Heptavalent equine (A, B, C, D, E, F, G) (eBAT NP-018) is an equine hyperimmune product that is prepared from plasma obtained from horses that have been immunized with a specific serotype of botulinum toxin. Botulism Antitoxin Heptavalent is indicated for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin serotypes A, B, C, D, E, F or G. Botulinum neurotoxin serotypes A, B, E and F are the most common cause of food-borne, wound and infant botulism.

Currently in North America, there are no available licensed products to treat botulism except for Botulism Immune Globulin Intravenous (BabyBIG or BIG-IV), which is a human immune globulin product used to treat infant botulism (< one year of age) caused by BoNT serotypes A and B. To meet this unmet medical need, Cangene Corporation has developed Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) – (Equine) for the treatment of symptomatic botulism following documented or suspected exposure to BoNT serotype A, B, C, D, E, F or G.

Since the evaluation of human efficacy of Botulism Antitoxin Heptavalent is unethical and not feasible, Cangene Corporation is using the “Animal Rule” (Title 21 Code of Federal Regulation (CFR) 601 Subpart H) to seek approval for its equine heptavalent botulism antitoxin product.

In order to evaluate the efficacy and pharmacokinetics of NP-018, the sponsor conducted the following studies:

- Therapeutic efficacy of NP-018 was conducted in guinea pigs and was subsequently used to determine the efficacy of all seven botulism antitoxin serotypes. Rhesus monkey was also used to evaluate the efficacy of NP-018 although only for serotype A. The effect of NP-018 was evaluated in humans in preventing paralysis of the extensor digitorum brevis (EDB) muscle following Botox or Myobloc administration.
- Pharmacokinetic studies were conducted in guinea pig, monkey, and humans.
- A population PK/PD model was developed to support the human dosing regimen for NP-018.

ANIMAL EFFICACY STUDIES

GUINEA PIGS

Treatment Group:

Efficacy in the guinea pig was demonstrated when animals were intoxicated with one of the seven BoNT serotypes (A-G) at a dose equivalent to 1.5x guinea pig intramuscular lethal dose 50% (GPIMLD₅₀). At the onset of four consecutive moderate or severe clinical signs, groups of thirty four animals per serotype were administered either 1x scaled human dose eBAT NP-018 or placebo intravenously and observed for twenty one days. Nearly all treated animals (97% or 100% per serotype) survived following intoxication at the target 1.5x GPIMLD₅₀ dose level. In contrast, Serotypes A, B, C, D, E and F placebo control groups experienced $\geq 85\%$ mortality following intoxication at the target 1.5x GPIMLD₅₀ dose level, and the Serotype G placebo control group (G2) experienced 50% mortality. No NP-018 treated animals in Serotype G died, so increased survival in the treated group was statistically significant. The incidence of most clinical signs was substantially reduced, with later times-to-onset and shorter durations in NP-018 treated animals when compared to controls in all serotypes.

Table 1: Survival results in Guinea Pig

BoNT Serotype	Treatment	Survival (%)	Fisher's Exact Test (p-value)
A	1x eBAT NP-018	34/34 (100%)	p<0.0001
	Placebo Control	0/34 (0%)	
B	1x eBAT NP-018	34/34 (100%)	p<0.0001
	Placebo Control	1/34 (3%)	
C	1x eBAT NP-018	33/34 (97%)	p<0.0001
	Placebo Control	4/34 (12%)	
D	1x eBAT NP-018	33/34 (97%)	p<0.0001
	Placebo Control	5/34 (15%)	
E	1x eBAT NP-018	34/34 (100%)	p<0.0001
	Placebo Control	0/34 (0%)	
F	1x eBAT NP-018	34/34 (100%)	p<0.0001
	Placebo Control	4/34 (12%)	
G	1x eBAT NP-018	34/34 (100%)	p<0.0001
	Placebo Control	17/34 (50%)	

BBRC = Battelle Biomedical Research Center; BoNT = Botulinum Neurotoxin; eBAT NP-018 = Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) – (Equine).

Postexposure Prophylaxis Group:

Five groups of 20 guinea pigs (10 males and 10 females) were administered with the toxin of the appropriate serotype at a toxin dose equivalent to 4x GPIMLD₅₀ via a single intramuscular injection. A single intravenous treatment of either NP-018 antitoxin at $\times 1$, $\times 0.2$, $\times 0.04$, or $\times 0.008$ scaled human dose or Placebo was administered approximately 12 hours after the toxin administration for Serotypes A, B, C, D, F, G and at approximately 6 hours post-intoxication for Serotype E. Animals were monitored frequently for clinical signs and mortality throughout the

study until termination of study on day 21. Results for 1x and 0.2x the human dose levels and placebo are shown in Table 2

Table 2: Survival results in Guinea Pigs

BoNT Subtype	Treatment Dose Level¹	Survival	Mean Time To Death² (d)
A	1x (0.2x)	20/20 (19/20)	
	placebo	0/20	3.5
B	1x (0.2x)	19/20 (20/20)	
	placebo	0/20	3.6
C	1x (0.2x)	20/20 (18/20)	
	placebo	0/20	3.5
D	1x (0.2x)	20/20 (20/20)	
	placebo	0/20	2.1
E	1x (0.2x)	19/19 (20/20)	
	placebo	0/20	0.9
F	1x (0.2x)	20/20 (20/20)	
	placebo	0/20	2.2
G	1x (0.2x)	19/20 (20/20)	
	placebo	0/20	2.4

¹ compared to proposed clinical NP-018 dose (mL/kg basis)

² Kaplan-Meier estimates

Control groups in all Serotypes had 100% mortality, confirming the lethality of the toxin dose used (4x GPIMLD₅₀). A decrease in mortality was observed among most NP-018 treated animals compared to the control group. The mean and median time to death in the NP-018 treated groups at all dose levels were longer than control animals intoxicated with the same Serotype. Treatment with any dose level of NP-018 resulted in a delayed mean time to onset of clinical signs when compared to control animal values.

NON-HUMAN PRIMATES (Rhesus Monkey)

Treatment Group:

Efficacy of eBAT NP-018 in the Rhesus macaque (n =30) was determined where animals were intoxicated intravenously with BoNT serotype A dose equivalent to 1.7x non-human primate lethal dose 50% (NHP LD₅₀/kg). eBAT was administered as either 1x scaled human dose of NP-018 or as placebo intravenously at the onset of clinical signs. Following treatment all animals were provided supportive care (nutritional) and observed for twenty one days. Results are compiled in Table 3.

Table 3: Survival results in Non Human Primates (NHP)

Group	Survival (%)	p-value	Median Time to Death (days)
NP-018	14/30 (47%)	< 0.0001 ^a	7.9
Placebo	0/30 (0%)		3.1

^a Fisher's Exact Test

Treatment with NP-018 resulted in statistical significant improvement in survival as compared to placebo controls. Out of 30 monkeys, 14 monkeys survived in the treated group whereas, all 30 monkeys died in placebo group.

Postexposure Prophylaxis Group:

Three groups of ten (10) rhesus monkeys received an intravenous injection of approximately 4x NHP LD₅₀ of Botulism toxin Type A. Two groups of ten rhesus monkeys per group received NP-018 (0.1 or 1.0 scaled human dose) intravenously about 4 hours following the intravenous intoxication. A third group of 10 rhesus monkeys received botulism antitoxin placebo. The animals were evaluated for 14 days post dose. Survival results are computed in Table 4. In order to determine the terminal half-life of NP-018 in the presence of BoNT/A blood samples were taken in Group 1 and Group 2.

Table 4: Survival in Non Human Primates (NHP)

Groups (Number of animals)	Number Survived to Study End	Median Survival Time (95% Confidence Interval) [*]
Treatment Group 1 (n=10)	10	> 362 hours (. . .)
Treatment Group 2 (n=10)	10	> 362 hours (. . .)
Treatment Group 3 (n=10)	0	36.5 hours (28.0, 39.0)

* The limits are presented except when the estimated survival distribution of the group did not cross 0.50, in which case they are shown as (. . .).

All animals in Treatment Groups 1 and 2 survived until scheduled sacrifice at study termination on days 14 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. For 1 exemption, 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.

Only animals in Treatment Group 1 had titers above the limit of detection of the mouse neutralization assay (MNA). The mean terminal half life (HL) for the antitoxin titers in serum was 4.7 hours with a range between 2.6 to 6.7 hours. This HL is similar to the results of 1x NP-018 in rhesus monkeys (Study # FY07-056), but without presence of the toxin BoNT/A (HL = 3.0, SD = 0.8). It is noteworthy, that in the 0.1x NP-018 Group 2 all animals survived, although all titers were below the limit of detection. This indicates that although not detectable by the MNA there was still sufficient botulism antitoxin present to protect animals from BoNT intoxication.

HUMAN EFFICACY STUDY

This clinical study was designed as an exploratory pharmacodynamic (PD) study to evaluate the ability of NP-018 in neutralizing botulinum toxins types A and B (Botox® and Myobloc®, respectively) in a validated local muscle model in healthy subjects. There were 26 subjects in this study. The subjects were randomized to receive either a single intravenous (IV) infusion of NP-018 over 150 minutes or placebo (0.9% saline solution) one day prior to intramuscular administration of botulinum toxin types A (5 U, Botox®) and B (500 U, Myotox®). The sites of injection for Botox® and Myotox® were the extensor digitorum brevis (EDB) muscles of the left and right foot, respectively.

Pharmacodynamic assessments were based primarily on the preservation of muscle function in both feet following the administration of toxins. The pharmacodynamic endpoints of this study were the percent muscle function based on the preservation of the compound muscle action potential (CMAP) M wave amplitude and area of the EDB muscle..

The results of the study indicated that subjects given NP-018 prior to exposure to botulinum toxins A and B (Botox® and Myobloc®, respectively) had very little to no loss of percent muscle function in the EDB muscles of both feet over the 28 day study period. On the other hand, subjects in the control group (placebo) had a significant decrease in percent muscle function following exposure to botulinum toxins A and B. This reduction in muscle function was maintained over the 28 day study period.

PHARMACOKINETIC STUDIES

Pharmacokinetic (PK) studies of NP-018 were conducted in guinea pig, rhesus monkey, and humans. The following is the summary of PK studies in these species.

Guinea Pigs:

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

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

Rhesus Monkey:

Twelve non-human primates were randomly assigned to two treatment groups. On Day 0, all animals received a single NP-018 IV dose. Group 1 animals were dosed at x5 scaled human dose, and Group 2 animals were dosed at x1 scaled human.

Blood samples were collected from all animals prior to dose administration and up to 20 post-dose. NP-018 concentrations (only serotype A) were measured in serum using a mouse neutralization assay. The PK parameters were estimated by non-compartmental analysis.

Results showed that the pharmacokinetics of NP-018 (serotype A) is non-linear (AUC did not increase proportionally with dose). The half-life of NP-018 is about 2 hours longer at the high dose as compared to low dose. There was no gender difference in the PK of NP-018.

Humans:

This was a Phase 1, single-center, randomized, double-blind, parallel arm study. NP-018 was intravenously administered to healthy, male and female volunteers between the ages of 19 and 52 years. Forty subjects were randomized to receive either one or two vials of NP-018, representing a single or double dose of botulinum antitoxin. Each dose was administered by slow intravenous infusion over 2.5 hours. The infusion rate was incremental, starting slowly and increasing if no safety related events were evident.

Blood samples for pharmacokinetic study were collected after NP-018 administration for botulinum toxin up to Day 28. Pharmacokinetic parameters were calculated by non-compartmental analysis (Table 5) using the concentration-time data generated from the mouse neutralization assay.

Table 5: Pharmacokinetic parameters for antitoxin serotypes A-G in subjects following intravenous administration of one or two vials of NP-018. Coefficient of variation in parenthesis.

Serotype	Treatment Group	AUC ₀₋₄ (U* ^h /mL)	AUC _{0-∞} (U* ^h /mL)	AUC ₀₋₄ / AUC _{0-∞} (%)	C _{max} (U/mL)	T _{max} (h)	Half-life (h)	λ _z (1/h)	Cl (mL/h)	V _d (mL)
A	1 Vial	21.40 (20.1)	26.00 (13.1)	86.3 (4.5)	2.685 (28.2)	0.698 (115.0)	8.64 (15.5)	0.0821 (15.8)	293 (13.5)	3637 (17.1)
	2 Vials	51.92 (27.4)	56.09 (24.7)	92.5 (5.6)	6.234 (22.0)	0.528 (4.1)	10.2 (34.7)	0.0759 (32.8)	285 (27.1)	3993 (27.7)
B	1 Vial	27.16 (23.0)	29.30 (19.2)	95.8 (1.7)	1.897 (42.7)	0.888 (125.0)	34.2 (40.4)	0.0250 (52.0)	196 (24.0)	9607 (48.1)
	2 Vials	58.53 (16.6)	62.55 (16.4)	95.5 (2.4)	4.282 (33.8)	0.540 (8.9)	57.1 (69.4)	0.0185 (60.8)	181 (19.3)	14865 (71.5)
C	1 Vial	36.63 (25.2)	37.34 (27.3)	94.8 (1.8)	2.263 (38.1)	1.90 (150.0)	29.6 (44.9)	0.0282 (42.3)	144 (28.1)	6066 (52.8)
	2 Vials	78.75 (29.0)	86.25 (28.8)	93.2 (3.9)	4.890 (41.7)	0.525 (4.1)	45.6 (34.0)	0.0169 (35.9)	127 (33.9)	8486 (52.4)
D	1 Vial	5.578 (48.5)	7.616 (23.3)	90.0 (5.8)	0.812 (55.0)	0.891 (124.0)	7.51 (24.4)	0.0968 (22.6)	137 (19.9)	1465 (25.8)
	2 Vials	13.27 (33.7)	14.83 (39.4)	89.3 (5.4)	1.603 (38.8)	0.704 (111.0)	7.77 (21.6)	0.0932 (21.7)	151 (30.5)	1653 (30.5)
E	1 Vial	6.653 (32.4)	7.162 (23.9)	89.8 (5.0)	0.938 (41.6)	0.518 (3.9)	7.75 (19.1)	0.0926 (20.8)	1250 (25.1)	14172 (32.1)
	2 Vials	13.47 (20.2)	15.66 (15.6)	90.4 (5.3)	1.749 (32.9)	0.534 (9.4)	7.32 (23.9)	0.0997 (23.1)	1110 (16.4)	11596 (22.4)
F	1 Vial	29.12 (29.1)	31.40 (25.4)	96.7 (2.7)	2.367 (25.8)	1.07 (122.0)	14.1 (16.7)	0.0508 (20.1)	169 (25.5)	3413 (29.3)
	2 Vials	59.63	63.19	96.5	4.285	1.63	18.2	0.0434	168	4334

Serotype	Treatment Group	AUC ₀₋₂₄ (U*h/mL)	AUC _{0-∞} (U*h/mL)	AUC ₀₋₂₄ / AUC _{0-∞} (%)	C _{max} (U/mL)	T _{max} (h)	Half-life (h)	λz (1/h)	Cl (mL/h)	V _d (mL)
		(26.5)	(25.0)	(4.0)	(31.0)	(149.0)	(42.9)	(33.3)	(26.4)	(50.9)
G	1 Vial	6.332 (26.2)	7.047 (23.3)	92.8 (5.1)	0.586 (27.7)	1.28 (154.0)	11.7 (38.1)	0.0689 (40.8)	149 (22.8)	2372 (29.5)
	2 Vials	13.40 (27.3)	14.66 (23.6)	97.0 (1.8)	1.193 (43.8)	0.704 (111.0)	14.7 (16.5)	0.0483 (17.9)	144 (23.8)	3063 (29.4)

The estimated pharmacokinetic parameters AUC, clearance, and half-life varied based upon the antitoxin serotype measured (Table 5). Both AUC_(0-∞) and C_{max} values increased in a dose proportional manner as NP-018 doses increased from one to two vials. The half-lives of the different antitoxin serotypes varied with the serotype. Antitoxin serotypes D and E had the shortest mean half-lives, ranging from 7.5 to 7.8 hours whereas, antitoxin serotypes B had the longest mean half-lives, ranging from 34.2 (one vial) to 57.1 (2 vials) hours. Comparison of the pharmacokinetic parameters between male and female subjects for antitoxin serotypes A through G showed that there were no gender related differences following a single intravenous administration of either one or two vials of NP-018.

Comparison of Clearance among Species:

The clearance of NP-018 appears to be much slower in humans than guinea pig and monkey for serotype A. Based on per kg basis; human clearance for serotype A is approximately 3.5-fold lower than guinea pig and monkey. The human clearance of serotypes B to G is almost 2.7 to 6.2 fold lower than guinea pigs. This comparison is helpful in justifying the proposed human dose.

POPULATION PK/PD MODELING AND SIMULATIONS TO SUPPORT HUMAN DOSING REGIMEN OF NP-018

The objective of this study was to present pharmacokinetic (PK) and pharmacodynamic (PD) modeling results using data related to guinea pigs, non-human primates (NHP) and humans in order to support the human dosing regimen of NP-018.

PK and PD information of NP-018 collected in a total of 5 studies (3 PK, 2 post-exposure prophylaxis PD) performed by Cangene Corporation that were included in the analysis.

Population PK analysis of NP-018 for all serotypes were best fitted using a 3-compartment model, with the exception of Serotype E which was fitted using a 2-compartment model. The estimated population PK parameters of NP-018 derived for all serotypes are summarized in Table 6.

Table 6: Final Population PK Parameters of NP-018 for All Serotypes

Parameter	PK Parameters of NP-018 (Geometric Mean)						
	Serotype A	Serotype B	Serotype C	Serotype D	Serotype E	Serotype F	Serotype G
CL (mL/h/kg)	14.75	10.82	8.08	4.44	7.72	12.25	10.01
CLd (mL/h/kg)	2.70	7.24	16.17	5.29	0.88	43.40	2.84
CLdt (mL/h/kg)	8.15	136.03	4.56	2.63	NA	2.53	40.48
Vc (mL/kg)	31.88	21.32	56.45	0.66	0.25	32.44	20.56
Vp (mL/kg)	21.17	145.29	25.44	14.93	4.19	44.23	67.04
Vdt (mL/kg)	23.38	34.39	1227.12	5.44	NA	40.94	28.03
Proportional Error (%)	24%	34%	45%	30%	38%	35%	35%

NA = Not Applicable

Exposure-Response Model:

Logistic regression was used to explore the relationship between NP-018 exposure (AUC) predicted by the population PK model and the probability of survival. The human projected probabilities of survival for serotypes A to G following a human dosing of 1x NP-018 ranged from 96% to 99.9%.

Comments:

- Overall, the studies are acceptable from Clinical Pharmacology perspective.
- The submitted exposure-response model only takes into account data from 2 “post-exposure prophylactic” animal studies. The results of additional 2 “treatment” studies have not been incorporated into the model. However, based on their design those two “treatment” studies are considered relevant, because the results can be directly linked to the proposed indication in the label.
- The study results in NHP only allow an assessment of the Botulism Antitoxin Heptavalent (Equine) Type A. The effects of antitoxins type B, C, D, E, F, and G on corresponding BoNT serotypes have not been investigated.
- Clinical study BT-002 Part B was designed as an exploratory pharmacodynamic study employing a local in-vivo model. Because the BoNT intoxication is expected to occur in patients systemically not locally, the observed clinical responses can only be considered supportive for the overall risk/benefit assessment.
- The pharmacodynamic study results of the clinical study BT-002 Part B only allow an assessment of the Botulism Antitoxin Heptavalent (Equine) Type A-and Type B. The effects of antitoxins type C, D, E, F, and G on corresponding BoNT serotypes have not been investigated.

OVERALL COMMENTS

- The study results in NHP only allow an assessment of the Botulism Antitoxin Heptavalent (Equine) Type A. The effects of antitoxins type B, C, D, E, F, and G on corresponding BoNT serotypes have not been investigated.
- Clinical study BT-002 Part B was designed as an exploratory pharmacodynamic study employing a local in-vivo model. Because the BoNT intoxication is expected to occur in patients systemically not locally, the observed clinical responses can only be considered supportive for the overall risk/benefit assessment.
- The pharmacodynamic study results of the clinical study BT-002 Part B only allow an assessment of the Botulism Antitoxin Heptavalent (Equine) Type A-and Type B. The effects of antitoxins type C, D, E, F, and G on corresponding BoNT serotypes have not been investigated.
- In the --(b)(4)-- CANG-RAS-001 report the therapeutic efficacy animal studies in Rhesus Macaques (No. FY10-066) and in Guinea Pigs (No. 1180-G005630) have not been included in the exposure-response and risk analysis. These 2 treatment studies are considered relevant for approval.

CLINICAL PHARMACOLOGY DOSE JUSTIFICATION

H-BAT [Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) – (Equine)] is indicated for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin (BoNT) serotypes A, B, C, D, E, F or G. The proposed dose of NP-018 in adults is 11.2 mL/vial, with a final fill volume depending on actual drug potencies. There are no human efficacy data for NP-018. As a result the human efficacious dose was derived from animal pharmacokinetics and efficacy studies and a human pharmacokinetic study.

In guinea pigs, efficacy (post-exposure prophylaxis) of NP-018 was assessed following a single intravenous (IV) dose of NP-018 at $\times 1$, $\times 0.2$, $\times 0.04$, or $\times 0.008$ scaled human doses. Not more than 1 death, out of a total of 20 animals per serotype, was noted in the NP-018 group for all 7 serotype at a scaled human dose of $\times 1$. A similar observation was noted in a separate pivotal efficacy (treatment) study.

In non-human primates (NHP), efficacy (post-exposure prophylaxis) of NP-018 was assessed following a single intravenous dose of NP-018 at $\times 1$, and $\times 0.1$ scaled human dose. In both dose groups, all animals survived. In a separate treatment study, animals received NP-018 at $\times 1$ scaled human dose. Only 47% of animals survived. All efficacy studies in NHP only assessed serotype A.

Pharmacokinetic studies in guinea pig, NHP, and humans indicated that the clearance of NP-018 ($\times 1$ scaled human dose) in humans is 3 to 5 times slower than guinea pigs and NHP, indicating higher exposure of NP-018 in humans than animals. This indicates that the humans will have higher protective drug levels against botulinum toxin compared to animals.

Pharmacokinetic and pharmacodynamic modeling and simulation supported the efficacy of 11.2 mL/vial NP-018 in humans. Based on the results of an exposure-response analysis, the predicted probabilities of human survival for the serotypes A to G following a dosing of 1x NP-018 ranged from 95.9%, to 99.9%.

In humans, a pharmacokinetic study of NP-018 was analyzed following a single intravenous dose of NP-018 at $\times 1$, and $\times 2$ scaled human doses. The sponsor's goal stated that NP-018 serum concentrations after IV administration of 1 vial ($\times 1$) should be able to counter BoNT serum concentrations of 400 to 20,000 MIPLD₅₀/mL. The following table (Table 1) displays the relationships between the 7 BoNT subtypes, measured (observed) maximal serum drug concentrations (at 30 min after start of the IV infusion) using actual potencies, and the calculated neutralizing capacity (NC) against the different BoNT subtypes. The NC were calculated as follows: 1 U of NP-018 neutralizes 10,000 MIPLD₅₀/mL BoNT for all serotypes except for serotype E, which equates 1 U of NP-018 to 1,000 MIPLD₅₀/mL. The range of the NC based on observed drug concentration is between 940 and 26,900 MIPLD₅₀/mL. Even with 40% less potency/vial the NC against BoNT is still between 400 and 20,000 MIPLD₅₀/mL. Compared to an actual measurement of BoNT serotype E in patients serum (≈ 160 MIPLD₅₀/mL, foodborne intoxication) the predicted NC of 1 vial NP-018 appears to be more than sufficient to counter expected BoNT concentrations in humans.

Table 1. NP-018 Observed C_{max} and Neutralizing Capacity against BoNT

BoNT Subtype	NP-018 Observed C_{max} (U/mL)	Neutralizing Capacity against BoNT (MIPLD₅₀/mL)
A	2.69	26,900
B	1.90	19,000
C	2.26	22,600
D	0.81	8,100
E	0.94	940
F	2.37	23,700
G	0.59	5,900

In short, based on the animal and human pharmacokinetic studies and animal efficacy studies, it appears that 11.2 mL or one vial NP-18 will be efficacious in humans against botulinum antitoxin.

CLINICAL PHARMACOLOGY LABELING COMMENTS

Dosing in Pediatric Population:

The infant dose (<1 year) for H-BAT is 10% of the adult dose regardless of body weight

Sponsor: Please provide the rationale for your dosing proposal in infants. Please note and comment to the following regarding dosing of Botulinum antitoxin derived from equine to the infants as mentioned by the Center of Drug Control (CDC), 1998:

“Equine antitoxin rarely has been used in infant botulism because of the risk of inducing lifelong hypersensitivity to equine antigens and lack of evidence of its benefit. Also, because of early concerns that anaphylactic reactions with the equine-derived product might be more severe in infants, few infants have been given the product”.

2.4 Administration

Please add the total duration of infusion time in Table 1.

8.4 Pediatric Use

~~Safety, and effectiveness of H-BAT has not been established in pediatric patients.~~

Replace with:

Safety, pharmacokinetics, and effectiveness of H-BAT has not been established in pediatric patients.

8.5 Geriatric Use

~~Safety and effectiveness of H-BAT has not been established in geriatric patients~~

Replace with:

Safety, pharmacokinetics, and effectiveness of H-BAT has not been established in geriatric patients

12.2 Pharmacodynamics

A ~~pharmacodynamic~~ clinical dose-response trial was conducted using the extensor digitorum brevis (EDB) muscle of the foot as a model for measuring muscle paralysis after exposure to botulism toxin.

RECOMMENDATION

The study design and conclusions of pharmacokinetic and pharmacodynamic studies are acceptable and the sponsor's proposed dose of 1 vial (0.16 mL/kg) appears to be adequate for neutralizing botulinum toxins in adults.

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INTRODUCTION

Botulism is a rare but serious neuroparalytic illness resulting from the action of a neurotoxin produced by the bacterium *Clostridium botulinum*. *C. Botulinum* produces 7 serologically distinct neurotoxins identified as serotypes A, B, C, D, E, F, and G. Naturally occurring botulism is rare, with less than 200 cases reported in the USA annually. Botulism results from food poisoning, wound and intestinal botulism as well as inhalation, although this form of exposure does not occur naturally. In all cases, botulism toxin is absorbed into the circulation, carried in the bloodstream to the peripheral cholinergic synapses, where it binds irreversibly to block neurotransmitter release at neuromuscular junctions. The predominant clinical effect of exposure to botulism is flaccid muscle paralysis. In severe forms, this leads to paralysis of the respiratory muscles and causes the life-threatening condition of respiratory failure. Therapy for botulism consists of supportive care and administration of botulism antitoxins.

Cangene Corporation is using the “Animal Rule” (Title 21 Code of Federal Regulation (CFR) 601 Subpart H) to seek approval for its equine heptavalent (A, B, C, D, E, F, G) botulism antitoxin product (H-BAT NP-018), an equine hyperimmune product that is prepared from plasma obtained from immunized horses. The heptavalent botulism antitoxin is a sterile solution of purified F(ab')₂ plus Fab' and F(ab')₂ related immune globulin (Ig) fragments produced from equine immune globulins modified by pepsin digestion. The effective antitoxin Ig fragment is F(ab')₂. Botulism antitoxin heptavalent (A, B, C, D, E, F, G) – (equine) is formulated with 10% maltose and 0.03% polysorbate 80 (PS80). The formulated bulk material contains approximately 6g% (60 milligrams/milliliter) protein.

The manufacturing process for each antitoxin type includes cation-exchange chromatography to purify the immune globulin fraction, digestion with pepsin to produce F(ab')₂ plus Fab and F(ab')₂ related fragments, anion exchange chromatography to remove the pepsin as well as other impurities and filtration. In addition, the manufacturing process includes two viral inactivation/removal steps; solvent/detergent (S/D) treatment and virus filtration.

The product potency is expressed in units based on the mouse neutralization assay (MNA). Each unit of H-BAT is designed to neutralize 10,000 MIPLD₅₀ (Mouse Intraperitoneal LD₅₀) of botulinum neurotoxin for serotype A, B, C, D, F, and G and 1,000 MIPLD₅₀ of serotype E.

H-BAT is supplied in either 20 milliliter or 50 milliliter glass vials sealed with a butyl rubber stopper and an aluminum seal with a plastic flip-top cap. Each vial, regardless of size (20 milliliter or 50 milliliter) or fill volume (10 to 22 milliliters), contains a minimum potency of >4,500 U serotype A antitoxin, >3,300 U serotype B antitoxin, >3000 U serotype C antitoxin, >600 U serotype D antitoxin, >5,100 U serotype E antitoxin, >3,000 U serotype F antitoxin and >600 U serotype G antitoxin.

The mechanism of action of H-BAT is through passive immunization with equine polyclonal antibody fragments F(ab)₂ against botulinum neurotoxin (BoNT) A through G. In the circulation the polyclonal antibody fragments bind to free BoNT. This prevents the BoNT from interacting with ganglioside anchorage sites and protein receptors on the cholinergic nerve endings. In turn, this prevents BoNT internalization into the target cells. The antibody/antigen complexes are then cleared from the circulation by the organs involved in processing immune complexes.

Study #1: Post exposure prophylaxis study of NP-018 in botulinum neurotoxin-challenged guinea pigs. **Study Number:** 731-G005630

Objective: The objective of this study was to assess the post-exposure prophylactic efficacy of NP-018 in Guinea Pigs (GP) when administered following an intramuscular botulinum neurotoxin (BoNT) intoxication equivalent to 4x GPIMLD50 of Serotypes A to G. NP-018 was administered by intravenous injection prior to the onset of clinical signs, at a range of doses (1x to 0.008x scaled human dose) in order to determine the minimum effective prophylactic dose.

Trial Design: The efficacy of NP-018 against each BoNT serotype was tested in a separate study Phase (total 7 Phases to evaluate Serotypes A, B, C, D, E, F, and G, respectively). In each Phase, five groups of 20 guinea pigs (10 males and 10 females) were administered with the toxin of the appropriate serotype at a toxin dose equivalent to 4x GPIMLD50 via a single intramuscular injection. A single intravenous treatment of either NP-018 antitoxin at $\times 1$, $\times 0.2$, $\times 0.04$, or $\times 0.008$ scaled human dose (groups 1, 2, 3, 4) or Placebo control (group 5) was administered approximately 12 hours after the toxin administration for Serotypes A, B, C, D, F, G and at approximately 6 hours post-intoxication for Serotype E. These time-points were selected based on the results of a previous study (study# 670-G005630). Animals were monitored frequently for clinical signs and mortality throughout the study until termination of study on day 21.

Progression of intoxication as determined by the onset and the increase in severity of clinical signs was determined for all groups. Time to onset of clinical signs, duration of clinical signs and time to death were determined for each serotype.

Results: Summarized group mortality, mean time to death, and mean time to onset of any moderate clinical sign are presented in Table 1.

Control groups in all Serotypes had 100% mortality, confirming the lethality of the toxin dose used (4x GPIMLD50). A decrease in mortality was observed among most NP-018 treated animals compared to the control group. A statistically significant ($p < 0.05$) improvement in survival rate was observed in NP-018 treated animals at all dose levels ($> 0.008x$ scaled human dose) for Serotype A, B, C, F, and G, and at $\geq 0.2x$ for Serotype D, and $\geq 0.04x$ for Serotype E. The mean and median time to death in the NP-018 treated groups at all dose levels was longer than control animals intoxicated with the same Serotype (Table 1).

Mortality was compared between treated and control groups. Statistical evaluation of these results was performed and the dose of NP-018 that would result in 80% survival for each Serotype was calculated. The lower 95% confidence interval of this calculated dose, defined as the Minimum Effective Dose, is presented in Table 2.

Treatment with any dose level of NP-018 resulted in a delayed mean time to onset of clinical signs when compared to control animal values.

Table 1: Mortality, Kaplan-Meier Mean Time to Death, and Kaplan-Meier Mean Time to Onset of Moderate Clinical Signs

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 2: Minimum Effective Dose of NP-018 per Serotype

Serotype	A	B	C	D	E	F	G
Minimum effective dose of NP-018 *	0.027×	0.054×	0.052×	0.078×	0.039×	0.013×	0.051×

* Compared to proposed human clinical NP-018 dose (1×) on a volume/kg basis, 11.17 mL (1 vial) per 70 kg.

For Serotypes A, E, F, and G, a dose response was noted with the incidence and severity of clinical signs highest in Groups 4 (lowest NP-018 dose) and 5 (control). Serotypes B, C and D did not show a clear dose-dependency, but the incidence and severity of clinical signs were markedly lower at $>0.04x$ for Serotypes B and C, and at $>0.2x$ scaled human dose for Serotype D when compared to control animals. All (100%) control (group 5) animals in all Serotypes showed moderate clinical signs, and in all serotypes except for Serotypes D and G, 100% of group 5 animals showed severe clinical signs.

The progression of botulism intoxication observed in control animals clearly indicates that irrespective of serotype, in the absence of prophylactic NP-018 treatment, intoxication will continue to increase in severity and progress to death. However, prophylactic treatment of NP-018 doses as low as $0.2x$ scaled human dose can prevent death (at least 90% survival in groups 1 and 2) in all 7 serotypes.

Conclusion: this study confirmed the efficacy of Heptavalent Botulism Antitoxin (Equine) A, B, C, D, E, F, and G NP-018 in preventing the occurrence/progression of clinical signs of BoNT intoxication and death, when administered prior to the onset of clinical signs (prophylactic). The calculated minimum effective dose levels resulting in 80% survival of treated animals following intoxication at $4x$ GPIMLD50 for all seven serotypes (A, B, C, D, E, F, G) are between $0.013x$ and $0.078x$ the scaled human NP-018 dose.

Comments:

- Overall, the pharmacodynamic results, analyses and conclusions of this study are acceptable from a Clinical Pharmacology perspective.

Study #2: Pivotal Therapeutic Efficacy Study of NP-018 Botulinum Antitoxin Heptavalent in Guinea Pigs Intoxicated with Botulinum Toxin. **Study Number:** 1180-G005630

The goal of this study was to evaluate the therapeutic efficacy (treatment) of NP-018 Heptavalent Botulinum Antitoxin (Equine) A, B, C, D, E, F, G when administered as a single intravenous injection to botulinum neurotoxin (BoNT) intoxicated guinea pigs after the onset of clinical signs of BoNT intoxication.

Objectives: The primary objective of the study was to determine any statistically significant improvement in survival between treatment (NP-018) and placebo control groups.

Secondary objectives included comparisons of (1) time to death, (2) incidence of clinical signs, (3) time to onset of clinical signs, and (4) resolution of clinical signs between treatment (NP-018) and placebo control groups.

Trial Design: Guinea pigs were randomized to fourteen (14) groups, each group targeted to contain 34 animals (seventeen (17) animals per sex). The appropriate BoNT serotype was administered at a dose equivalent to 1.5x GPIMLD₅₀ as a single intramuscular (IM) injection to the right hind limb of each animal. Animals were monitored frequently for clinical signs/mortality for 21 days following intoxication. Immediately after the fourth consecutive observation of any moderate/severe clinical sign of intoxication, animals received either NP-018 antitoxin treatment or placebo control as a single intravenous injection. All study personnel involved in observations or treatments were blinded to the study groups and treatment materials. In the majority of animals, the first four consecutive moderate/severe clinical sign observations were solely right hind limb weakness (RHLW).

Results: The study results show a statistically significant improvement in survival between treatment (NP-018) and placebo control groups in all serotypes (Table 1). Also shown in Table 1, time to death is statistically greater in treated (NP-018) groups of all serotypes compared to their respective placebo control groups. Administration of NP-018 did not result in immediate cessation of the disease progression. Most NP-018 treated animals, along with all control animals, continued to progress from the trigger (RHLW) to develop other systemic clinical signs such as change in breathing and weak limbs. The median duration of most clinical signs (incidence, time to onset, progression and resolution) was shorter in NP-018 treated animals than controls (Table 1).

The clinical severity scores show that for all serotypes, the clinical progression was initially very similar between treated and control animals but then appeared to diverge substantially at approximately 21-58 hours post-treatment, depending upon the serotype. This is an indication that intoxication tended to progress for a time after treatment, with recovery (reduction in clinical severity score) starting substantially later.

Table 1: Summary of Survival with Fisher’s Exact Test Comparisons, and Kaplan-Meier Median Time to Death with Log-Rank Test Comparisons between Treated and Control Groups in Guinea Pigs Intoxicated with 1.5x GPIMLD₅₀ Botulinum Toxin Serotypes A, B, C, D, E, F and G

BoNT Serotype	Group	Treatment Dose Level	Survival (percent)	Two-Sided Fisher’s Exact Test Comparison (p-value)	Kaplan-Meier Median Time to Death (95% Confidence Interval) in Hours	Log-Rank Test Time-to-Death Comparison (p-value)
A	A1	1.0x NP-018 ¹	34/34 (100%)	<0.0001*	--(--)	<0.0001*
	A2	Placebo Control ²	0/34 (0%)		99 (87, 113)	
B	B1	1.0x NP-018 ¹	34/34 (100%)	<0.0001*	--(--)	<0.0001*
	B2	Placebo Control ²	1/34 (3%)		94 (94, 112)	
C	C1	1.0x NP-018 ¹	33/34 (97%)	<0.0001*	--(--)	<0.0001*
	C2	Placebo Control ²	4/34 (12%)		114 (111, 141)	
D	D1	1.0x NP-018 ¹	33/34 (97%)	<0.0001*	--(--)	<0.0001*
	D2	Placebo Control ²	5/34 (15%)		156 (141, 180)	
E	E1	1.0x NP-018 ¹	34/34 (100%)	<0.0001*	--(--)	<0.0001*
	E2	Placebo Control ²	0/34 (0%)		29 (27, 30)	
F	F1	1.0x NP-018 ¹	34/34 (100%)	<0.0001*	--(--)	<0.0001*
	F2	Placebo Control ²	4/34 (12%)		58 (45, 68)	
G	G1	1.0x NP-018 ¹	34/34 (100%)	<0.0001*	--(--)	<0.0001*
	G2	Placebo Control ²	17/34 (50%)		168 (143, --) ³	

¹ Compared to proposed human clinical NP-018 dose (mL/kg basis)

² Normal Equine Immune Globulin

--Either animal death was not observed (groups A1, B1, E1, F1 and G1) or the Kaplan-Meier estimates could not be calculated due to censoring (groups C1 and D1).

³ The upper bound of the 95 percent confidence interval could not be estimated due to the high incidence of censoring.

* Comparison significant at the 0.05 level of significance.

Conclusions: Nearly all treated animals (97% or 100% per serotype) survived following intoxication at the target 1.5x GPIMLD₅₀ dose level. In contrast, Serotypes A, B, C, D, E and F placebo control groups experienced ≥ 85 % mortality following intoxication at the target 1.5x GPIMLD₅₀ dose level, and the Serotype G placebo control group (G2) experienced 50% mortality. No NP-018 treated animals in Serotype G died, so increased survival in the treated group was statistically significant.

Treatment with NP-018 resulted in a statistically significant improvement in survival when compared to control animals for all serotypes. Although treatment with NP-018 resulted in virtually complete survival it did not immediately halt disease progression. Despite intervention soon after clinical signs began, most NP-018 treated animals continued to demonstrate similar clinical signs at rates consistent with placebo control animals for a time before beneficial effects were observed. Overall, however, the incidence of most clinical signs was substantially reduced,

with later times-to-onset and shorter durations in NP-018 treated animals when compared to controls in all serotypes.

Comments:

- The pharmacodynamic results, analyses and conclusions of this study are acceptable from a Clinical Pharmacology perspective.

Study #3: Efficacy of post-exposure prophylactic administration of NP-018 Botulism Antitoxin Heptavalent in rhesus macaques (*Macaca mulatta*). **Study Number:** FY08-061.

Objectives:

The primary objective of this study was to demonstrate that NP-018 Botulism Antitoxin Heptavalent administered prophylactic at about 4 hours post-injection of Botulism Neurotoxin Complex Type A prevents the occurrence of mortality and clinical signs of toxicity in male and female rhesus macaques (*Macaca mulatta*).

The secondary objective was to assess the PK of eBAT NP-018 following administration of BoNT serotype A.

Serotype A was selected as the appropriate serotype for intoxication because it is considered a typical serotype and has been the most prevalent serotype found in cases of food-borne botulism in human adults in the United States since 1955.

The primary endpoint of this study was the comparison of survival rates between groups treated with test article and the placebo treated group.

Trial Design: The experimental design is shown in Table 1. Three groups of ten (10) rhesus monkeys (5 males and 5 females) received an intravenous injection of ~104 MIPLD₅₀/kg (approximately ~4LD₅₀ for rhesus monkeys) of Botulism toxin Type A. Two groups of 10 rhesus monkeys (5 males and 5 females) per group received Test Article (NP-018 Botulism Antitoxin Heptavalent) intravenously about 4 hours following the intravenous intoxication.

The dose selected was directly related to the intended human clinical dose scaled on a mL/kg basis. Treatment Group 1 received the projected scaled human dose or 0.16mL/kg (149 U/kg) of botulism antitoxin 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 received botulism antitoxin placebo. All animals were dosed at about 4 hours following the same dose of botulism neurotoxin complex Type A.

Blood was collected for pharmacokinetic analysis prior to exposure to antitoxin and at 4, 8, 12, and 24 hours post administration of antitoxin. Using Noncompartment Analysis (WinNonlin Version 5.0.1) only the terminal serum half life was calculated.

Table 1 Experimental Design

Groups (Number of animals) ^(a)	Toxin Challenge IV (IV)	Antitoxin Dose (IV)
Treatment group 1 (n=10)	Type A toxin at 104 MIPLD ₅₀ /kg (~4 LD ₅₀)	0.16 mL/kg (x 1 the scaled human dose) or 149 U/kg or 9 mg protein/kg
Treatment group 2 (n=10)		0.016 mL/kg (x 0.1 the scaled human dose) or 14.9 U/kg or 0.9 mg protein/kg
Treatment group 3 (n=10)		0.18 mL/kg or 9 mg protein/kg Botulism Antitoxin Placebo (equivalent to protein dose of Group 1)

^(a) equal number of males and females

The animals were evaluated for 14 or 15 days post dose. Clinical observations were made on each animal about hourly for 5 days and then about every 4 hours until the end of the study on Day 14 or 15.

A sample size of 10 per group was considered adequate to achieve at least 80% power for finding a difference in two groups when probability of death is (approximately) 0 vs. 70%. Two Tailed Fisher's Exact Test was used to determine if there is a statistically significant difference between survival rates for both of the Test Article treated groups (Group1 and Group 2) and the placebo treated group (Group 3).

Results: Survival results are summarized in Table 2. 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 $p < 0.001$.

Table 2: Summary of mortality data

Groups (Number of animals)	Number Survived to Study End	Median Survival Time (95% Confidence Interval)*
Treatment Group 1 (n=10)	10	> 362 hours (. . .)
Treatment Group 2 (n=10)	10	> 362 hours (. . .)
Treatment Group 3 (n=10)	0	36.5 hours (28.0, 39.0)

* The limits are presented except when the estimated survival distribution of the group did not cross 0.50, in which case they are shown as (. . .).

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. The signs of botulism intoxication in Treatment Group 3 had an onset at about 27.5 hours (median time to ptosis) 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 short (about 9 hours).

The exposure to botulism toxin did not appreciably alter body weights or organ weights between the groups given different doses. There were no significant lesions at gross necropsy of the animals.

PK Results: Only animals in Treatment Group 1 had titers above the limit of detection of the mouse neutralization assay (MNA). The mean terminal half life (HL) for the antitoxin titers in serum was 4.7 hours with a range between 2.6 to 6.7 hours. This HL is similar to the PK results of 1x NP-018 in rhesus monkeys (Study # FY07-056), but without presence of the toxin BoNT/A (HL = 3.0, SD = 0.8).

Conclusion: In conclusion, the treatment with intravenous NP-018 Botulism Antitoxin Heptavalent at about 4 hours post injection of botulism neurotoxin complex Type A was highly effective at preventing the lethal effects and clinical signs of botulism intoxication.

It is noteworthy, that in the 0.1x NP-018 Group 2 all animals survived, although all titers were below limit of detection. This indicates that although not detectable by the MNA there was still sufficient botulism antitoxin present to protect animals from BoNT intoxication.

Comments:

- The PK/PD results of this study are acceptable from a Clinical Pharmacology perspective.
- The study results only allow an assessment of the Botulism Antitoxin Heptavalent (Equine) Type A. The effects of antitoxins type B, C, D, E, F, and G on corresponding BoNT serotypes have not been investigated.

Study #4: Therapeutic efficacy of NP-018 Botulinum Antitoxin Heptavalent in combination with minimal supportive care in rhesus macaques (*Macaca mulatta*) intoxicated with botulinum toxin.

Study Number: FY10-066

Objective: The objective of this study was to confirm the therapeutic effect (treatment) of a single intravenous dose of Botulinum Antitoxin Heptavalent NP-018 when administered at the onset of clinical signs and in combination with minimal (nutritional) supportive care, in preventing mortality among Rhesus macaques (*Macaca mulatta*) intoxicated with Botulinum Neurotoxin Serotype A Complex.

Serotype A was selected as the appropriate serotype for intoxication because it is considered a typical serotype and has been the most prevalent serotype found in cases of food-borne botulism in human adults in the United States since 1955.

The primary endpoint of this study was the comparison of survival rates between groups treated with the test article and the placebo treated group.

Trial Design: The experimental design is shown in Table 1. Sixty (60) rhesus macaques (29 males and 31 females) were surgically implanted with an indwelling central venous catheter and were used in 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. On Day 0, all animals received a dose equivalent to 1.7 x LD₅₀/kg (~44 mouse intraperitoneal median lethal dose [MIPLD₅₀]/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.

The selected Botulinum Antitoxin dose was directly related to the intended human clinical dose scaled on a mL/kg basis. 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 Antitoxin Placebo (0.31 mL/kg, based on equivalent protein dose) was administered intravenously via the central venous catheter. Study personnel were blinded to treatment group allocations. Animals were observed for 21 days.

Minimal supportive care (nutrition, fluids) was initiated in all animals (Groups 1 and 2) within 23 minutes of antitoxin or placebo administration and was either administered parenterally through the central venous catheter via constant rate infusion by use of an ambulatory pump, or via oral gavage of Liquid Rhesus monkey diet.

A total of 29 nonhuman primates per group were necessary to achieve at least 80% power to detect the assumed difference in survival rates between the treatment group and control group at alpha level of 5%.

Table 1 Experimental Design

Treatment Group	Toxin Challenge (IV)	Treatment Administration (IV)	Minimal Supportive Care
Group 1 (n = 30) ^a	Serotype A toxin at ~1.7 LD ₅₀ /kg (44 MIPLD ₅₀ /kg)	~121 U/kg NP-018 (1.0x scaled human dose) or 0.26 mL/kg	Yes
Group 2 (n = 30) ^b		0.31 mL/kg Botulinum Antitoxin Placebo (equivalent protein dose)	

^a No. of males = 14, No. of females = 16

^b No. of males = 15, No. of females = 15

IV = Intravenous

For the primary analysis, 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 1 error set at $\alpha = 0.05$. For the secondary analysis, the median time to death was calculated using the product-limit method. The survival curves were compared between the treatment and placebo groups using the log-rank test. Time to onset of clinical signs and time from onset of clinical signs to recovery were analyzed using the same survival techniques as described for the time to death endpoint. The proportion of animals recovering after the onset of clinical signs in each group was calculated using the exact binomial distribution. The proportions of animals that recovered after the onset of clinical signs in each treatment group were compared using Fisher's Exact test.

Results: For the primary analysis survival results are summarized in Table 2. None of the animals 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 Botulinum Antitoxin Hepatavalent NP-018 survived to the end of the study. This difference in survival rate between the two groups was statistically significant.

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)	

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

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 Botulinum Antitoxin Hepatavalent NP-018, compared with animals treated with Placebo control. There was no significant difference in the time to onset of any clinical signs except for nasal discharge, which was significantly delayed in nonhuman primates treated with Botulinum Antitoxin Hepatavalent NP-018. The median time interval

between the onset of clinical signs and recovery (resolution of clinical signs) for Group 1 animals was 137 hr.

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 (ie, 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. Therefore, treatment with Botulinum Antitoxin Heptavalent NP-018 delayed the progression of botulinum intoxication resulting in euthanasia, when compared with Botulinum Antitoxin Placebo. Analysis of these results confirmed a statistically significant improvement in survival of NP-018-treated animals.

The spectrum of gross pathology did not vary substantively among animals receiving Test or Control Article. The majority of the gross findings observed were expected with botulinum intoxication. Based on gross pathologic and histopathologic examination results, no findings were considered to be Test Article-related.

Conclusion: 1x scaled human dose (0.26 mL/kg) of NP-018, administered in combination with supportive care at the onset of clinical signs resulted in a significant improvement in survival of Rhesus macaques intoxicated with botulism neurotoxin serotype A complex.

Comments:

- The pharmacodynamic results, analyses and conclusions of this study are acceptable from a Clinical Pharmacology perspective.
- The study results only allow an assessment of the Botulism Antitoxin Heptavalent (Equine) Type A. The effects of antitoxins type B, C, D, E, F, and G on corresponding BoNT serotypes have not been investigated.

Study #5: Intravenous NP-018 pharmacokinetics determination in guinea pigs (**Study Number:** 684-G005630).

The objective of this study was to determine the pharmacokinetics (PK) of NP-018, a botulism antitoxin heptavalent (ABCDEFG) following intravenous (IV) administration of NP-018 to naïve male Hartley guinea pigs. NP-018 is a combination of 7 antitoxins (one per Botulism Serotype), with each product blended at different concentrations. Therefore, the study was designed to analyze all 7 serotypes at each time point. Due to the large number of animals required in the study, dosing (and subsequent sample collection) was divided into two identical Phases (A and B) separated by 1 week. Each Phase contained equal numbers of animals from both the High and the Low dose groups.

Two hundred and sixty-four animals (mean body weight 0.5 kg) were dosed intravenously at two doses (x0.2 (low) and x1.0 (high) human dose of one vial per 70 kg person). The proposed human clinical dose of NP-018 is 0.16 mL/kg (149 U/kg). The following Table describes the dosing characteristics in terms of volume and protein contents.

Table 1 Study Design to Determine Pharmacokinetics of NP-018

Group (n=132)	Dose Volume of NP-018 (Human dose)	Total Protein Dose ¹	Phase ² (n)	Dose Day	Serum Collection Time Points ³
1	0.160 mL/kg (x1)	8.96 mg/kg	A (66) B (66)	Day 0	0, 10 minutes, 4, 8, 12, 24, 48 hours, 3, 5, 8 and 12 days post-dose
2	0.0320 mL/kg (0.2x)	1.79 mg/kg	A (66) B (66)		

¹ Undiluted NP-018 total protein concentration is 56 mg/mL.

² Each Phase (A and B) consisted of 66 animals per group and each animal's IV dosing day was Day 0 for that animal.

³ For each Phase, and at each time point, 6 animals per group were bled for a total of 12 animals per Phase per time point. At each time point, serum was pooled from 3 animals per group. For both Phases combined, there were a total of 4 samples per time point per group. The 0 time point animals were not dosed.

Blood samples were collected from twelve (12) animals per group by terminal bleeds at 11 time points (0, 10 minutes, 4, 8, 12, 24, 48 hours, 3, 5, 8 and 12 days post-dose). At each time point 10-20 mL of blood was collected. Serum was separated and samples from three (3) animals were pooled to generate four (4) serum samples per group per time point. All serum samples were stored frozen ($\leq -70^{\circ}\text{C}$) at Battelle Biomedical Research Center, pending analysis by mouse neutralization assay (MNA). Serum samples were analyzed to quantify the neutralizing antibody concentrations (NAC) for each serotype (U/mL). A significant portion of the serum samples were hemolytic (53%) and a few were lipemic (5%). These sample conditions occurred throughout the study (at early and late time points). The reason for this hemolysis remains unknown. Cangene assessed the impact of hemolysis on the subsequent MNA and concluded that analysis of hemolyzed samples did not appear to affect the accuracy of the MNA results. For each serotype, the pre-dose NAC values were below lower limit of quantification (LOQ). NAC-time profile for the Serotype D and E low dose groups were incomplete due to an insufficient number of measurable NAC values, which thereby prevented adequate PK analysis. PK parameters were

assessed by non-compartmental analysis. The pharmacokinetics parameters of NP-018 in male guinea pigs are summarized in Table 2.

Table 2: Pharmacokinetic parameters of NP-018 in male guinea pigs for different serotypes

Serotype	Dose (U/kg)	NAC _{0min} (U/mL)	NAC _{max} (U/mL)	T _{max} (hr)	NAC Decay Half-Life (hr)	AUC _{last} (hr*U/mL)	AUC _∞ (hr*U/mL)
A	29.8	0.461	0.430	0.167	2.11	1.30	1.39
	149	3.14	2.92	0.167	6.24	10.0	10.3
B	20.3	0.417	0.386	0.167	7.44	1.37	1.45
	101	1.49	1.42	0.167	15.3	8.18	8.39
C	20.4	0.268	0.259	0.167	3.76	1.30	1.46
	102	1.98	1.86	0.167	4.84	6.97	7.98
D	4.20	ND	ND	ND	ND	ND	ND
	20.8	1.26	1.17	0.167	3.31	3.77	3.97
E	30.6	ND	ND	ND	ND	ND	ND
	153	0.592	0.563	0.167	2.81	2.15	2.25
F	18.8	ND	ND	ND	ND	ND	ND
	93.8	1.90	1.79	0.167	10.4	7.68	7.83
G	3.50	0.113	0.105	0.167	3.14	0.340	0.355
	17.6	0.518	0.481	0.167	5.54	1.69	1.73

ND Not determined due to insufficient data points

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

Comment: Overall, the PK study design, blood sampling scheme, and conclusions drawn from this study for NP-018 in male guinea pigs are acceptable.

Study #6: NP-018 Antitoxin: Intravenous Pharmacokinetic Study in Rhesus Macaques (Study Number: FY07-056).

The objective of the study was to determine the pharmacokinetics of two doses of NP-018, Botulism Antitoxin Heptavalent (x1 and x5 the proposed human clinical dose), in male and female rhesus macaques (*Macaca mulatta*) following a single intravenous (IV) administration. The proposed human clinical dose of NP-018 is 0.16 mL/kg (149 U/kg). Therefore, the monkeys received 0.16 mL/kg (149 U/kg) or 0.8 mL/kg (745 U/kg). Twelve non-human primates (six males and six females), were randomly assigned to two treatment groups, each of three (3) males and three (3) females. On Day 0, all animals received a single NP-018 IV dose. Group 1 animals were dosed at x5 scaled human dose (0.8 mL/kg, or 745 ± 16 U/kg), and Group 2 animals were dosed at x1 scaled human (0.16 mL/kg, or 149 ± 1 U/kg).

Blood samples were collected from all animals prior to dose administration and at 0.5, 2, 4, 6, 8, 12, 18, 24 hours, days 3, 6, 9, 12, 14 and 20 post-dose. NP-018 concentrations (only serotype A) were measured in serum using a validated neutralizing antibody assay. The concentrations of NP-018 could be detected by 8 and 18 hours following 0.16 mL/kg and 0.8 mL/kg dose, respectively. The PK parameters were estimated by non-compartmental analysis and are summarized in the following Table. Plasma concentration-time data for individual monkey is shown in Figures 1 and 2 for two doses of NP-018.

Pharmacokinetic parameters of NP-018 (serotype A) in rhesus monkey following two doses

Dose	PK Parameters			
	AUC _(0-t)	Half-life (hrs)	CL (mL/hr/kg)	V _d (mL/kg)
0.16 mL/kg	9.7 ± 1.6	3.0 ± 0.8	13.1 ± 3.2	57.2 ± 21.9
0.80 mL/kg	80.2 ± 14.2	5.2 ± 1.3	9.1 ± 1.4	68.5 ± 21.9

Unit of AUC (hr*U/mL); V_d = volume of distribution by area or at terminal phase

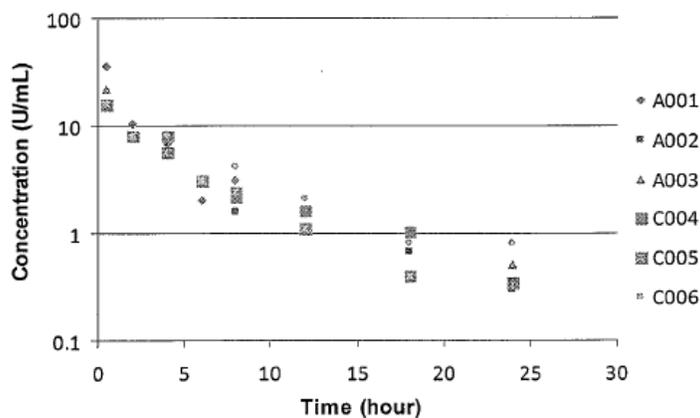


Figure 1. NP-018 Concentration (U/mL) in serum after a single 745 U/kg IV dose.

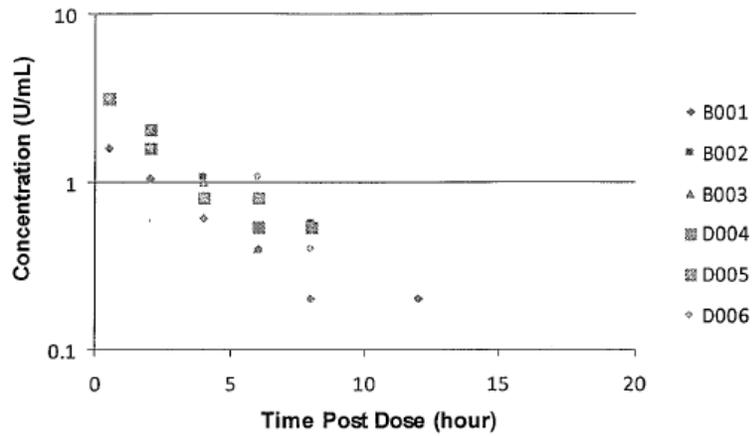


Figure 2. NP-18 Concentration (U/mL) in serum after a single 149 U/kg IV dose.

The pharmacokinetics of NP-18 (serotype A) is non-linear (AUC did not increase proportionally with dose). The half-life of NP-18 is about 2 hours longer at the high dose as compared to low dose. There was no gender difference in the PK of NP-18.

Comment: Overall, the PK study design, blood sampling scheme, and conclusions drawn from this study for NP-18 in male and female monkeys are acceptable.

Study #7: Pharmacokinetics of a heptavalent equine-derived botulinum antitoxin (NP-018) in humans (**Study Number:** BT-001).

The primary objective of this study was to evaluate the safety of NP-018 based upon clinical observations, adverse events (AEs) and laboratory assessments. The secondary objective was to assess the pharmacokinetics (PK) of the seven botulinum antitoxin serotypes contained in NP-018 following intravenous (IV) administration.

This was a Phase 1, single-centre, randomized, double-blind, parallel arm study. NP-018 was intravenously administered to healthy, male and female volunteers between the ages of 19 and 52 years. Forty subjects were randomized to receive either one or two vials of NP-018, representing a single or double dose of botulinum antitoxin. Each vial had a nominal potency of serotype A = 7500 U, serotype B = 5500 U, serotype C = 5000 U, serotype D = 1000 U, serotype E = 8500 U, serotype F = 5000 U and serotype G = 1000 U. Each vial contained 11.17 mL botulinum antitoxin. Each dose was administered by slow intravenous infusion **over** 2.5 hours. The infusion rate was incremental, starting slowly and increasing if no safety related events were evident.

Blood samples (50 mL) for pharmacokinetic study were collected after NP-018 administration for botulinum toxin at the following time points: 0.5, 4 and 8 h; days 1, 3, 7, 14, 21, 28. Of the 40 subjects enrolled, 39 subjects completed the study as per protocol and were included in the pharmacokinetic analysis. One subject (Subject 1) did not complete NP-018 dosing due to adverse events judged to be possibly or probably related to study treatment. All 40 subjects were included in the safety assessment. Pharmacokinetic parameters were calculated by non-compartmental analysis (Table 1) using the data generated from the mouse neutralization assay. Mean serum concentrations of Botulinum Antitoxins (serotypes A-G) vs time plots are shown in Figures 1-7.

The pharmacokinetic parameters such as AUC, clearance, and half-life varied based upon the antitoxin serotype measured (Table 1). Both $AUC_{(0-\infty)}$ and C_{max} values increased in a dose proportional manner as NP-018 doses increased from one to two vials. The half-lives of the different antitoxin serotypes varied with the serotype. Antitoxin serotypes D and E had the shortest mean half-lives, ranging from 7.5 to 7.8 hours. Whereas antitoxin serotypes B had the longest mean half-lives, ranging from 34.2 (one vial) to 57.1 (2 vials) hours. Comparison of the pharmacokinetic parameters between male and female subjects for antitoxin serotypes A through G showed that there were no gender related differences following a single intravenous administration of either one or two vials of NP-018.

Comment: The results and analysis of this study are acceptable.

Table 1
Arithmetic Mean (%CV) of Pharmacokinetic Parameters for Antitoxin Serotypes A-G in
Subjects Following Intravenous Administration of One or Two Vials of NP-018

Serotype	Treatment Group	AUC ₀₋₄ (U* ^h /mL)	AUC _{0-∞} (U* ^h /mL)	AUC ₀₋₄ / AUC _{0-∞} (%)	C _{max} (U/mL)	T _{max} (h)	Half-life (h)	λz (1/h)	Cl (mL/h)	V _d (mL)
A	1 Vial	21.40 (20.1)	26.00 (13.1)	86.3 (4.5)	2.685 (28.2)	0.698 (115.0)	8.64 (15.5)	0.0821 (15.8)	293 (13.5)	3637 (17.1)
	2 Vials	51.92 (27.4)	56.09 (24.7)	92.5 (5.6)	6.234 (22.0)	0.528 (4.1)	10.2 (34.7)	0.0759 (32.8)	285 (27.1)	3993 (27.7)
B	1 Vial	27.16 (23.0)	29.30 (19.2)	95.8 (1.7)	1.897 (42.7)	0.888 (125.0)	34.2 (40.4)	0.0250 (52.0)	196 (24.0)	9607 (48.1)
	2 Vials	58.53 (16.6)	62.55 (16.4)	95.5 (2.4)	4.282 (33.8)	0.540 (8.9)	57.1 (69.4)	0.0185 (60.8)	181 (19.3)	14865 (71.5)
C	1 Vial	36.63 (25.2)	37.34 (27.3)	94.8 (1.8)	2.263 (38.1)	1.90 (150.0)	29.6 (44.9)	0.0282 (42.3)	144 (28.1)	6066 (52.8)
	2 Vials	78.75 (29.0)	86.25 (28.8)	93.2 (3.9)	4.890 (41.7)	0.525 (4.1)	45.6 (34.0)	0.0169 (35.9)	127 (33.9)	8486 (52.4)
D	1 Vial	5.578 (48.5)	7.616 (23.3)	90.0 (5.8)	0.812 (55.0)	0.891 (124.0)	7.51 (24.4)	0.0968 (22.6)	137 (19.9)	1465 (25.8)
	2 Vials	13.27 (33.7)	14.83 (39.4)	89.3 (5.4)	1.603 (38.8)	0.704 (111.0)	7.77 (21.6)	0.0932 (21.7)	151 (30.5)	1653 (30.5)
E	1 Vial	6.653 (32.4)	7.162 (23.9)	89.8 (5.0)	0.938 (41.6)	0.518 (3.9)	7.75 (19.1)	0.0926 (20.8)	1250 (25.1)	14172 (32.1)
	2 Vials	13.47 (20.2)	15.66 (15.6)	90.4 (5.3)	1.749 (32.9)	0.534 (9.4)	7.32 (23.9)	0.0997 (23.1)	1110 (16.4)	11596 (22.4)
F	1 Vial	29.12 (29.1)	31.40 (25.4)	96.7 (2.7)	2.367 (25.8)	1.07 (122.0)	14.1 (16.7)	0.0508 (20.1)	169 (25.5)	3413 (29.3)
	2 Vials	59.63	63.19	96.5	4.285	1.63	18.2	0.0434	168	4334

Serotype	Treatment Group	AUC ₀₋₄ (U* ^h /mL)	AUC _{0-∞} (U* ^h /mL)	AUC ₀₋₄ / AUC _{0-∞} (%)	C _{max} (U/mL)	T _{max} (h)	Half-life (h)	λz (1/h)	Cl (mL/h)	V _d (mL)
		(26.5)	(25.0)	(4.0)	(31.0)	(149.0)	(42.9)	(33.3)	(26.4)	(50.9)
G	1 Vial	6.332 (26.2)	7.047 (23.3)	92.8 (5.1)	0.586 (27.7)	1.28 (154.0)	11.7 (38.1)	0.0689 (40.8)	149 (22.8)	2372 (29.5)
	2 Vials	13.40 (27.3)	14.66 (23.6)	97.0 (1.8)	1.193 (43.8)	0.704 (111.0)	14.7 (16.5)	0.0483 (17.9)	144 (23.8)	3063 (29.4)

Mean serum concentrations of Botulinum Antitoxins (U/mL)

Figure 1: Botulinum Antitoxins A

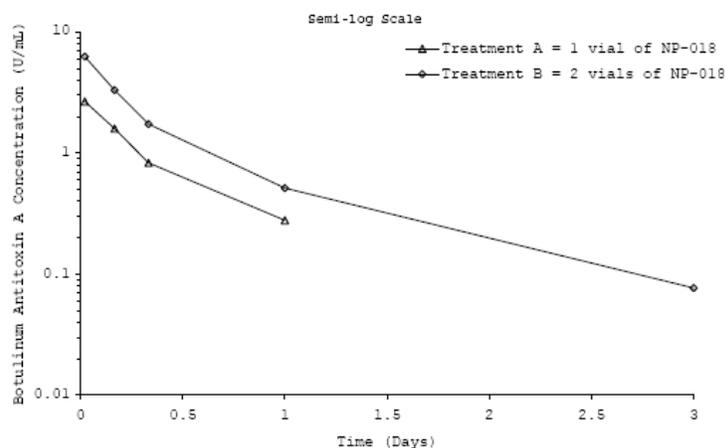


Figure 2: Botulinum Antitoxins B

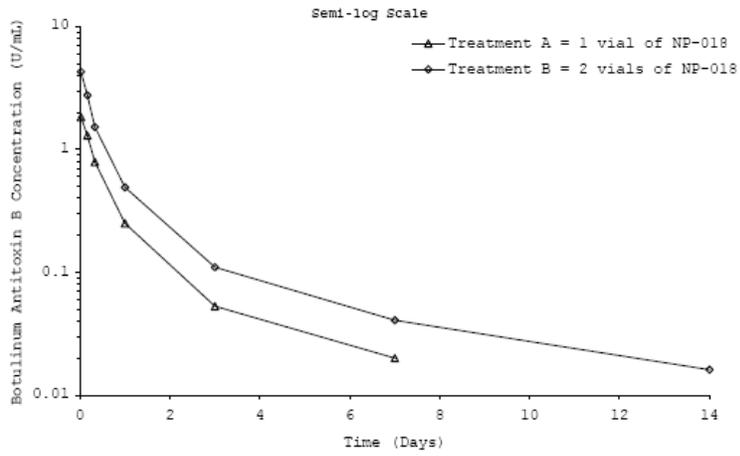


Figure 3: Botulinum Antitoxins C

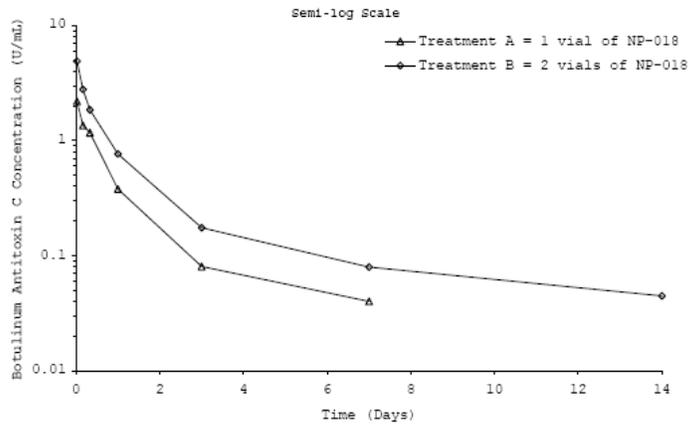


Figure 4: Botulinum Antitoxins D

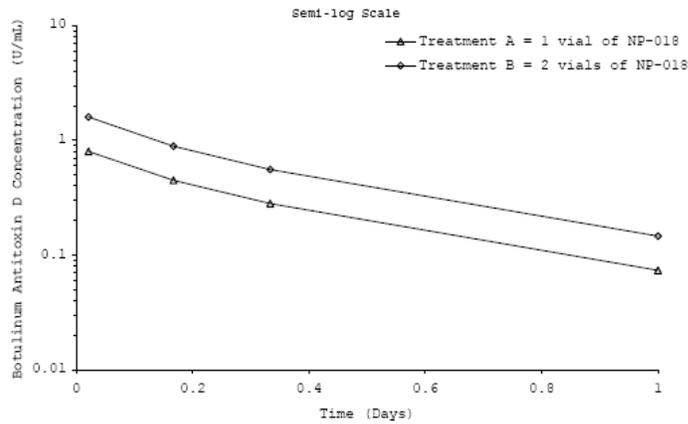


Figure 5: Botulinum Antitoxins E

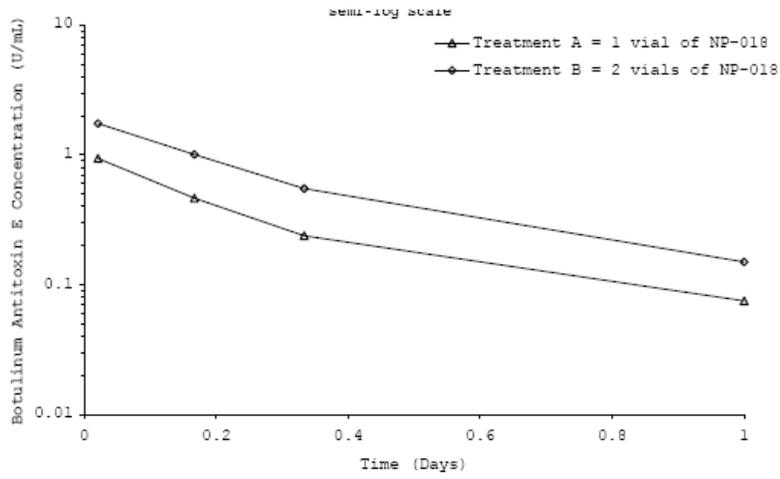


Figure 6: Botulinum Antitoxins F

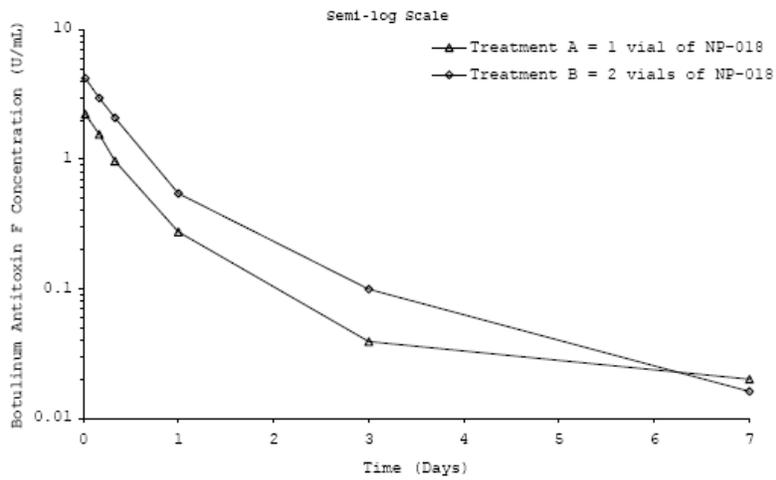
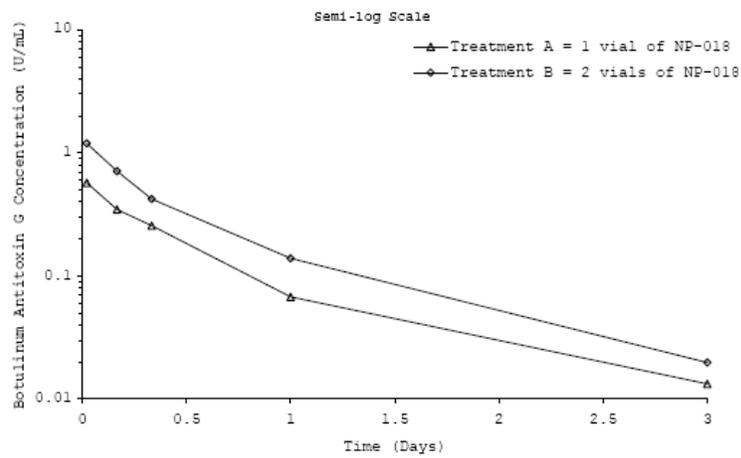


Figure 7: Botulinum Antitoxins G



Study #8: Botulism antitoxin effects on paralysis induced by Type A and Type B botulinum neurotoxin in the extensor digitorum brevis (EDB) muscle. **Study Number: BT-002 Part B**

The clinical study BT-002 is an exploratory pharmacodynamic (PD) study in healthy volunteers that is divided into two stages (BT-002 Stage A and BT-002 Stage B). The precursor clinical study BT-002 Stage A was designed to evaluate the ability of the licensed botulism antitoxin bivalent (Equine) Types A and B (Aventis Pasteur) to neutralize botulism toxin in the EDB model of paralysis in healthy subjects versus placebo. The results of this study report will not be reviewed. Since the principle effect of exposure to botulism in humans is muscle paralysis, inhibition of muscle paralysis induced by BoNT serotypes A (Botox®) or B (Myobloc®), was used as a surrogate endpoint to demonstrate the effectiveness of eBAT NP-018 in humans.

BT-002 Stage B will evaluate the ability of an investigational heptavalent (equine) botulism antitoxin [(NP-018) Cangene Corporation]) to neutralize botulism toxin in an equivalent muscle paralysis model.

Objectives: The primary objective of this study is to evaluate the effect of botulism antitoxins (bivalent and heptavalent) in preventing paralysis of the extensor digitorum brevis (EDB) muscle following Botox® or Myobloc® administration.

The secondary objective of this study is to evaluate the safety of botulism antitoxin types in healthy subjects.

This Clinical Pharmacology review is focused on the primary objective of BT-002 B clinical trial in healthy volunteers.

Trial Design: BT-002 Stage B of this two stage study was a phase 1b/2a, single center, randomized, placebo-controlled, double-blind study with two parallel arms. The study was designed as an exploratory pharmacodynamic (PD) study to evaluate the ability of botulism antitoxin heptavalent (equine) types A-G (NP-018, Cangene Corporation) in neutralizing botulism toxins types A and B (Botox® and Myobloc®, respectively) in a validated muscle model in healthy subjects. In this stage, 26 healthy adult subjects (13 females, 13 males), aged between 19-49 years (median 24 years) were randomized 8:5 to receive either a single intravenous (IV) infusion of NP-018 over 150 minutes or placebo (0.9% saline solution) one day prior to intramuscular administration of botulinum toxin types A and B.

Botox® (botulinum toxin type A, Allergan Inc.) was administered as a single intramuscular (IM) injection of 5 U the day following antitoxin administration. The site of injection for Botox® was the EDB muscle of the left foot. Myobloc® (botulinum toxin type B, Elan Pharmaceuticals, Inc.) was administered as a single IM injection of 500 U the day following antitoxin administration. The site of injection for Myobloc® was the EDB muscle of the right foot. NP-018 was administered as a slow IV infusion over 150 minutes of a single dose containing the following nominal levels: 7500 U anti-A, 5500 U anti-B, 5000 U anti-C, 1000 U anti-D, 8500 U

anti-E, 5000 U anti-F and 1000 U anti-G (one 18.51 mL vial of NP-018 diluted 1:10 with saline). The dose specified corresponds to an established treatment dose of a naturally occurring Botulism intoxication of serotypes A and B. The concentrations of NP-018, Botox® and Myobloc® were not measured in this study.

Pharmacodynamic assessments were based primarily on the preservation of muscle function in both feet following NCS. During NCS, the compound muscle action potential (CMAP) M wave amplitude and area of the EDB muscle were recorded at screening, before antitoxin infusion (baseline Day 0) and on days 3, 4, 7, 14, 21 and 28 post-administration of the botulinum toxins. The CMAP M wave amplitude and area recordings of the EDB muscle post-administration of toxin were compared to recordings obtained at baseline and used to determine the degree of preservation of muscle function of the EDB muscle.

The pharmacodynamic endpoints for Stage B of this study are the percent muscle function based on the preservation of the CMAP M wave amplitude and area of the EDB muscle (with a reference electrode at either the standard or inactive locations) following exposure to Botox® and Myobloc® after treatment with botulism antitoxins or placebo.

Treatment effect over time was evaluated using an exploratory repeated-measures analysis of variance (ANOVA) model that was fitted separately to the percent muscle function following exposure to Botox® (botulinum toxin Type A) and Myobloc® (botulinum toxin Type B).

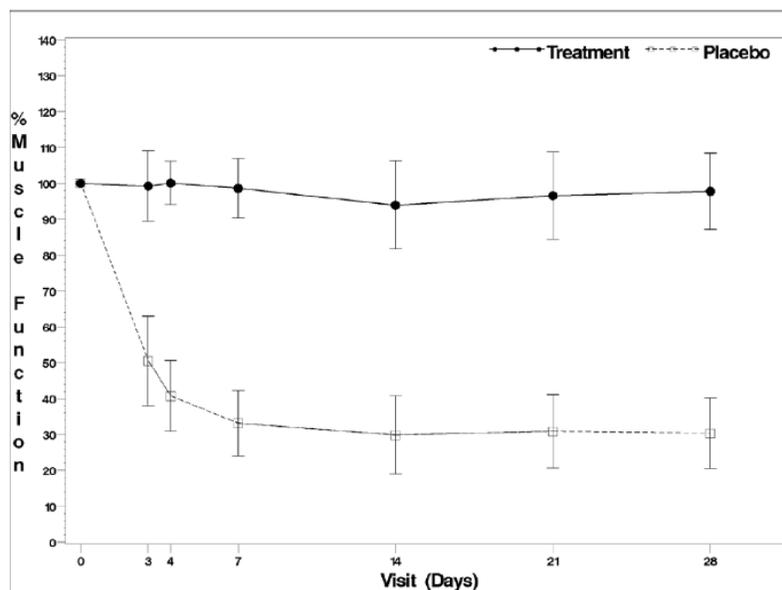
Data collected on an initial ten subjects enrolled in Stage A of this study was used to re-evaluate the sample size for Stage B to ensure there is enough statistical power for the pharmacodynamic analysis. Based on Stage A data, the proposed sample size of 26 subjects was adequate to provide more than 90% statistical power for the Stage B pharmacodynamic analysis. Therefore, no sample size revision was necessary.

Results: Fifteen subjects in the treatment arm completed the study. One female subject was withdrawn from the study due to AEs on Day 0. A comparison of the percent muscle function of the EDB muscle following exposure to Botox® between the treatment and placebo group (between groups test) indicates that the observed preservation of muscle function in the treatment group is significant (P-value < 0.0001). Table 1 and Figure 1 show that the treatment arm presents with no decrease in percent muscle function following IM administration of Botulinum toxins (99% muscle function), the placebo arm shows a loss of 50% muscle function within 3 days post-exposure to botulinum toxin type A). Figure 1 shows that there is no decrease in percent muscle function in subjects exposed to NP-018 (treatment arm) from baseline (Day 0) to the end of the study (Day 28). In the same figure, there is a significant loss of percent muscle function in the placebo arm within the first 7 days following IV infusion. The residual muscle function appears to be stable at approximately 30% muscle function for the remainder of the study (Days 7 to 28).

Table 1 Mean and standard deviation of the percent muscle function (CMAP M wave amplitudes) of the EDB muscle for both treatment arms following exposure to BOTOX® over time (ITT population). Reference electrode at the ‘standard’ location

Study Visit	Botulism Antitoxin Heptavalent (Equine) Types A-G			placebo (0.9% saline solution)		
	Number of subjects (N)	Mean percent muscle function	SD	Number of subjects (N)	Mean percent muscle function	SD
Day 3	15	99	10	10	50	12
Day 4	15	100	6	10	41	10
Day 7	15	99	8	10	33	9
Day 14	15	94	12	10	30	11
Day 21	15	97	12	10	31	10
Day 28	15	98	11	10	30	10

Figure 1: The percent muscle function over time (CMAP M wave amplitudes) of the EDB muscle following exposure to BOTOX® over time (ITT population). The reference electrode at the ‘standard’ location



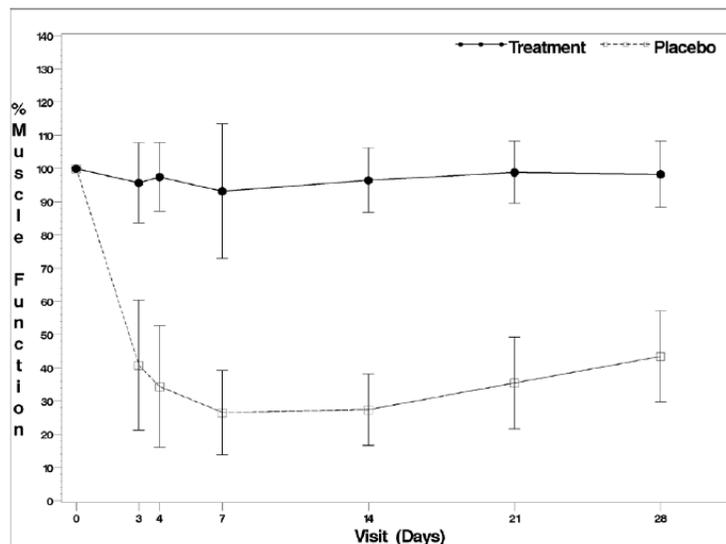
A comparison of the percent muscle function of the EDB muscle following exposure to Myobloc® (botulinum toxin type B), between the treatment and placebo group indicates that the observed preservation of muscle function in the treatment group is significant (P-value < 0.0001). Table 2 and Figure 2 show that, whereas the treatment arm presents with no decrease in percent muscle function following IM administration of botulinum toxins (96% muscle function), the placebo arm shows a loss of approximately 60% muscle function within 3 days post-exposure to

botulinum toxin B. Table 2 and Figure 2 also show that there is no decrease in percent muscle function in subjects exposed to NP-018 (treatment arm) from baseline (Day 0) to the end of the study (Day 28). In the same figure, however, there is a rapid decrease in percent muscle function in the placebo arm over the first 7 days. Thereafter, a gradual increase in muscle function is observed from Day 14 until the end of the study (Day 28) suggesting that Myobloc® (botulinum toxin Type B) is unable to sustain muscle paralysis to the same extent as Botox® (botulinum toxin type A) over time. Similar results on the duration of effect were observed in Stage A of the study.

Table 2 Mean and standard deviation of the percent muscle function (CMAP M wave amplitudes) of the EDB muscle for both treatment arms following exposure to Myobloc® over time (ITT population). Reference electrode at the ‘standard’ location

Study Visit	Botulism Antitoxin Heptavalent (Equine) Types A-G			placebo (0.9% saline solution)		
	Number of subjects (N)	Mean percent muscle function	SD	Number of subjects (N)	Mean percent muscle function	SD
Day 3	15	96	12	10	41	20
Day 4	15	97	10	10	34	18
Day 7	15	93	20	10	27	13
Day 14	15	96	10	10	27	11
Day 21	15	99	9	10	35	14
Day 28	15	98	10	10	43	14

Figure 2: The percent muscle function over time (CMAP M wave amplitudes) of the EDB muscle following exposure to Myobloc® over time (ITT population). The reference electrode at the ‘standard’ location



An additional analysis was also performed on similar data collected for the reference electrode in the 'inactive' location. Similar results were observed as with the reference electrode in the 'standard' position.

Conclusions:

This exploratory study was designed to evaluate a pharmacodynamic model for the prevention of Botulism intoxication in healthy human subjects.

In BT-002 Stage B, subjects given Botulism Antitoxin Heptavalent (Equine) Types A-G prior to exposure to botulism toxins A and B (Botox® and Myobloc®, respectively) presented with little to no loss of percent muscle function in the EDB muscles of both feet over the 28 day study period. Subjects in the control group (placebo) presented with a significant decrease in percent muscle function following exposure to botulism toxins A and B. This reduction in muscle function was maintained over the 28 day study period. Similar results were observed in BT-002 Stage A.

In this study the investigational product botulism antitoxin heptavalent (equine) types A-G manufactured by Cangene Corporation demonstrated comparable results in its ability to neutralize Botulinum toxins Types A and B and preserve muscle function (preventing muscle paralysis) as the currently licensed botulism antitoxin bivalent (equine) from Aventis Pasteur.

Comments:

The pharmacodynamic results, analyses and conclusions are acceptable from a Clinical Pharmacology perspective.

- Study BT-002 was designed as an exploratory pharmacodynamic clinical study employing a local in-vivo model. Because the BoNT intoxication is expected to occur in patients systemically not locally the study results can only be considered supportive for the overall risk/benefit assessment.
- The pharmacodynamic study results only allow an assessment of the Botulism Antitoxin Heptavalent (Equine) Type A-and Type B. The effects of antitoxins type C, D, E, F, and G on corresponding BoNT serotypes have not been investigated.

Study #9: Population PK/PD modeling and simulations to support human dosing regimen specification of NP-018. **Study Number:** CANG-RAS-001

Objective: The objective of the current report is to present pharmacokinetic (PK) and pharmacodynamic (PD) modeling results using data related to guinea pigs, non-human primates (NHP) and humans in order to support the human dosing regimen of NP-018.

- A compartmental PK model to assess the PK profile of NP-018 for each species, based on data collected from the Cangene Corporation pre-clinical/clinical studies was established. A traditional allometric power model was used to scale compartmental PK parameters according to the corresponding body weight of each species.
- Subsequently, a body burden-response model was constructed using NHP and guinea pig prophylaxis data in order to assess the response to BoNT in the presence of various body burdens of NP-018 across species. Exposure to NP-018 was simulated using the PK model and response was compiled from available post-exposure prophylaxis studies. This model was then leveraged to justify the proposed clinical dose levels.

PK and PD information of NP-018 collected in a total of 5 studies performed by Cangene Corporation that were included in this analysis are presented in Table 1

Table 1: Summary of PK and PD Studies Performed by Cangene Corporation

Title	Year
Efficacy of Post-exposure Prophylactic Administration of NP-018 Botulinum Antitoxin Heptavalent in Rhesus Macaques (<i>Macaca mulatta</i>)	2008
Intravenous NP-018 Pharmacokinetics Determination in Guinea Pigs	2007
NP-018 Antitoxin: Intravenous Pharmacokinetic Study in Rhesus Macaques	2007
Pharmacokinetics of a Heptavalent Equine-derived Botulinum Antitoxin (NP-018)	2006
Post-exposure Prophylaxis Study of NP-018 in Botulinum Neurotoxin - Challenged Guinea Pigs	2010

Pharmacokinetic Model:

The following four single-dose datasets across three animal species were available for population PK analysis of NP-018 (Table 2):

Table 2: Summary of PK Datasets

Species	# of Single Dose PK Datasets	Serotypes	NP-018 Alone	BoNT Challenge
Guinea Pig	1	7	Yes	No
Non-human Primate	2	1 (A)	Yes	Yes
Human	1	7	Yes	No

A total of 264 guinea pigs, 42 NHP, and 40 humans were sampled for PK of NP-018. Overall, 502 profiles were included in the PK analysis of this study. Guinea pig samples were pooled

Table 4: Final Population PK Parameters of NP-018 for All Serotypes

Parameter	PK Parameters of NP-018 (Geometric Mean)						
	Serotype A	Serotype B	Serotype C	Serotype D	Serotype E	Serotype F	Serotype G
CL (mL/h/kg)	14.75	10.82	8.08	4.44	7.72	12.25	10.01
CLd (mL/h/kg)	2.70	7.24	16.17	5.29	0.88	43.40	2.84
CLdt (mL/h/kg)	8.15	136.03	4.56	2.63	NA	2.53	40.48
Vc (mL/kg)	31.88	21.32	56.45	0.66	0.25	32.44	20.56
Vp (mL/kg)	21.17	145.29	25.44	14.93	4.19	44.23	67.04
Vdt (mL/kg)	23.38	34.39	1227.12	5.44	NA	40.94	28.03
Proportional Error (%)	24%	34%	45%	30%	38%	35%	35%

NA = Not Applicable

Table 5: Allometric Exponent Values of CL and Vc of NP-018 for All Serotypes

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

Only Serotype E displayed a CL that was directly proportional to BW (slope of 1.033; Table 5). The remaining estimates were close to 0.75 or 0.66 in all cases but for Serotype D clearance, which was significantly lower (0.376). Volume slope estimates were close to 1 for all serotypes except for Serotypes A and D, where slopes were significantly different from 1 (0.460 for Serotype A and 0.193 for Serotype D).

Exposure-Response Model:

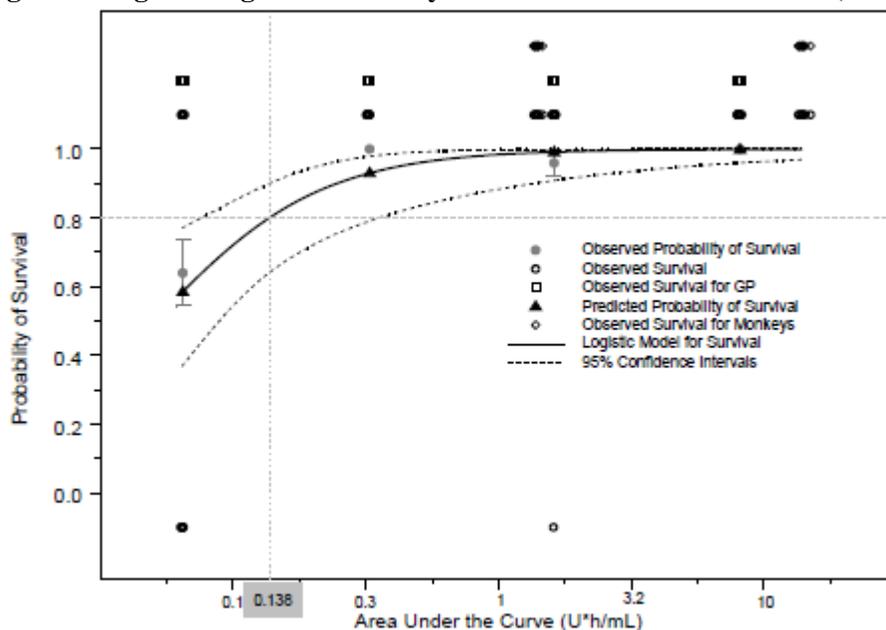
Given the assumption that the interaction between NP-018 and BoNT in the systemic circulation (i.e., the pharmacodynamics) remains the same across species and thus combining the

relationship between body burden (exposure) and effect in animals and estimated body burden of NP-018 in humans is sufficient to predict efficacy for a specific body burden in man.

A key component to this assessment is the relationship between NP-018 exposure (AUC) and effect, which can be constructed using available animal data. Following simulations of the NP-018 exposure (AUC) in animals showing various clinical signs (salivation, lacrimation, weak limbs, and change in breathing sounds) a logistic regression was performed. This analysis was used to explore the relationship between the NP-018 exposure measures (AUC) predicted by the population PK model and the probability of survival. In addition the AUC calculated from the PK model was used to predict various relevant moderate clinical signs.

Logistic regression was used to explore the relationship between NP-018 exposure measures (AUC) predicted by the population PK model and the probability of survival. Using serotype A as an example, the relationship between the body burden (AUC) of NP-018 (Serotype A) and survival derived with the logistic regression model is presented in Figure 1.

Figure 1. Logistic Regression of body burden of NP-018 vs. Survival (Serotype A)



Parameter estimates (logit scale) for the regression model (slope and intercept) were estimated for each exposure-survival relationship. Based on the results, the predicted probabilities of human survival for the serotypes A to G following a dosing of 1x NP-018 were ranging between 95.9%, and 99.9%. In addition predicting human survival, logistic regression models of moderate clinical signs were performed with the human-specific body burden data following administration of 1 x NP-018. Results showed, that the 1x NP-018 dose is expected to result in a significant protection against all clinical signs in humans for all BoNT serotypes.

Conclusions:

- Population PK Modeling: A population PK model including allometric components was used to assess the PK of NP-018 in guinea pigs, NHP, and humans. Following development of this PK model, the NP-018 body burden in humans at the proposed clinical dose (0.160 mL/kg) was estimated and compared against BoNT-treated animals (guinea pig and NHP). Since the PK model allowed for extrapolation across multiple species, simulation of the body burden (AUC) was predicted in animals for which no pharmacokinetic samples were collected. As such, exposure-response curves were constructed to correlate NP-018 exposure to the probability of survival and other clinical signs of interest.
- Exposure-Response models were used to explore the relationship between NP-018 exposure measures predicted by the population PK model (AUC) and the probability of survival as well as relevant moderate clinical signs observed during the pre-clinical development of NP-018. The predicted probability of survival in humans for all serotypes of BoNT was more than 95.9% following IV administration of 1x NP-018. Furthermore, the 1x NP-018 dose will be expected to result in a significant protection against salivation and lacrimation clinical signs in human for all BoNT serotypes.

Comments:

- This study is acceptable from Clinical Pharmacology perspective.
- The submitted exposure-response model only takes into account data from 2 “prophylactic” animal studies. The results of additional 2 “treatment” studies have not been incorporated into the model. Based on their design we consider those two studies relevant, because the results can be directly linked to the proposed indication in the label.