CLINICAL PHARMACOLOGY BLA REVIEW
Division of Hematology
Office of Blood Review & Research

BLA 125562/0

Product: Anthrax Immune Globulin Intravenous (Human) (AIGIV)

Sponsor: Cangene Corporation

Indication: Treatment of adult and pediatric patients with toxemia associated with inhalational anthrax

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EXECUTIVE SUMMARY

Inhalational anthrax occurs after the inhalation of aerosolized *B. anthracis* spores. It has a short incubation, rapid progression and high mortality rate. Early symptoms of infection appear after a 1 to 5 day incubation period. The disease is usually fatal within 24-36 hours after the onset of respiratory symptoms. The rapid course of symptomatic inhalation anthrax makes early antibiotic administration desirable and antibiotics are indicated for post-exposure prophylaxis to *B. anthracis* spores.

Anthrax Immune Globulin Intravenous (Human) (AIGIV or NP-015), is being developed for the treatment of toxemia associated with inhalational anthrax disease. The product is a human polyclonal antisera produced from the serum of individuals vaccinated with Anthrax Vaccine. NP-015 contains antibodies against Protective Antigen (PA) and has toxin neutralizing capabilities. It is anticipated that AIGIV will be used in conjunction with antimicrobial therapy in patients with symptomatic anthrax. The treatment of toxemia with AIGIV is expected to complement the bactericidal actions of antimicrobial therapy in patients with symptomatic anthrax disease.

The effective human dose of AIGIV is currently unknown and was determined based on pre-clinical efficacy studies and human dose scaling techniques. An initial estimate of 420 U TNA for the theoretical human effective dose was proposed by Cangene based on literature data from animal studies and vaccination data in humans.

Since the evaluation of human efficacy of AIGIV 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 human immunoglobulin antitoxin product.

In order to find the human efficacious dose and pharmacokinetics of AIGIV, the applicant conducted the following studies:

- Pharmacokinetic studies were conducted in healthy as well as in inhalational anthrax exposed rabbits and monkeys.
- A safety and pharmacokinetic study in healthy humans was conducted.
- Therapeutic efficacy studies of AIGIV were conducted in rabbits and monkeys.
- A population PK/PD model was developed to support the human dosing for AIGIV.
- Modeling and simulation was used to project AIGIV dose in obese subjects.
- Allometric scaling was used to project AIGIV dose in children (from neonates to adolescents).

PHARMACOKINETIC STUDIES IN HEALTHY ANIMALS AND HUMANS

Pharmacokinetic (PK) studies of Anthrax Immune Globulin Intravenous (Human) (AIGIV) were conducted in rabbits, cynomolgus monkeys, and humans. The analytical methods used to

determine serum anti-protective antigen (anti-PA) levels were validated anti-PA (b) (4) and validated Toxin Neutralization Assay (TNA). However, Cangene and the FDA had agreed that TNA would be the primary assay for product potency, dosing and PK analysis, as it measures neutralizing antibodies as opposed to the anti-PA (b) (4) Therefore, the PK parameters reported in this summary are based on TNA analytical method. PK parameters of AIGIV were calculated by non-compartmental analysis. The following is the summary of the PK studies conducted in rabbits, cynomolgus monkeys, and humans.

New Zealand White Rabbits:

Rabbits received intravenous infusion of AIGIV at the doses of 5, 10, 15, 30 and 40 U/kg body weight. Blood samples for PK study were drawn before infusion (day -7) and at 1, 8, 24, 48 hours, and days 3, 5, 8, 11, 14, 21, and 28. At 40 U/kg dose of NP-015, rabbits did not survive due to toxicity of the dose hence, no PK could be assessed in rabbits at this dose. The PK parameters of AIGIV in rabbit are shown in Table 1.

Cynomolgus Monkey:

Monkeys received intravenous infusion of AIGIV at the doses of 5 and 30 U/kg body weight. Blood samples were collected on day -7 or day -8 (prior to infusion), day 0 (1 h and 12 h post-infusion), and days 1, 3, 5, 7, 14, 21, 28, 35, 45 and 56. PK parameters of AIGIV in monkey are shown in Table1.

Humans:

A dose-ranging study was designed to assess the PK and safety of three doses of AIGIV (210 U, 420 U and 840 U/kg body weight) after intravenous administration to healthy volunteers. This study was double-blinded and randomized and included placebo controls. A total of 72 healthy adult male and female subjects aged 19-55 were recruited in three cohorts of 24. Subjects were randomized to receive a 210 U (cohort 1), 420 U (cohort 2) or 840 U (cohort 3) dose of AIGIV (N = 18/dosing group) or an equal volume of saline placebo (N = 6/dosing group). Blood samples were drawn from the study subjects in cohorts 1-3 at the following times after drug administration: 1, 3, and 8 hours, and days 1, 3, 5, 7, 9, 11, 14, 21, 28, 42, 56 and 84 or at early withdrawal. The PK parameters of AIGIV in humans are summarized in Table 1.

The clearance (CL) of AIGIV in rabbits (9.46 to 13.2. mL/day/kg) and cynomolgus monkey (12.5 mL/day/kg at the 30 U/kg dose) was at least 4-5 fold faster (based on per kg basis) than in humans (2.33 to 2.48 mL/day/kg). The volume of distribution is relatively similar in all species when normalized for body weight. The half-life of AIGIV in humans is four to five times longer than in rabbits and cynomolgus monkeys primarily due to slower clearance of AIGIV in humans.

Table 1: Interspecies Comparison of AIGIV PK Parameters (TNA)

PK Parameter	Rabbit			Cynomolgus Macaque		Human		
	5 U/kg	15 U/kg	30 U/kg	5 U/kg	30 U/kg	210 U	420 U	840 U
C_{max} (mU/mL)	111 ±9	420 ±15	559 ±29	101 ±10	532 ±14	82.3 ±13.7	152.9 ±22.4	311.8 ±18.2
$t_{1/2}$ (days)	4.61 ±0.58	4.56 ±0.38	4.43 ±0.49	9.8[a]	6.71 ±1.02	24.3 ±33.3	28.3 ±19.9	28.0 ±25.2
Cl (mL/day/kg)	11.5 ±0.7	9.46 ±0.41	13.2 ±0.9	6.55[a]	12.5 ±1.2	2.34[b]	2.33[b]	2.48[b]
V_d (mL/kg)	75.6 ±9.9	55.2 ±2.70	80.2 ±5.9	92.6[a]	112.0 ±12.0	76.8[b]	93.9[b]	95.4[b]

PK Parameter	Rabbit			Cynomolgus Macaque		Human		
	5 U/kg	15 U/kg	30 U/kg	5 U/kg	30 U/kg	210 U	420 U	840 U
AUC_{0-inf} (day*mU/mL)	475 ±32	1710 ±80	2450 ±180	752[a]	2437 ±200	1239.4 ±26.5	2507.5 ±16.4	4624.2 ±28.5

Values for rabbits and NHPs are means ±SEM (standard error of the mean); values for humans are means ±SD (standard deviation).

[a] Data represent a single animal.

[b] Cl and V_d were calculated per kg body weight for human subjects using the following average weights:
210 U – 74.4 kg; 420 U – 72.8 kg; 840 U – 75.9 kg

Impact of immunogenicity on PK in normal healthy animals:

Based on a sample size of 28 rabbits, there was a decrease in AIGIV $AUC_{(0-last)}$ in anti-human IgG positive animals compared to negative animals. There was increase in clearance (30%) and decrease in half-life (50%) in anti-human IG positive animals. The impact of immunogenicity on the PK of AIGIV could not be assessed in normal healthy non-human primates because there was only one animal that showed positive anti-human IG response. Immunogenicity to AIGIV is not anticipated to occur in humans as AIGIV is a human IG product.

PHARMACOKINETIC STUDIES IN ANTHRAX EXPOSED ANIMALS

Rabbits:

The PK profile of AIGIV in anthrax exposed rabbits was determined at AIGIV dose of 15 U/kg. Rabbits were exposed to 200 x LD50 anthrax spores via aerosol route. In this study, 34 rabbits out of 110 were excluded from the PK analysis because they did not survive to Day 7 post-exposure. The $AUC_{(0-7)}$ and $AUC_{(0-14)}$ were different in the group of rabbits with aerosolized anthrax spores than the group of rabbits who were not given the toxin. PK parameters (with the exception of AUC and CL) could not be assessed in this study. The clearance of AIGIV was more than 2-fold higher in the rabbits who received the toxin than those rabbits who were not administered the toxin. The clearance of AIGIV in normal healthy rabbits based on $AUC_{(0-7)}$ and

$AUC_{(0-14)}$ was 12.6 and 9.7 mL/day/kg, respectively. The clearance of AIGIV in the exposed rabbits, based on $AUC_{(0-7)}$ and $AUC_{(0-14)}$ was 26.3 and 23.1 mL/day/kg, respectively.

Monkeys:

The pharmacokinetic profile of AIGIV in anthrax exposed monkeys was determined at AIGIV doses of 7.5, 15 or 30 U/kg. Monkeys were exposed to 200 x LD_{50} anthrax spores via aerosol route. Due to the nature of the study (exposure to aerosol spore), animals died prior to their scheduled termination time. For the assessment of AUC (both AUC_{0-7} and AUC_{0-14}), all animals that survived to seven or fourteen days post-infusion were included in the analysis. The clearance of AIGIV was not different in the group of monkeys with aerosolized anthrax spores than the group of monkeys who were not given the toxin. The clearance of AIGIV in normal healthy monkey based on $AUC_{(0-7)}$ and $AUC_{(0-14)}$ was 18.5 and 13.9 mL/day/kg (30 U/kg), respectively. The clearance of AIGIV in the exposed monkey, based on $AUC_{(0-7)}$ and $AUC_{(0-14)}$ was 21 and 13 mL/day/kg, respectively.

Conclusions: The PK study in healthy animals and humans indicate that the clearance of AIGIV in rabbits and monkeys is at least 4-5 fold faster (based on per kg basis) than in humans. The volume of distribution is relatively similar in all species when normalized for body weight. The half-life of AIGIV in humans is four to five times longer than in rabbits and monkeys primarily due to slower clearance of AIGIV in humans.

In anthrax exposed animals, the PK assessment was difficult due to deaths in the animals. However, it was possible to estimate AUC(0-7 days) and AUC(0-14 days) for animals who survived till days 7 and 14. The clearance of AIGIV in anthrax exposed rabbits based on $AUC_{(0-7)}$ and $AUC_{(0-14)}$ was at least 2-fold higher than the healthy rabbits. On the other hand, no difference in the CL of AIGIV was found between healthy and anthrax exposed monkeys.

Impact of immunogenicity on PK in anthrax exposed animals:

When comparing rabbits by immunogenicity status at day 21 (n = 4), the mean half-life reduced by 32% (clearance increased by 10-15%) for animals with anti-human IgG antibodies compared to the levels observed in animals without anti-human IgG antibodies.

In study (b) (4) 1182-100011472, there was no significant difference in $AUC_{(0-last)}$ or $AUC_{(0-\infty)}$ or clearance or half-life between animals positive or negative for anti-human IG based on (b) (4) data. For TNA data, clearance increased by 18% based on immunogenicity status on day 14; however, there were only a few animals (n = 2) available for the analysis.

EFFICACY STUDIES IN RABBITS AND MONKEYS

Rabbit:

Study #1: The objective of this study was to assess the efficacy of AIGIV at three dose levels given either at 20 or 30 hours post-exposure in rabbits exposed to lethal doses of *B. anthracis* (Ames strain) spores by the aerosol route. In this study, 122 rabbits were randomized into eight treatment groups and one untreated control group. Rabbits received a target aerosol spore dose of 200 x LD_{50}. Following exposure, rabbits received either IGIV (placebo) or one of three doses of AIGIV at 20 or 30 hours post-exposure. The doses of AIGIV tested were 7.5, 15 and 30 U/kg. The primary efficacy endpoint for this study was survival at day 28 post-exposure. All untreated and placebo (IGIV) control animals died. A statistically significant improvement in survival was observed with all three doses of NP-015 given at 20 hours post-exposure compared to placebo treatment. Survival of 57, 79 and 93% was attained for the 7.5, 15 and 30 U/kg dose levels, respectively. When the treatments were delayed to 30 hours post-exposure, the survival rates decreased to 29, 43 and 36%, respectively, but were still significantly higher than the 0% survival observed in the placebo and control group.

Comments: Although the 30 U/kg dose produced higher survival rate than the 15 U/kg (93 vs. 79%, respectively), there was no statistically significant difference in survival between the two dose levels. It should be however, noted that 30 U/kg dose may not be statistically different than the 15 U/kg, the 30 U/kg dose may be clinically relevant. Based on this study, the applicant decided to select 15 U/kg AIGIV dose as a minimum efficacious dose (MED).

Study #2: New Zealand white rabbits (n =110, 55 male, 55 female) were challenged with *Bacillus anthracis* (Ames strain) spores via aerosol exposure. The average challenge dose received by the animals was 194 ± 33 x LD_{50}. The exposed animals were treated with either a single intravenous infusion of 15 U/kg of AIGIV or an equivalent volume of IGIV (50 rabbits per treatment group) at the onset of toxemia. The remaining 10 animals served as controls. Survival was the primary endpoint used to determine the efficacy of AIGIV over placebo. Of the animals that were bacteremic prior to treatment, 26% (13/50) that received AIGIV survived to the end of the study while only 2% (1/48) rabbits that received IGIV survived. The median times to death were 75.8 and 148.5 hours post-challenge for IGIV and AIGIV-treated animals, respectively. The overall survival and time to death were significantly greater in the AIGIV group. A logistic regression model for animals having the same toxin levels prior to infusion indicated that the odds of survival in the AIGIV group were 57 times those in the IGIV group.

Comments: AIGIV, when administered therapeutically at the onset of toxemia, significantly increased survival and the clearance of circulating PA and bacteremia compared to the placebo in rabbits exposed to aerosolized anthrax spores. However, it should be recognized that only 26% (13/50) rabbits that received AIGIV survived to the end of the study while only 2% (1/48) rabbits that received IGIV survived. Compared with IGIV, the survival rate was higher in the rabbits

who received AIGIV yet, 74% of the total number of rabbits did not survive. One may attribute the low survival of rabbits in the treated group either due to lack of appropriate dose or lack of efficacy of the product.

Cynomolgus monkey:

In this study, 64 cynomolgus monkeys randomized to four groups of 16 animals each were exposed to a target aerosol spore dose of 200 x LD_{50}. Upon detection of toxemia, the animals were treated intravenously with one of three doses (7.5, 15 or 30 U/kg) of AIGIV or a single dose of IGIV (placebo). The primary endpoint was survival on day 28 post anthrax exposure. Only 6% (1/16) of placebo-treated animals survived the lethal anthrax exposure. Survival rates of 27% (4/15), 44% (7/16) and 71% (10/14) were observed with 7.5, 15 and 30 U/kg doses of AIGIV, respectively. Both 15 and 30 U/kg doses of AIGIV had significantly higher survival rates than the placebo treated group. There was no statistically significant difference in the efficacy between the 15 and 30 U/kg doses of AIGIV.

Comments: Although, 30 U/kg dose in terms of efficacy was not statistically significant than 15 U/kg, the data suggest that 30 U/kg dose was superior to 15 U/kg dose in producing efficacy (71% vs 44%). Not only total mortality rate was higher in 15 U/kg dose group (56% vs 29%) but recurrence of toxin was also noted in this dose group.

AIGIV COMBINATION THERAPY IN THE RABBIT

Study #1: In this study, therapeutic efficacy of AIGIV given in combination with levofloxacin was assessed in comparison to placebo (IGIV) given in combination with levofloxacin when treatment was administered at up to 60 hours after exposure to lethal doses of *B. anthracis*. Seventy-two rabbits were divided into nine groups of eight animals. All animals were aerosol challenged with a target of spore dose of 200 x LD50 *B. anthracis* in three cohorts with animals from each group. Four groups were administered with 15 U/kg of AIGIV via slow intravenous infusion in combination with 50 mg/kg levofloxacin (given orally, once a day for three days) starting at 30, 36, 48, or 60 hours post-exposure. Four other groups were administered placebo (IGIV) via slow intravenous infusion in combination with the same regimen of levofloxacin at the same time points post-exposure. AIGIV or placebo infusion was initiated within 30 minutes of the first levofloxacin dose. One group of eight animals remained untreated following exposure. Animals were observed to 30 days post-challenge for survival, body temperature, clinical observations, hematology, bacteremia and toxemia.

All of the untreated control animals died following anthrax exposure. A survival rate of 100% (8/8) was observed in the IGIV plus levofloxacin groups that received treatments starting at 30, 36 and 48 hours post-exposure. A survival rate of 87.5% (7/8) was observed for the group that received treatment at 60 hours post exposure.

Treatment with AIGIV plus levofloxacin resulted in similar survival rates of 87.5, 100, 100 and 75% in the 30, 36, 48 and 60 hour treatment groups, respectively. The data demonstrated the efficacy of levofloxacin when given in combination with IGIV or AIGIV as 94% (59/63) of treated animals from all groups survived, even when treatment was significantly delayed. The difference in the survival rates between the IGIV (placebo) plus levofloxacin and AIGIV plus levofloxacin was not statistically significant at any of the time points tested.

Comments: The study indicates the efficacy of levofloxacin alone in toxin treated rabbits and AIGIV does not appear to be more efficacious than levofloxacin alone or provided added benefit to levofloxacin treatment.

Added Benefit Study #1: The objective of this study was to evaluate the therapeutic efficacy of AIGIV over placebo (IGIV) when either was administered with levofloxacin at different time points post-challenge with 200 x LD_{50} aerosolized spores of *B. anthracis*. Treatment groups received 15 U/kg NP-015 or IGIV with levofloxacin starting 60, 72, 84, or 96 hours post-challenge. Pre-treatment mortality (i.e., animals that died before completing the infusion) ranged from 10 to 70%, and increased with lag time when the treatments were delayed to 60, 72, 84 and 96 hours post exposure.

Survival rates observed for the IGIV plus levofloxacin treated groups were 90%, 50%, 25% and 25% when treatments were initiated at 60, 72, 84 and 96 hours post exposure, respectively. At these same time points, survival rates among the AIGIV plus levofloxacin groups were 100, 65.2, 40 and 71.4%, respectively. Differences in the survival rates and time to death observed between IGIV plus levofloxacin and AIGIV plus levofloxacin treatments were not statistically significant at any of the time points tested.

The majority of AIGIV treated animals became negative for PA (toxemia) within one hour post-infusion of AIGIV and remained negative, even with the delayed treatment from 60 to 96 hours post-anthrax challenge and high levels of toxemia pretreatment. In contrast, IGIV placebo treated animals remained toxemic up to three days after initiating antibiotic treatment.

Added Benefit Study #2: In this study, the therapeutic efficacy of the combination treatment of AIGIV and levofloxacin was evaluated over that of IGIV (placebo) and levofloxacin when either treatment was initiated 96 hours after aerosol exposure. All animals (n =336) were aerosol challenged with a target dose of 200 x LD50 *B. anthracis*. Animals surviving to 96 hours post-challenge (n = 81) were randomized to treatment with IGIV and 50 mg/kg levofloxacin or 15 U/kg NP-015 and 50 mg/kg levofloxacin. Three doses of levofloxacin were given once daily for three days starting at infusion. Animals were observed over a 36 day period. Survival was the primary endpoint used to assess added benefit of AIGIV at 96 hours post-exposure.

Out of 64 animals that were positive for toxemia and bacteremia prior to treatment, 31 of them received AIGIV and 33 of the rabbits received IGIV. Of these 33 animals in the IGIV

group, 13 animals survived through 36 days post-exposure (13/33, 39% survival). Out of 31 animals who received AIGIV, 18 animals survived through day 36 (58% survival). The 19% absolute difference in survival rate between the AIGIV and IGIV groups did not reach statistical significance however, that difference may be clinically meaningful.

Due to the delay in the treatment time of 96 hours post exposure in this study, there was a significant pre-treatment mortality, suggesting that animals were in their late stages of the systemic disease at the time of treatment. Following treatment, the mortality continued in treated animals, suggesting that toxin levels at the time of treatment may play a role in the outcome.

AIGIV **COMBINATION THERAPY IN THE MONKEY**

A total of 72 cynomolgus monkey were divided into four groups; 12 animals were untreated controls and the remaining animals were randomized to three treatment groups of 20 animals each treated at 64 hours post-exposure with ciprofloxacin and either IGIV placebo or 15 or 30 U/kg AIGIV. All animals were aerosol challenged with a target dose of 200 x LD50 *B. anthracis*. Sixty-four hours post-challenge, animals received a loading dose of 32 mg/kg ciprofloxacin followed by nine maintenance doses (16 mg/kg) every 12 hours. Animals also received either a placebo infusion of IGIV, 15 or 30 U/kg AIGIV. Animals were observed till day 73. The results of the study indicated that antibiotic treatment alone successfully treated anthrax infection (Table 2) and the survival rate was comparable between IGIV + ciprofloxacin and AIGIV + ciprofloxacin treated groups.

Table 2: Survival in Cynomolgus Macaques after Treatment with Combination Therapy 64 Hours Post-exposure

Group	Survival	
	All Animals	**Bacteremic Animals**
Untreated	1/12 (8%)	1/12 (8%)
IGIV + Ciprofloxacin	12/19 (63%)	9/12 (75%)
AIGIV (15 U/kg) + Ciprofloxacin	11/18 (61%)	10/12 (83%)
AIGIV (30 U/kg) + Ciprofloxacin	12/19 (63%)	11/14 (79%)

Overall Comments: The efficacy studies of AIGIV in rabbits and monkeys do indicate that AIGIV is efficacious against anthrax challenge. However, the survival rate indicates that the protection provided by AIGIV in animals is not high and ranged from 26% to <80% at 15 U/kg dose. On the other hand, levofloxacin alone provided >80% survival in animals and was comparable with AIGIV in its protective characteristics against anthrax challenge.

COMMENTS

1. AIGIV DOSING IN ANTHRAX EXPSED HUMANS

Throughout the submission, the applicant focused on justifying a 420 U dose in humans rather than trying to find out other suitable doses (besides 420 U). The applicants' justification of 420 U human dose as optimal is not convincing.

The efficacy studies of AIGIV in rabbits and monkeys do indicate that AIGIV is efficacious against anthrax challenge. However, the survival rate indicates that the protection provided by AIGIV in animals is not high and ranged from 26% to <80% at 15 U/kg dose. Although, data in Table 1 indicate that an improved therapeutic benefit was observed from 30 U/kg dose than 15 U/kg dose in animals, the applicant chose 15 U/kg animal dose, citing that there was no statistical difference between the two doses.

Table 1: Survival rate (%) against dose (monotherapy)

Species	7.5 U/kg	15 U/kg	30 U/kg
Rabbit	57	79	93
Monkey	27	44	71

The applicant used the following methods to project AIGIV human dose:

Table 2: Summary of different dose scaling methods for the estimation of human dose

Source	Estimated Human AIGIV Dose	
	U/kg	U/70 kg Patient
Human Equivalent Dose; rabbit or NHP dose of 15 U/kg x 0.32 conversion based on body surface area)	4.8	336
Rabbit 15 U/kg dose, AUC threshold of ~1400 mU*day/mL, and human PK scaled from rabbit	4.5	315
Rabbit 15 U/kg dose, AUC threshold of ~1400 mU*day/mL, and human AIGIV PK data	3.9	273
Cynomolgus macaque 15 U/kg dose, AUC threshold of ~1300 mU*day/mL, and human AIGIV PK date	3.6	252
Range	3.6–4.8	252–336

Please note that the maximum projected dose in Table 2 in humans from these methods is 336 U and the applicant suggests a dose of 420 U.

Based on the exposure response relationship presented in Table 3 and Figure 1, the 420 U mono-therapeutic AIGIV dose is predicted to result in 27% probability of survival in 90% of the population. From Table 3 and Figure 1, it is evident that as AIGIV dose increases the survival rate also increases. Based on the exposure-response analysis, a threshold target of 80% survival

would require a dose of >2127 U or 35 vials. This corresponds to 5-fold increase over proposed dose of 420 U.

Table 3: Exposure-response: Survival probability as a function of predicted AIGIV exposure (AUC) as monotherapy

Survival Probability		AUC (U*h/L)	Typical CL in Unexposed Humans (L/h)	Predicted Dose (U)	No. of Vials[a]
Predicted	95% Confidence Interval Lower–Upper	Predicted			
50%	42.7–57.2 %	196308	0.00519	1019	17
55%	46.4–63.1 %	227183	0.00519	1179	20
60%	50.8–68.7 %	258733	0.00519	1343	22
65%	54.8–74.0 %	291608	0.00519	1513	25
70%	59.1–79.0 %	326749	0.00519	1696	28
75%	63.7–83.7 %	365449	0.00519	1897	32
80%	68.6–88.0 %	409749	0.00519	2127	35
85%	74.0–91.9 %	463374	0.00519	2405	40
90%	80.2–95.2 %	534599	0.00519	2775	46
95%	87.7–98.1 %	649674	0.00519	3372	56
99%	96.1–99.7 %	904024	0.00519	4692	78

[a] Based on ≥60 U/vial.

Figure 1: Survival probability as a function of predicted AIGIV exposure (AUC) as monotherapy
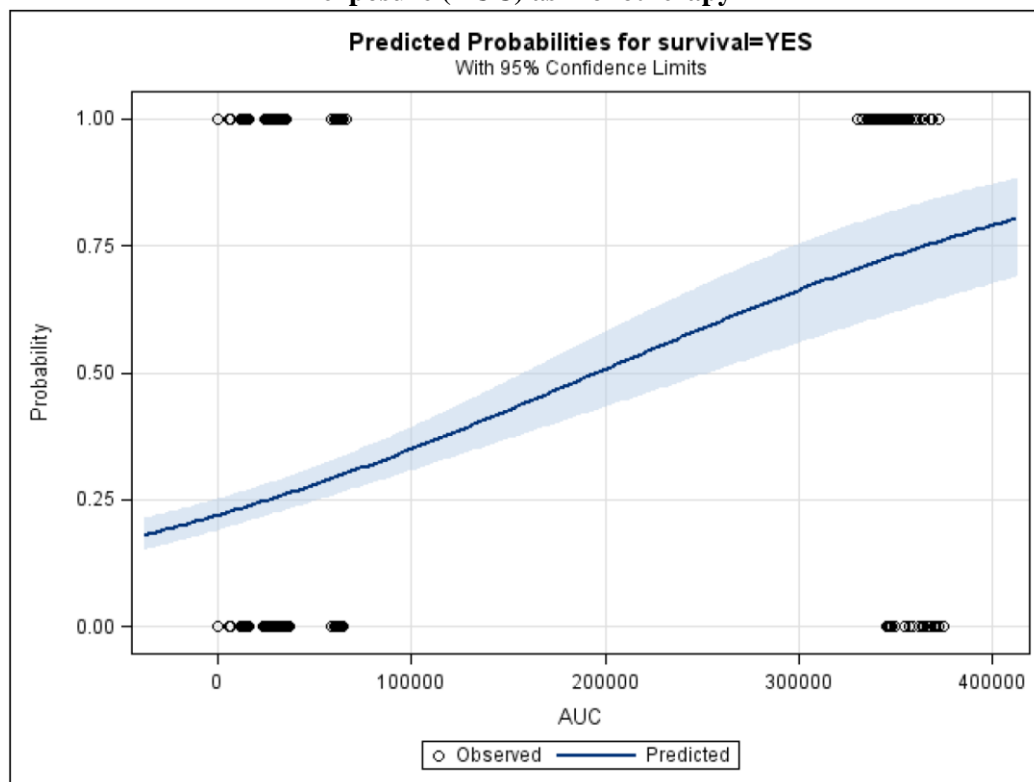
In combination with antibiotics, the 420 U dose is expected to provide 60% probability of survival in 90% of the population (Table 4).

Table 4: Exposure-response: Survival probability as a function of predicted AIGIV exposure (AUC) as co-administration

Survival Probability		AUC (U*h/L)	Typical CL in Unexposed Humans (L/h)	Predicted Dose (U)	No. of Vials[a]
Predicted	95% Confidence Interval Lower–Upper				
50%	38.9–61.1 %	(-88543)	0.00519	N/A	N/A
55%	46.2–63.5 %	(-6567.5)	0.00519	N/A	N/A
60%	52.9–66.7 %	77108	0.00519	400	7
65%	57.8–71.6 %	164408	0.00519	853	14
70%	61.1–77.6 %	257658	0.00519	1337	22
75%	63.6–83.7 %	360358	0.00519	1870	31
80%	65.9–89.2 %	477883	0.00519	2480	41
85%	68.3–93.7 %	620208	0.00519	3219	54
90%	71.1–97.1 %	809233	0.00519	4200	70
95%	75.2–99.2 %	1114558	0.00519	5785	96
99%	82.5–100 %	1789508	0.00519	9288	155

[a] Based on ≥60 U/vial.

The applicants' projection of survival in humans against anthrax is based on a clearance value which is even lower (5.19 mL/hr) than the mean clearance value of 7.1 mL/hr at 420 U dose. As shown below, the clearance of AIGIV ranges from 5.1 to 13.5 mL/hr in healthy subjects. It is expected that in anthrax exposed humans the clearance of AIGIV will be higher than the healthy subjects and the variability around the clearance will also be higher than the healthy subjects. If one accounts for higher clearance in anthrax exposed humans (in fact even in healthy subjects with variability in clearance) the human survival rate for 420 U dose will be much lower than the projected survival rate by the applicant.

Clearance (CL) in Healthy subjects:

Dose 420 U = Mean = 170 mL/day, Range = 146-240 mL/day
              OR 7.1 mL/hr, Range = 6.1-10 mL/hr

Dose 840 U = Mean = 189 mL/day, Range = 123-324 mL/day;
              OR 7.9 mL/hr, Range = 5.1-13.5 mL/hr

It should be noted that the pharmacokinetics for these two doses are linear.

Comments: The proposed human dose of 420 U by the applicant for the management of anthrax does not seem to be therapeutically effective in majority of the subjects as monotherapy. The antibiotic treatment alone appears to provide a similar therapeutic benefit as AIGIV.

Projection of AIGIV clearance in Adult humans exposed to Anthrax
(FDA Analysis)

There are only two animal species (rabbit and monkey) in which PK studies were conducted both in healthy animals and anthrax infected animals. A two-species allometric scaling may not be adequate to predict human PK parameters but can be useful and occasionally may give comparable results obtained from three or more species. For AIGIV, even the two-species scaling to predict clearance in anthrax exposed humans was not possible because the body weights of rabbit and monkey were almost similar (3 kg). Therefore, a single species with fixed exponent of 0.75 approach (not necessarily scientifically correct approach nevertheless, a useful approach) was used to predict AIGIV clearance in anthrax exposed humans. In order to test the robustness of the method first the clearance of AIGIV was predicted in healthy humans. Clearances of AIGIV calculated from partial AUCs (0-7 days and 0-14 days) were also predicted in humans because the AUC values in infected animals were only available till day 14. AIGIV clearance was predicted in humans as follows:

Predicted human CL = Monkey or rabbit clearance X (Human body weight 70 kg/ monkey or rabbit body weight 3 kg)^0.75

Prediction of AIGIV clearance in healthy humans

Monkey Dose = 30 U/kg (selected because in healthy monkey PK study was not conducted at 15 U/kg dose)

From monkey (based on $AUC_{(0\text{-infinity})}$ = monkey CL = 1.6 mL/hr
Predicted human CL = 17 mL/hr
Observed human CL = 6.8 mL/hr (based on 420 U dose)
Prediction error = 150%

From monkey (based on $AUC_{(0\text{-}14d)}$ = monkey CL = 1.74 mL/hr
Predicted human CL = 18.5 mL/hr
Observed human CL = 13.4 mL/hr (based on 420 U dose)
Prediction error = 38%

From monkey (based on $AUC_{(0\text{-}7d)}$ = monkey CL = 2.3 mL/hr

Predicted human CL = 24.4 mL/hr

Observed human CL = 21.6 mL/hr (based on 420 U dose)

Prediction error = 13%

Observed CL ratio in humans:
Based on AUC(0-14)/AUC(0-inf) = 1.97
Based on AUC(0-7)/AUC(0-inf) = 3.18

Since rabbit and monkey clearance values and body weights are similar, the projected clearance in healthy humans will also be similar from rabbit and monkey clearance values.

Prediction of AIGIV clearance in exposed humans

Monkey Dose = 15 U/kg in infected monkey
Observed monkey CL = 3.3 mL/hr: based on $AUC_{(0-7d)}$
Observed monkey CL = 1.9 mL/hr: based on $AUC_{(0-14d)}$

Predicted human CL (based on monkey CL based on $AUC_{(0-7d)}$ = 35 mL/hr
Predicted human CL (based on monkey CL based on $AUC_{(0-14d)}$ = 20 mL/hr

Rabbit Dose = 15 U/kg in infected rabbit
Observed rabbit CL = 3.3 mL/hr: based on $AUC_{(0-7d)}$
Observed rabbit CL = 2.9 mL/hr: based on $AUC_{(0-14d)}$

Predicted human CL (based on rabbit CL based on $AUC_{(0-7d)}$ = 35 mL/hr
Predicted human CL (based on rabbit CL based $AUC_{(0-14d)}$ = 31 mL/hr

Based on the ratios of AUC(0-14) or AUC(0-7) (3.18 or 1.97), the projected mean CL (based on AUC (0-inf)) of AIGIV in exposed humans is expected to be 11 mL/hr (range may be 10-15 mL/hr). However, a high variability (CV may be even 100%) should be envisioned.

Projected dose in exposed humans based on projected CL of 11 mL/hr or 264 mL/day (assuming targeted AUC is 1400 mU*day/mL):
A dose of 420 Units will produce an AUC value = (420x1000/264 mL/day) = 1591 mU*day/mL.

Projected dose in exposed humans based on projected CL of 15 mL/hr or 360 mL/day (assuming targeted AUC is 1400 mU*day/mL):
A dose of 420 Units will produce an AUC value = (420x1000/360 mL/day) = 1167 mU*day/mL.

Projected dose in exposed humans based on projected CL of 11 mL/hr or 264 mL/day (assuming targeted AUC is 1400 mU*day/mL):
A dose of 840 Units will produce an AUC value = (840x1000/264 mL/day) = 3182 mU*day/mL.

Projected dose in exposed humans based on projected CL of 15 mL/hr or 360 mL/day (assuming targeted AUC is 1400 mU*day/mL):
A dose of 840 Units will produce an AUC value = (840x1000/360 mL/day) = 2333 mU*day/mL.

Conclusions: Based on applicant' proposed effective AUC (1400 mU*day/mL), a dose of 420 U in humans appears to provide protection against anthrax toxicity. There may be however, a small percentage of people who may require higher doses of AIGIV. Considering the high variability in human CL especially in anthrax exposed humans, it is anticipated that 840 U dose will provide therapeutic benefit to more patients than 420 U dose.

Based on the exposure response relationship (survival probability as a function of predicted AIGIV (AUC) presented in Table 3 and Figure 1, the 420 U mono-therapeutic AIGIV dose is predicted to result in 27% probability of survival in 90% of the population. From Table 3 and Figure 1, it is evident that as AIGIV dose increases the survival rate also increases. In combination with antibiotics, the 420 U dose is expected to provide 60% probability of survival in 90% of the population (Table 4).

2. Modeling and Simulation of AIGIV to Support Dosing in Obese Subjects

Obesity is associated with physiological changes and in most of the time obesity impacts the PK of a drug. There are also instances when obesity has no impact on the PK of drugs but this can only be determined after conducting a PK study. The FDA requested that the applicant conduct a PK study of AIGIV in obese subjects to evaluate PK differences (if any) between normal healthy and obese healthy subjects. The following is the applicant's response.

In an emergency scenario such as a broad exposure event, it is anticipated that it would be advantageous for a fixed dose of AIGIV to be deployed and administered. FDA had asked Cangene to evaluate a weight based dose in addition to the proposed fixed dose and as such, modeling was conducted to examine whether the proposed fixed dose would be adequate for the obese population to supplement the initial population PK modeling. This modeling was not conducted to support any specific label claims regarding obese patients. As requested, the AIGIV exposure (420 U TNA dose) for the obese population using the revised model estimates are presented below in the following Table.

Table: AIGIV (420 U) Exposure in Obese Population – Combined Model

Body Weight (kg)	Dose (U)	CL (L/h)	AUC (mU*day/mL)
77.3	420	0.0055	3193
100.1	420	0.0063	2779
125.5	420	0.0071	2461

AIGIV exposure ranged between 2461 and 3193 mU*day/mL for patient body weights greater than 77 kg. In comparison, the exposure derived with the previous Population PK (POPPK) human model provided an exposure range for obese population from 1547 to 3965 mU*day/mL. Therefore, using unfixed exponents and a combined model, a 420 U dose is still considered adequate in obese patients independent of which model is used to simulate the AIGIV exposure.

Comments: The applicant's modeling and simulation of AIGIV in the obese assumes that there is no impact of obesity on the PK of AIGIV. This assumption may or may not be true. Therefore, a PK study of AIGIV in obese subjects is needed for supporting the dose of AIGIV in this population. Based on the modeling, the exposure range in the obese is from 1547 to 3965 mU*day/mL. This exposure range indicates that a 420 U dose may not be therapeutically beneficial to majority of obese subjects. It should be noted that the applicant's choice of 420 U dose in humans is based on effective exposure of 1400 mU*day/mL derived from effective animal exposure.

Generally, immunoglobulins are administered on per kg body weight basis but AIGIV will be given as a fixed dose. In a clinical setting, the treating physician will adjust the dose based on the response of the patients. In a mass casualty where hundreds of people will be affected, it will not be possible to monitor the anthrax exposed patients as one will be able to do when there are only few patients. As a result, it is important to give the initial dose as optimum or therapeutic as possible. Even in non-obese subjects, an anthrax dose of 420 U (as a monotherapy) appears to be sub-therapeutic in majority of subjects and if the PK is different (higher clearance and volume of distribution) between obese and non-obese subjects then the 420 U anthrax dose may be even less effective in the obese than the normal weight subjects.

3. Modeling and Simulation of AIGIV to Support Dosing in Pediatrics

The applicant proposed dosing of AIGIV in children (neonates to adolescents) is based on allometric scaling (not on population PK as claimed by the applicant). The applicant used a single exponent of 0.75 across all age groups which is an incorrect approach because a single exponent does not describe the PK parameters across all age groups. Fixed exponent 0.75 on CL will substantially over-estimate the CL of a drug in children <5 years of age especially, in neonates and infants. The applicant's current approach will lead to over-estimation of AIGIV clearance in

neonates and infants and any dose selection based on these CL values may overestimate the dose of AIGIV and may cause toxicity in this age group. The applicant also ignored the possibility that the clearance of anthrax in exposed humans may be higher than the unexposed subjects.

Using an age dependent exponent (1.2 for ≤ 3 months, 1.0 from >3 months to 2 years, 0.9 from >2 to 5 years, and 0.75 for >5 years of age) AIGIV clearance (14 mL/hr) in anthrax exposed humans resulted in the similar projection of AIGIV pediatric dose as a fixed exponent of 0.75 across all age groups. This similarity in pediatric dosing by two methods is by chance.

CLINICAL PHARMACOLOGY LABELING

Mechanism of Action

The polyclonal immune globulin G in ANTHRASIL is a passive immunizing agent that neutralizes anthrax toxin. ANTHRASIL binds to protective antigen (PA) to prevent PA mediated cellular entry of anthrax edema factor and lethal factor. ANTHRASIL is administered in combination with appropriate antibiotic therapy as the product by itself is not known to have bactericidal activity against anthrax bacteria, which otherwise may continue to grow and produce anthrax toxins.

Pharmacokinetics

The mean TNA activities for three doses of ANTHRASIL (210, 420 and 840 units TNA) in the clinical trial in healthy volunteers [See *14 CLINICAL STUDIES*] are plotted on a semi-log scale in Figure 1. The pharmacokinetics of ANTHRASIL after intravenous infusion of the three dose levels were characterized; the peak levels of ANTHRASIL were reached immediately after infusion and then declined over the duration of study (84 days). The mean TNA activity remained above the lower limit of quantitation (b) (4) over the entire 84-day post-dose period for the three doses studied.

Figure 1 Mean TNA Activities for Three Doses of ANTHRASIL
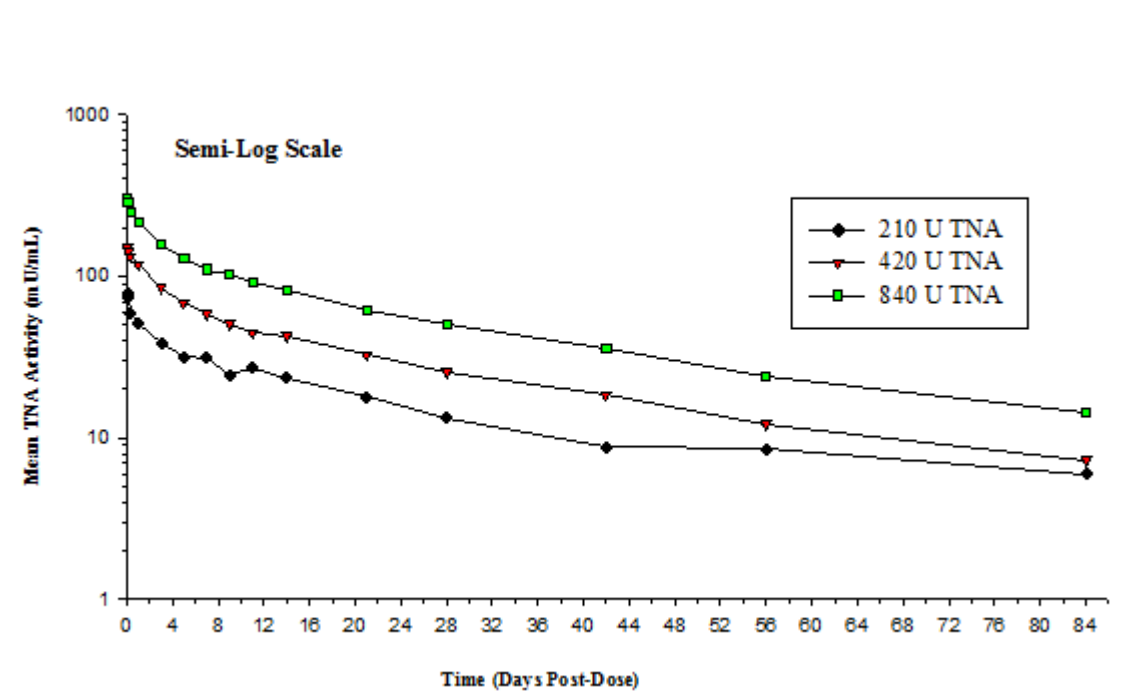
A summary of the mean pharmacokinetic results for the TNA data collected in the healthy volunteer study is presented in Table 3.

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Table 1 Summary of Mean PK Results by Treatment (TNA Data)

PK Parameters	Dose Levels					
	210 U TNA	N	420 U TNA	N	840 U TNA	N
Arithmetic Mean (CV%)						
AUC_{0-t} (mU·d/mL)	1031.8 (23.3)	15	2176.7 (18.9)	17	4271.0 (22.3)	16
$AUC_{0-\infty}$ (mU·d/mL)	1277.5 (27.7)	7	2536.7 (14.7)	16	4788.8 (26.5)	15
C_{max} (mU/mL)	83.0 (13.4)	15	156.4 (21.7)	17	316.7 (18.3)	16
$t_{1/2}$ (d)	24.3 (33.3)	7	28.3 (19.9)	16	28.0 (25.2)	15
CL (mL/d)	174.2 (24.1)	7	169.7 (17.9)	16	188.6 (29.5)	15
Vd (mL)	5714.8 (11.4)	7	6837.2 (20.4)	16	7238.2 (19.4)	15
Median (Min-Max)						
T_{max} (d)	0.116 (0.109–1.068)	15	0.120 (0.120–0.412)	17	0.169 (0.165–0.459)	16

In comparison to healthy subjects, patients with inhalational anthrax are expected to initially have greater clearance of anti-PA antibodies and lower AUC from ANTHRASIL administration due to the presence of PA antigen.

Mean PK results (TNA data) were evaluated by sex and revealed no sex-related differences over the dose range studied. Systemic exposure of ANTHRASIL increased in a dose-proportional manner over the dose range studied. ANTHRASIL has a serum elimination half-life of 24 to 28 days in healthy humans.

Inhalational anthrax patients, concomitantly treated with antibiotics and a single dose of 420 units TNA of ANTHRASIL, exhibited increases in serum and pleural anti-PA levels; these levels remained at >50% of the peak anti-PA levels over the next five days. The peak serum anti-PA levels in these patients following ANTHRASIL administration (132 to 160 mcg/mL, mean 145 mcg/mL) overlapped with those obtained with the 420 unit dose in healthy volunteers (135 to 250 mcg/mL, mean 190 mcg/mL, median 192 mcg/mL), although mean levels were approximately 25% lower in the inhalational anthrax patients. In the three inhalational anthrax patients, serum and pleural levels of lethal factor declined after initiation of antibiotics and further decreased over the period of five days following ANTHRASIL administration; however, due at least in part to ANTHRASIL targeting the PA component of lethal toxin, plasma and pleural fluid lethal factor levels remained detectable when measured two to five days following ANTHRASIL administration.

Because the effectiveness of ANTHRASIL cannot ethically be tested in placebo-controlled trials in humans, a comparison of ANTHRASIL exposures achieved in healthy human subjects to those observed in animal models of inhalational anthrax in therapeutic efficacy studies was necessary to support the dosage regimen. A dose of 420 units has a similar exposure to the efficacious dose of

15 U/kg administered to New Zealand white rabbits and cynomolgus macaques. In cynomolgus macaques treated with ANTHRASIL monotherapy, a higher dose of 30 U/kg, with a similar exposure to a human dose of 840 units, may result in improved survival.

INTRODUCTION

Inhalational anthrax occurs after the inhalation of aerosolized *B. anthracis* spores. It has a short incubation, rapid progression and high mortality. Early symptoms of infection appear after a 1 to 5 day incubation period and are consistent with a mild respiratory tract infection and include malaise, fever, fatigue, myalgias, non-productive cough, lethargy, nausea and vomiting. These early symptoms usually persist for 2-3 days and may temporarily improve, only to be followed by an acute, sudden onset of severe respiratory symptoms. The disease is usually fatal within 24-36 hours after the onset of respiratory symptoms.

Most naturally occurring strains of *B. anthracis* are susceptible to antibiotics, including doxycycline, ciprofloxacin, and penicillin. The rapid course of symptomatic inhalation anthrax makes early antibiotic administration desirable and antibiotics are indicated for post-exposure prophylaxis to *B. anthracis* spores. Anthrax pathogenesis depends on both the prevalent bacteremia and toxin production, so delayed antimicrobial therapy and/or overwhelming exposure may result in toxemia despite attenuation of bacterial proliferation.

NP-015, Anthrax Immune Globulin Intravenous (Human), is being developed for the treatment of toxemia associated with inhalational anthrax disease. The product is a human polyclonal antisera produced from the serum of individuals vaccinated with Anthrax Vaccine Adsorbed (AVA, BioThrax™). NP-015 contains antibodies against Protective Antigen (PA) and has toxin neutralizing capabilities. Individuals vaccinated with 4-6 injections of AVA develop significant anti-PA titers, and anti-PA titers of ≥ 25 μg/mL are typically correlated with protective immunity. According to the applicant, intravenous administration of NP-015 offers the same immunity provided by the vaccine, but is immediately effective whereas it may take weeks to months to confer protection through vaccinations. The likely mechanism of action for NP-015 is one where antibodies to the toxin components prevent cell binding or toxin internalization. However, the polyclonal nature of NP-015 may target many different anthrax antigens for broad biological activity. Anti-PA antibodies may also have anti-spore activities which could prevent the further germination of anthrax spores remaining in the lung after initial exposure. It is anticipated that NP-015 will be used in conjunction with antimicrobial therapy in patients presenting with symptomatic anthrax. The treatment of toxemia with NP-015 is expected to complement the bactericidal actions of antimicrobial therapy in patients with symptomatic anthrax disease.

NP-015 is a sterile solution of purified human gamma globulin prepared from plasma donated by individuals immunized with AVA (BioThrax™). The plasma is purified by an anion exchange column chromatography method with two added viral reduction steps; a solvent detergent treatment step that effectively inactivates lipid-enveloped viruses, and a filtration step using a Planova® (b) (4) virus filter that effectively removes potential lipid enveloped and non-enveloped viruses based on size. NP-015 is formulated with 10% maltose and 0.03% polysorbate 80 and contains (b) (4) protein (b) (4)

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The effective human dose of NP-015 is currently unknown and will ultimately be determined based on preclinical efficacy studies and human dose scaling techniques. An initial estimate of 420 U TNA for the theoretical human effective dose was proposed by Cangene based on literature data from animal studies and vaccination data in humans.

**Pharmacokinetics of NP-015 (AIGIV) in Healthy Human Subjects
And Healthy Animals (monkeys and rabbits)**

STUDY #1

Study Title: Safety and Pharmacokinetics of Anthrax Immune Globulin Intravenous (Human), NP-015, in Healthy Volunteers (AX-001).

The primary objective of the study was to assess the pharmacokinetics of three doses of NP-015 (210 U, 420 U and 840 U by TNA) in healthy volunteers. The secondary objectives of the study were to evaluate the safety of NP-015. An additional objective was to assess differences in blood glucose levels measured using glucose-specific point-of-care (GS-POC) versus glucose non-specific point-of-care (GNS-POC) using glucose monitoring devices. The monitoring was conducted at specified time points prior to, during, and after dosing to determine whether the maltose content in NP-015 interferes with accurate measurement of blood glucose by GNS-POC glucose monitoring devices. **This study consisted of two stages:**

The first stage was a sequential, dose-ranging study designed to assess the pharmacokinetics and safety of three doses of NP-015 (210 U, 420 U and 840 U by TNA) after intravenous administration to healthy volunteers. This study was double-blinded and randomized and included placebo controls. A total of 72 healthy adult male and female subjects aged 19-55 were recruited in three cohorts of 24. Subjects were randomized to receive a 210 U (cohort 1), 420 U (cohort 2) or 840 U (cohort 3) dose of NP-015 (N = 18/dosing group) or an equal volume of saline placebo (N = 6/dosing group).

Blood samples for pharmacokinetic analysis were drawn from the study subjects in cohorts 1-3 at the following times after drug administration: 1 hour, 3 hours, 8 hours, 1 day, 3days, 5 days, 7 days, 9 days, 11 days, 14 days, 21 days, 28 days, 42 days, 56 days and 84 days or at early withdrawal. Serum anti-protective antigen (anti-PA) levels were measured using a validated anti-PA (b) (4) and validated Toxin Neutralization Assay (TNA) at Cangene Corporation. Pharmacokinetic analysis was performed using both the (b) (4) and TNA data. However, Cangene and the FDA had agreed that TNA would be the primary assay for product potency, dosing and PK analysis, as it measures neutralizing antibodies as opposed to the anti-PA (b) (4), which measures only binding. This decision was based upon the fact that the TNA and (b) (4) assays did not correlate 100% for all lots. (b) (4) data are intended only to be supportive. Pharmacokinetic parameters were calculated by non-compartmental analysis.

NP-015 contains 10% maltose, which could potentially interfere with accurate measurement of blood glucose levels using non-specific glucose monitoring devices. Thus, differences in blood glucose levels measured by GS-POC and GNS-POC monitoring devices (finger-prick test), and serum glucose measured by laboratory test, were compared at specified time points prior to, during, and after dosing with NP-015.

The second stage of the study (cohort 4) was a randomized, open-label study in 20 healthy adult male and female volunteers aged 19-55 years. Subjects in cohort 4 were randomized to receive a dose of 840 U by TNA from one of two additional product lots (10 subjects per lot).

There was no placebo group. An open label design was selected since the volume of the 840 U dose from these two lots differed to the extent that blinding (b) (4)   and addition of saline to the lower volume dose to equalize the volumes would have diluted the product extensively. Dosing of cohort 4 was to proceed only if no safety concerns were noted for cohorts 1-3.

Cangene added cohort 4 to the study to address two issues; use of multiple product lots (based on FDA recommendation) and a change in dosage format. Subjects in cohort 4 received a 14-vial (double dose) from two additional lots of NP-015 (10 subjects per lot) versus the 12-vial (double dose) received by subjects in cohort 3. The two additional lots were selected based on specific criteria. Lot 10804812 was selected since it was a lot filled by volume (b) (4) extractable volume) and a 7-vial dosage format. Subjects in cohort 4 would receive (b) (4) of this lot, which is the largest possible volume that any patient requiring NP-015 would ever receive. Lot 10804816 was selected since it was filled by potency (the current practice) but contains the highest level of protein per mL (72 mg/mL) of any lot manufactured to date. Subjects receiving this lot in cohort 4 would therefore experience the highest rate of protein infusion/minute.

The lot of NP-015 (24906011) used for cohorts 1-3 was filled to volume (b) (4) extractable volume) and labeled with a potency of 1.4 mg anti-PA IG/mL by (b) (4). Subsequently, toxin neutralization, as measured using a functional bioassay (TNA), was felt to be a more appropriate method to determine potency so the label was changed to include a potency value in TNA Units per vial. The potency of lot 24906011 is 72.5 Units per vial based on the Certificate of Analysis but cohorts 1-3 were dosed by number of vials (3 vials for cohort 1, 6 vials for cohort 2, and 12 vials for cohort 3). The potency of the two additional product lots (10804812 and 10804816) used in cohort 4 is ≥ 60 Units/vial.

The infusion of drug began at a rate of 0.5 mL/min for the first 30 minutes. If well tolerated, the rate was gradually increased to 1 mL/min and then 2 mL/min, allowing 15-30 minutes before each increment. For infusion of larger volumes in the 420 U and 840 U by TNA dosing groups, the infusion rate was increased to a maximum of 4 mL/min, provided the 2 mL/min infusion rate was well tolerated by the subjects. The duration of the infusion was approximately 98 min for cohort 1, 113 min for cohort 2, 180 min for cohort 3, and 128 min or 202 min for cohort 4 (depending on the lot). Pharmacokinetic and statistical analyses were performed on data from 48 subjects (27 males and 21 females) who received the active treatment and completed the study.

The PK parameters of different doses of NP-015 in humans by TNA and (b) (4) methods are summarized in Tables 1 and 2. The concentration-time data for anti-PA levels and TNA activity are shown in Figures 1-2. Based on TNA activity the PK of NP-015 is linear over the dose range of 210 U TNA to 840 U TNA. The half-life ranged from 24 to 28 days and the clearance of NP-015 ranged from 170 to 189 mL/day over the dose range of 210 U TNA to 840 U TNA. There was no impact of gender on the PK of NP-015.

Table 1: Pharmacokinetics parameters of NP-015 in humans (TNA data)

| PK Parameters	Dose Levels		
	210 U TNA	**420 U TNA**	**840 U TNA**
AUC_{inf} (mUxd/mL)	1239 (27)	2507 (16)	4624 (29)
Half-life (days)	24.3 (33.3)	28.3 (19.9)	28.0 (25.2)
Clearance (mL/day)	174 (24)	170 (18)	189 (30)
V_d (mL)*	5715 (11)	6837 (20)	7238 (19)

*Volume of distribution; numbers in parenthesis are %CV

**Table 2: Pharmacokinetics parameters of NP-015 in humans (b) (4) data)**

PK Parameters	Dose Levels		
	250 mg	**500 mg**	**1000 mg**
AUC_{inf} (mUxd/mL)	1188 (n=2)	3330 (22)	5431 (31)
Half-life (days)	14.7 (n=2)	25.1 (28.8)	25.6 (28.6)
Clearance (mL/day)	212 (n=2)	154 (23)	192 (32)
V_d (mL)*	4374 (n=2)	5305 (19)	6708 (26)

*Volume of distribution; numbers in parenthesis are %CV

Based on (b) (4) assay the PK of NP-015 was non-linear over the dose range of 250 mg to 1000 mg. The half-life was 25 days and the clearance of NP-015 ranged from 154 to 212 mL/day over the dose range of 250 to 1000 mg. There was no impact of gender on the PK of NP-015.

Figure 1: Mean TNA activities (TNA data)
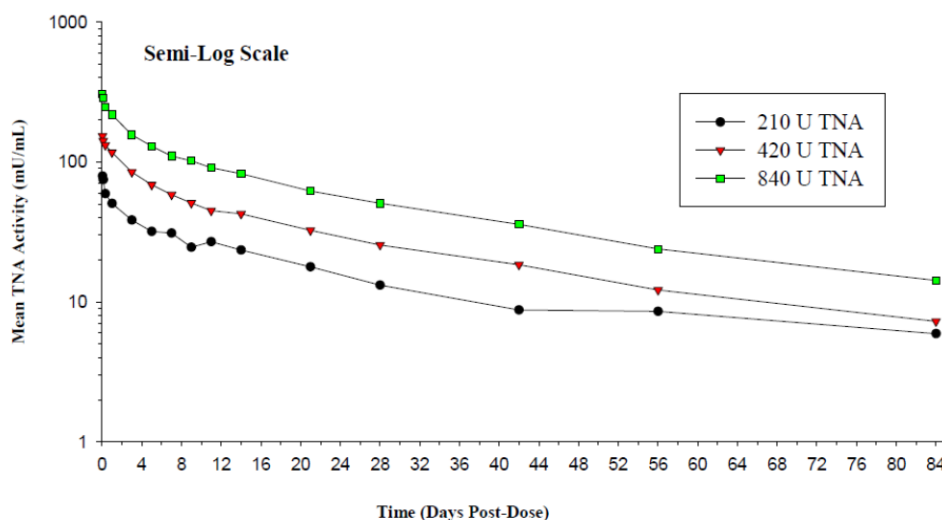
Figure 2: Mean serum anti-PA levels (b) (4) data)
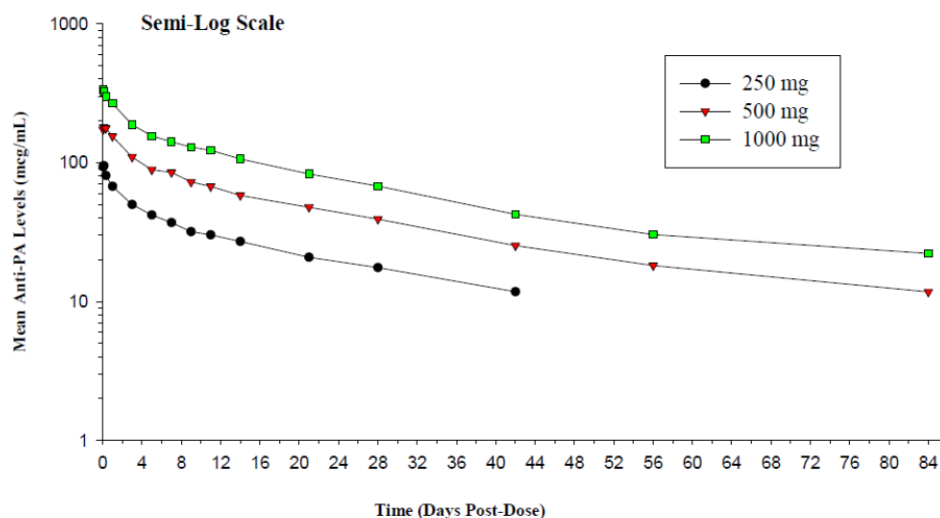
The comparison of the reading methods of GS-POC, GNS-POC, and serum glucose at each time point showed that there was a significant difference between GNS-POC and GS-POC for cohorts 1, 2 and 3 on Day -1. Also, there was a significant difference between GNS-POC and GS-POC at 1 hour post-dose for active subjects in cohorts 1, 2 and 3, between GNS-POC and serum glucose at 1 h post-dose for active subjects in cohorts 1, 2 and 3, and between GSPOC and serum glucose at 1 hour post-dose for active subjects in cohort 3.

Comparison of differences in readings between GS-POC and GNS-POC devices suggest that the 420 U and 840 U doses of NP-015 can cause falsely elevated blood glucose readings when blood glucose is tested using non-specific glucose monitoring devices. GNS-POC readings were significantly elevated only at the end of dosing, and this elevation persisted for 1 hour (420 U) or 2 hours (840 U) after the end of dosing. This safety data will be helpful to diabetic patients who are recipients of NP-015 to prevent inappropriate dosing with insulin or masking of hypoglycemia based on falsely elevated GNS-POC readings. This could also potentially affect urine glucose tests, as urine glucose was shifted from normal to abnormal in subjects receiving the 420 U or 840 U dose of NP-015.

Conclusions: The PK of NP-015 is linear over the dose range of 210-840 U based on TNA activity but not based on anti-PA levels (b) (4) assay). Gender has no impact on the PK of NP-015.

STUDY #2

Study Title: Pharmacokinetic Evaluation of Anthrax Immune Globulin (AIG), NP-015 in Cynomolgus Macaques Following Single Intravenous Infusion (695-G005780).

The primary objective of this study was to determine the pharmacokinetics (PK) of NP-015 (Anthrax-IGIV) in a non-human primate (NHP), cynomolgus macaques. A secondary objective of this study was to measure the immune response of the NHP to NP-015 (a human IG product) using an anti-Human IG (b) (4).

NHP were divided into two dose groups ($ male and 4 female monkeys in each dose group). The doses given to NHPs were 5 and 30 U toxin neutralization assay (TNA) activity/kg body weight. NHP were dosed by intravenous infusion of NP-015 at 1.5 mL/hr per kg for the low dose, and at 1.5 mL/hr per kg increasing at 0.5 mL/hr per kg hour increments at hourly intervals to a maximum of 3 mL/hr per kg for the high dose. The low dose, 5 U/kg body weight was infused over a period of approximately 1.25 hour and the high dose, 30 U/kg, over a period of approximately 4.75 hour. The individual doses to each NHP were within 14% of the target. Actual doses to individual animals were used for PK analysis.

From each animal, approximately 2.0 mL of blood was collected on Day -7 or Day -8 (prior to infusion), Day 0 (1 h and 12 h post-infusion), and Days 1, 3, 5, 7, 14, 21, 28, 35, 45 and 56. Serum samples were analyzed for the concentrations of anti-PA IG by (b) (4), for neutralizing antibodies by Toxin Neutralization Assay (TNA). Serum samples from Days -7 or -8, 7, 14, 21, 28, 45, and 56 were used to assess the immunogenicity of NP-015 product in NHP by anti-human IG (b) (4) assay. The results of the PK study are summarized in Tables 1 and 2 and concentration-time data are shown in Figures 1 and 2.

Table 1: Pharmacokinetics parameters of NP-015 in monkey (TNA data)

PK Parameters	5 U/kg (n =1)*	30 U/kg (n =7)
AUC_{inf} (mUxday/mL)	752	2490 (200)
Half-life (days)	9.8	6.7 (1.0)
Clearance (mL/day /kg)	6.5	12.5 (1.2)
V_z (mL)**	93	112 (12)

*Single animal data as there were insufficient data to calculate PK parameters from other animals; **Volume of distribution by area; number in parenthesis is standard error of mean

Based on TNA assay, a comparison of PK parameters was not possible since at the low dose the PK data were available only from one animal. Based on (b) (4) assay, the half-life of NP-015 was comparable between the two doses but clearance was 45% higher at higher dose (30 U/kg) than the low dose (5 U/kg). This indicates a non-linearity of NP-015 over the two doses. Gender had no impact on the PK of NP-015.

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**Table 2: Pharmacokinetics parameters of NP-015 in monkey (b) (4) data)**

PK Parameters	5 U/kg (n =7)	30 U/kg (n =7)
AUC_{inf} (µgxday/mL)	661 (42)	2810 (270)
Half-life (days)	7.4 (0.6)	7.4 (1.4)
Clearance (mL/day /kg)	7.7 (0.4)	11.6 (1.8)
V_z (mL)*	79 (3)	112 (13)

*Volume of distribution by area; number in parenthesis is standard error of mean

Figure 1: Anti-PA IG (TNA) concentration-time plot in cynomolgus macaque
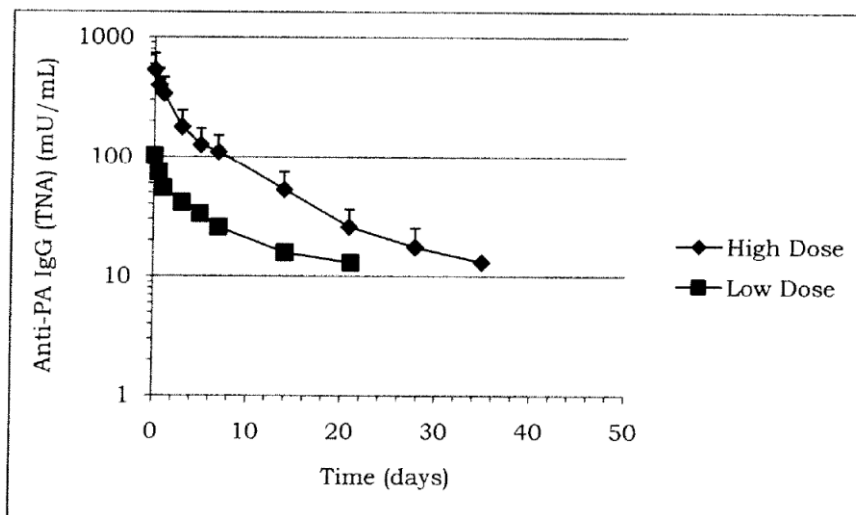
Figure 2: Anti-PA IG (b) (4) concentration-time plot in cynomolgus macaque

30
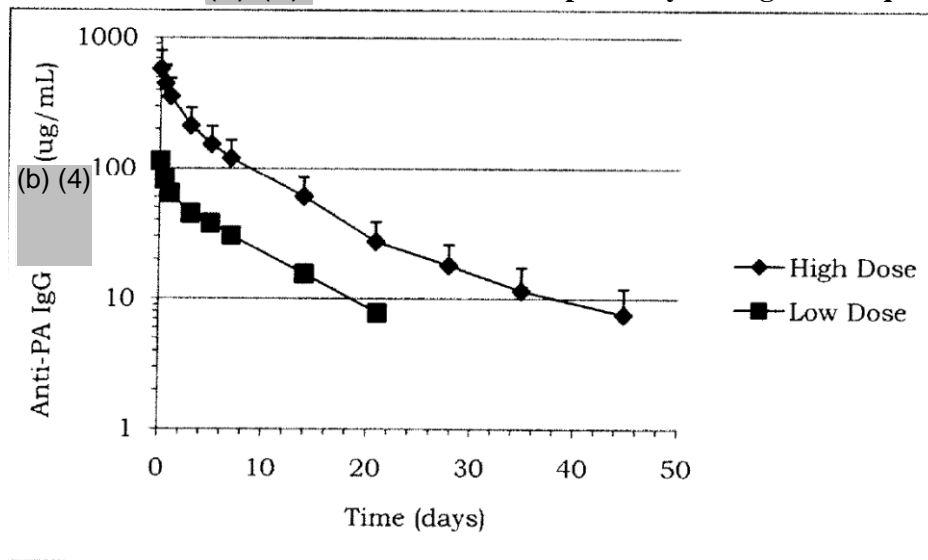
A small (<0.1 μg/mL) but positive antihuman IG response was detected in 2 NHP on days 45 and 56 post-infusion. One of these two NHPs was excluded from the PK analysis due to inaccurate dosing. With only a single animal demonstrating an anti-human IG response, it was not possible to determine the effect of an anti-human IG response on the NP-015 PK using either (b) (4) or TNA data.

Conclusions: The study demonstrated that based on (b) (4) assay the systemic exposure of NP-015 did not increase in a dose-proportional manner in cynomolgus macaques. Gender did not impact the PK of NP-015. Two NHP, (one at the low dose (5 U/kg) and the other at the high dose (30 U/kg)) of NP-015 demonstrated cross-reactivity with human IG with a positive response being observed on days 45 and 56 post-infusion. The semi-quantitative assay indicated that the anti-human IG values were < 0.1 μg/mL. This indicates that a single dose of NP-015, at the dose levels and protein concentrations used in this study, may not be antigenic in the cynomolgus macaque.

STUDY #3

Study Title: Determination of Pharmacokinetics of Anthrax Immune Globulin (AIG), NP-015 in Rabbits (694-G005681).

The objective of this study was to determine the pharmacokinetics (PK) of AIGIV (Human) (NP-015) in rabbits. A secondary objective of this study was to measure the immune response of the rabbit to human IG using an anti-Human IgG (b) (4)

Two groups of rabbits (15 males and 15 females per group) received two doses of 5 and 30 Units (U) lethal toxin neutralization activity (TNA) /kg body weight (Lot # 10602912). Rabbits were dosed by intravenous infusion of NP-015 at a constant flow rate of 1.5 mL/h per kg (low dose), or starting at 1.5 mL/h per kg increasing at 0.5 mL/hr per kg increments at hourly intervals to a maximum of 3 mL/h per kg (high dose). The low dose, 5 U TNA was infused over a period of approximately 1.25 hour and the high dose, 30 U/kg, over a period of approximately 4.75 hour. Blood samples for PK study were drawn before infusion (day -7) and at 1, 8, 24, 48 hours, and days 3, 5, 8, 11, 14, 21, and 28. Serum concentrations of NP-015 were measured either by (b) (4) measurement of anti-PA IG (NP-015) or a functional analysis of lethal toxin neutralization activity (TNA). The lower limits of quantitation (LLOQ) for (b) (4) and TNA analyses were (b) (4) , respectively. Serum concentration-time data were used to calculate PK parameters using a non-compartmental analysis. PK parameters are summarized in Table 1. Concentration-time plot are shown in Figures 1 and 2.

Table 1: Pharmacokinetic parameters of NP-015 in rabbits

Parameters	(b) (4)		TNA	
	5 mg/kg	30 mg/kg	5 mg/kg	30 mg/kg
C_{max} (µg/mL)	128	725	111	559
AUC (ugxday/mL)	552	3310	475	2450
CL (mL/day/kg)	10	9.5	11.5	13.2
Half-life (days)	4.4	4.2	4.6	4.4

The units for C_{max} and AUC for (b) (4) are (µg/mL) and µgxday/mL, respectively.
The units for C_{max} and AUC for TNA are mU/mL and mUxday/mL , respectively.

Impact of immunogenicity on the PK of NP-015 in healthy rabbits:

Anti-human IG analysis was used to assess the immunogenicity of NP-015 in rabbits. (only at low dose). In 2 animals, a positive anti-human IG response was detected prior to dosing and at all time points after dosing indicating that some rabbits have IG that will cross react with human IG in the absence of the delay observed in a typical humeral immune response. Eighty percent of the rabbits in Group 1 (low dose) displayed a positive anti-human IG response at 21 days post-infusion, and fifty percent at 28 days post-infusion (last time point analyzed).

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There were only eight animals in treatment Group 1 available for the analysis of anti-human IG antibodies so the ability to fully assess the effect that anti-human IG antibodies had on PK parameters was limited. The presence of anti-human IG antibodies did not have a statistically significant effect on most of the PK parameters in the anti-PA (b) (4) data except for a statistically significant effect on the $AUC_{(last)}$ on study day 28. For the TNA data, the presence of anti-human IG antibodies had a significant effect on clearance and $AUC_{(0-\infty)}$.

For (b) (4) data, the mean $AUC_{(0-last)}$ for animals that were anti-human IG positive on day 28 were reduced by 24% compared to the respective $AUC_{(0-last)}$ from animals without anti-human IG. For TNA data, animals that were positive for antihuman IG antibodies on Days -7, 8, or 14 had a higher mean clearance (28 to 32% higher) and a significantly lower mean $AUC_{(0-\infty)}$ (27 to 30% decrease) compared to those animals that were negative for anti-human IgG at these time points. PK parameter comparisons between animals with anti-human IG to animals without antihuman IgG should be interpreted with caution due to limited number of animals available (n=2 or 3) for comparison in any one group and variability associated with the analysis method.

Conclusions: Based on (b) (4) assay, the AUC of NP-015 increased proportionally but this proportional increase in AUC was not observed with TNA assay. Since NP-015 is human IG, as expected, the PK of NP-015 was influenced by immunogenicity in the rabbit.

Figure 1: Mean concentration-time plot Plots for Anti-PA IG (b) (4) in rabbits
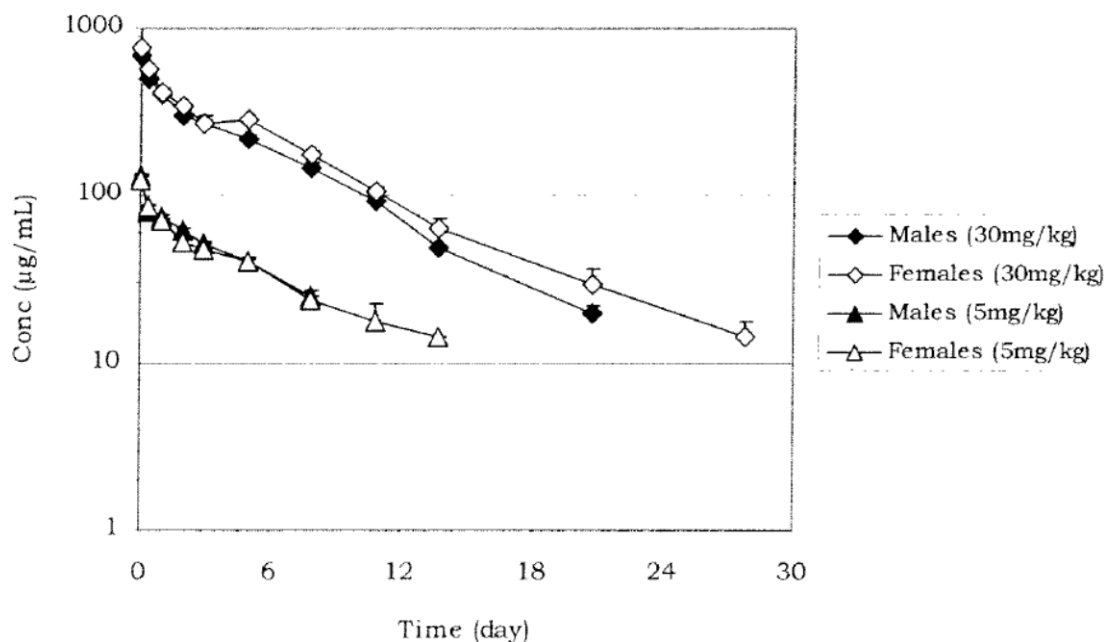
Figure 2: Mean concentration-time plot Plots for Anti-PA IG (TNA) in rabbits
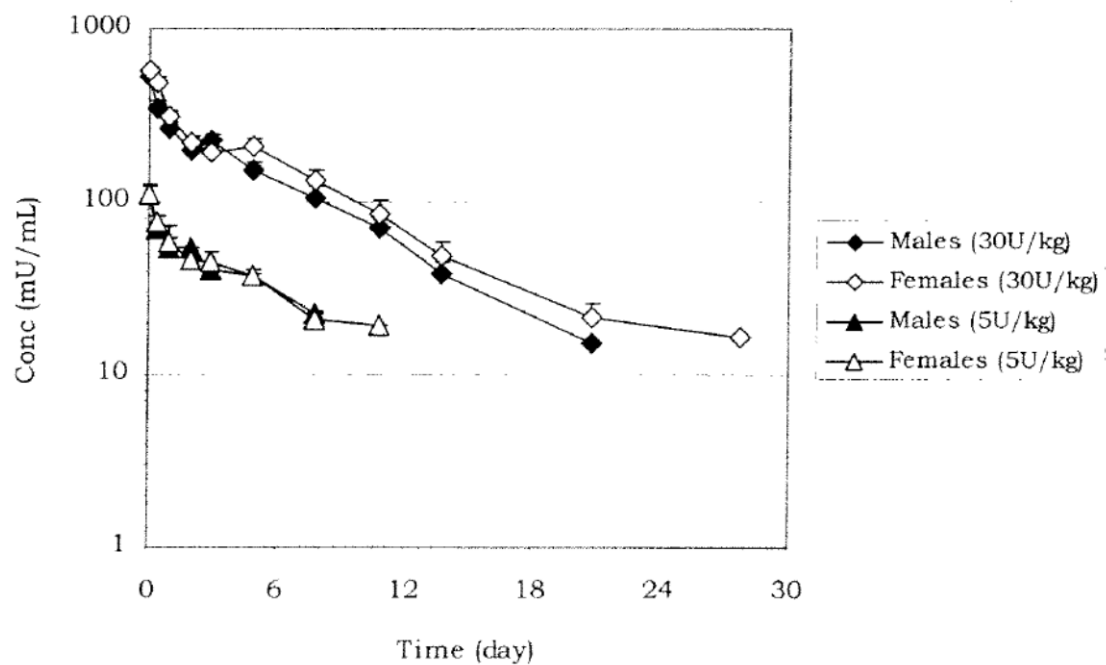
STUDY #4

Study Title: Determination of pharmacokinetics of anthrax immune globulin (AIG), NP-015 in rabbits following single intravenous infusion (b) (4) Study No. 658-G005681).

The objective of this study was to determine the pharmacokinetics (PK) of AIGIV (Human) (NP-015) in rabbits. A secondary objective of this study was to measure the immune response of the rabbit to human IgG using an anti-Human IgG (b) (4)

Due to adverse effects of the test article in the initial study which contained experimental Groups 1 and 2, this study was amended to include three additional groups (3, 4, and 5) to investigate the tolerability of the test article. Groups 3 to 5 were infused with a different lot of NP-015 that has higher specific activity of AIG than the previous one.

Rabbits in Groups 1 and 2 (5 males and 5 females per group) received two doses (10 and 40 U TNA/kg) of NP-015 (Lot #24405011) and were infused at an escalating dose rate from 2 mL/h per kg to 4 mL/h per kg changing in 1 mL/h per kg increments at 1 hour intervals. Dosing to rabbits at high dose was terminated prematurely due to toxicity. Blood samples for PK study were drawn before infusion (day -7) and at 1, 8, 24, 48 hours, and days 3, 5, 8, 11, 14, 21, and 28. Serum concentrations of NP-015 were measured either by (b) (4) measurement of anti-PA IgG (NP-015) or a functional analysis of lethal toxin neutralization activity (TNA). The lower limits of quantitation (LLOQ) for (b) (4) and TNA analyses were (b) (4) , respectively. Blood samples from Group 2 rabbits were not analyzed due to the toxicity and subsequently euthanization of the rabbits. Serum concentration-time data were used to calculate PK parameters using a non-compartmental analysis. PK parameters are summarized in Table 1. Mean concentration-time profiles of NP-015 in rabbits are shown in Figures 1 and 2.

**Table 1:Mean (±SD) pharmacokinetics parameters of NP-015 in rabbit (10 U/kg)**

PK Parameters	(b) (4)	TNA
AUC_{inf}	1450 ± 350	1210 ± 300
Half-life (days)	4.7 ± 2	5.5 ± 2.4
Clearance (mL/day/kg)	10.2 ± 2.6	8.8 ± 2.1
V_z (mL/kg)	62.5 ± 16.1	64.5 ± 18.1

The units for AUC for (b) (4) are µgxday/mL and for TNA mUxday/mL

Anti-human IgG analysis was used to assess the immunogenicity of NP-015 in rabbits. This qualitative analysis was only carried out for the low dose. In 2 animals, a positive anti-human IG response was detected prior to dosing; one of these animals (L08003) continued to show a positive response through day 28 while the other animal (L08031) was negative at days 14, 21 and 28. A positive result prior to dosing with AIG indicates that some rabbits have IG that will cross react with human IG in the absence of a typical humoral immune response. The positive

anti-human IG responses observed in three of the Group 1 rabbits starting 14 to 21 days after NP-015 dosing are consistent with an immune response to a foreign protein, in this case NP-015.

Figure 1: Mean concentration-time plot Plots for Anti-PA IgG (b) (4) in rabbits
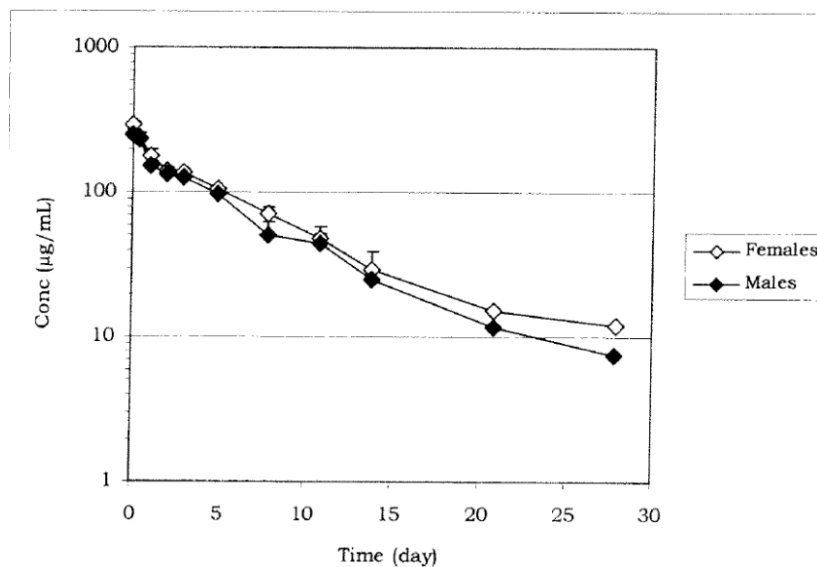
Figure 2: Mean concentration-time plot Plots for Anti-PA IgG (TNA) in rabbits
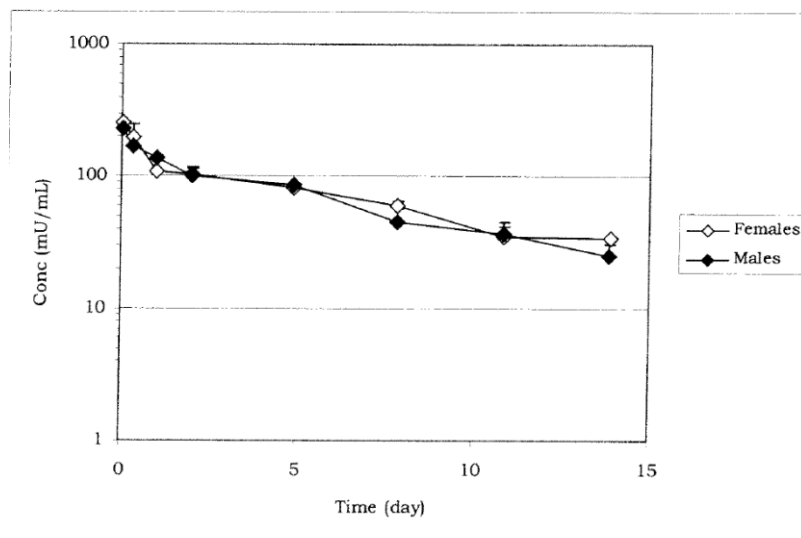
The amended study (Groups 3 to 5) used a new lot of NP-015 (Lot #10602912) with a higher specific activity of NP-015 (2.73 U TNA/mL and 59 mg protein/mL). It was anticipated that this formulation would be better tolerated since, for a given TNA dose, the total protein administered would be half as much as the formulation used for Groups 1 and 2. The dose levels for the

tolerability study were 20, 30, and 40 U TNA/kg. Results of the tolerability study indicated that NP-015, despite the lower dose of total IG protein, also caused hemolytic anemia, however, the hematocrit levels did not decrease as much as observed in groups 1 and 2, and RBC counts returned to normal, or close to normal, within 2 weeks after the infusion.

Conclusions: The high dose (40 TNA U/kg) caused toxicity and the PK assessment of NP-015 at this dose in rabbit was not possible. At the lower dose (10 TNA U/kg) PK assessment indicated no gender effect on the PK of NP-015 in rabbits.

STUDY #5

Study Title: Assessment of Pharmacokinetics of Anthrax Immune Globulin Intravenous (AIGIV), NP-015 Following a Single Intravenous Infusion in Healthy New Zealand White Rabbits (b) (4) Study 1206-G00568).

The objective of this study was to determine the pharmacokinetics (PK) of AIGIV (Human) (NP-015) in New Zealand White rabbits. A secondary objective of this study was to measure the immune response of the rabbit to human IG using an anti-Human IG (b) (4) (b) (4)   Forty rabbits (20 males, 20 females) received a single NP-015 infusion dose of 15 Units/kg starting at 1.5 mL/h per kg, increasing at 0.5 mL/h per kg increments at hourly intervals to a maximum of 2.5 mL/h per kg. Blood samples for PK study were drawn before infusion (day -7) and at 1, 12, 24, 48 hours, and days 3, 5, 7, 10, 14, 21, and 28. Serum concentrations of NP-015 were measured either by (b) (4) measurement of anti-PA IG (NP-015) or a functional analysis of lethal toxin neutralization activity (TNA). Serum concentration-time data were used to calculate PK parameters using a non-compartmental analysis. PK parameters are summarized in Table 1. The concentration-time profiles of NP-015 are shown in Figures 1-2. Gender had no impact on the PK of NP-015 in rabbits.

Table 1: Pharmacokinetic parameters of NP-015 in rabbits (dose = 15 U/kg)

Parameters	(b) (4)	TNA
C_{max} (µg/mL)	432 ± 10	420 ± 15*
$AUC_{(0-inf)}$ (ugxday/mL)	1863 ± 80	1710 ±80*
CL (mL/day/kg)	8.8 ± 0.4	9.5 ± 0.4
Half-life (days)	4.4 ± 0.4	4.6 ± 0.4

*the units for C_{max} and $AUC_{(0-inf)}$ from TNA assay are mU/mL and dayxmu/mL, respectively.

Figure 1: TNA concentration-time profile of NP-015 in rabbits
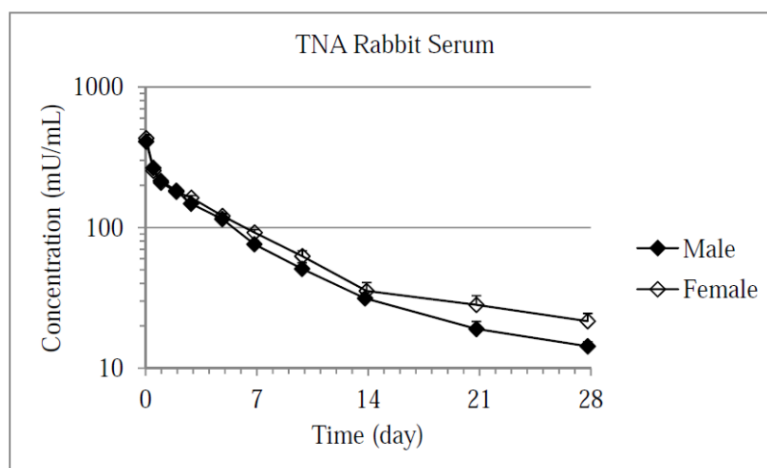
Figure 2: Anti-PA IgG (b) (4) concentration-time profile of NP-015 in rabbits

Anti-PA IgG (b) (4) Rabbit Serum
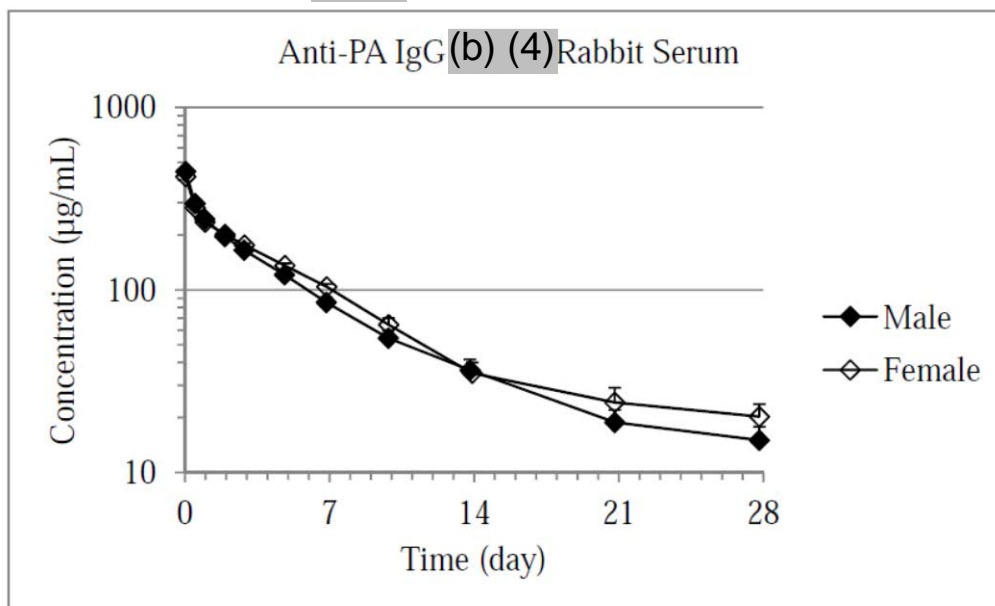
Impact of immunogenicity on the PK of NP-015 in healthy rabbits:

Five treated animals (12%) had reactive anti-human IG responses prior to infusion, but these animals did not remain reactive at all time-points after dosing, which suggests that some of the reactive results may be non-specific. The number of reactive anti-human IG responses increased after infusion; four animals on day 7, ten animals on day 14, 12 animals on day 21, and fifteen animals on day 28. The presence of anti-human IG antibodies did not have a significant effect on any of the PK parameters in the TNA and anti-PA (b) (4) data on days -1 or 7.

For TNA data, the mean half-life and $AUC_{(0-last)}$ for animals that were positive for anti-human IG antibodies at days 14 and 28 were less than the respective PK levels for those animals that were negative for antihuman IG antibodies. The mean $AUC_{(0-\infty)}$ for animals that were positive for anti-human IG antibodies on days 14, 21, and 28 were less than the respective PK levels for those animals that were negative for anti-human IG antibodies. The mean clearance for animals that were positive for anti-human IG antibodies on days 14 and 28 were greater (30%) than the clearance for those animals that were negative for anti-human IG antibodies. A similar observation was noted with (b) (4) data.

Pharmacokinetic Comparison across Species (Healthy Animals)

Pharmacokinetic parameters of AIGIV determined using a non-compartmental analysis for all three species are presented in the following Table.

Interspecies Comparison of AIGIV PK Parameters (HEALTHY)

PK Parameter	Rabbit			Cynomolgus Macaque		Human		
	5 U/kg	15 U/kg	30 U/kg	5 U/kg	30 U/kg	210 U	420 U	840 U
C_{max} (mU/mL)	111 ±9	420 ±15	559 ±29	101 ±10	532 ±14	82.3 ±13.7	152.9 ±22.4	311.8 ±18.2
$t_{1/2}$ (days)	4.61 ±0.58	4.56 ±0.38	4.43 ±0.49	9.8[a]	6.71 ±1.02	24.3 ±33.3	28.3 ±19.9	28.0 ±25.2
Cl (mL/day/kg)	11.5 ±0.7	9.46 ±0.41	13.2 ±0.9	6.55[a]	12.5 ±1.2	2.34[b]	2.33[b]	2.48[b]
V_d (mL/kg)	75.6 ±9.9	55.2 ±2.70	80.2 ±5.9	92.6[a]	112.0 ±12.0	76.8[b]	93.9[b]	95.4[b]

PK Parameter	Rabbit			Cynomolgus Macaque		Human		
	5 U/kg	15 U/kg	30 U/kg	5 U/kg	30 U/kg	210 U	420 U	840 U
AUC_{0-inf} (day*mU/mL)	475 ±32	1710 ±80	2450 ±180	752[a]	2437 ±200	1239.4 ±26.5	2507.5 ±16.4	4624.2 ±28.5

Values for rabbits and NHPs are means ±SEM (standard error of the mean); values for humans are means ±SD (standard deviation).

[a] Data represent a single animal.

[b] Cl and V_d were calculated per kg body weight for human subjects using the following average weights:
210 U – 74.4 kg; 420 U – 72.8 kg; 840 U – 75.9 kg

The clearance of NP-015 in rabbits (9.46 to 13.2. mL/day/kg) and cynomolgus macaque (12.5 mL/day/kg at the 30 U/kg dose) is at least five-fold faster (based on per kg basis) than in humans (2.33 to 2.48 mL/day/kg). The volume of distribution is relatively similar in all species when normalized for body weight. The half-life of AIGIV in humans is four to five times longer than in rabbits and cynomolgus macaques primarily due to slower clearance of AIGIV in humans.

Impact of immunogenicity on PK in normal healthy animals:

In study (b) (4) 694-G005681 (healthy rabbits), there was a very limited number of animals available for comparison between anti-human IG positive and anti-human IG negative animals. Moreover, only the low dose group (5 U/kg) was examined in this study, making any meaningful assessment of the impact of immunogenicity on PK difficult.

In study (b) (4) 1206-G005681 (healthy rabbits)), the sample size was large and it was possible to make any conclusion regarding the impact of immunogenicity on the PK of N-015 in healthy rabbits. Both (b) (4) and TNA data from this study indicated a significant decrease in

AUC$_{(0-last)}$ with anti-human IgG positive animals compared to negative animals. There was increase in clearance (30%) and decrease in half-life (50%) in anti-human IG positive animals.

The impact of immunogenicity on PK was not assessed in normal healthy non-human primates because there was only one animal that showed positive anti-human IG response.

Immunogenicity to AIGIV is not anticipated to occur in humans as AIGIV is a human IG product.

Impact of immunogenicity on PK in exposed animals:

In study (b) (4) 1207-100005104, there was a large sample size available for impact analysis. There was no observed impact of immunogenicity on PK parameters based on (b) (4) data. There was a significant decrease in half-life on Day 21 in anti-human IgG positive animals and there was an increase in clearance; however, this was not statistically significant.

In study (b) (4) 1182-100011472, there was no significant difference in AUC0-last or AUC0-∞ or clearance or half-life between animals positive or negative for anti-human IG based on (b) (4) data. For TNA data, clearance increased by 18% based on immunogenicity status on Day 14; however, there were only a few animals (n = 2) available for PK analysis.

Pharmacokinetics in Exposed Animals (Monkeys and Rabbits)

STUDY #6

Study Title: Pharmacokinetics of anthrax immune globulin intravenous (AIGIV) for anthrax exposed cynomolgus monkey (study 828-G005780 PK).

The objective of this study was to evaluate the survival rate in cynomolgus monkeys after a target aerosol spore dose of 200 x LD50. Another objective of the study was to determine the impact of anthrax toxin (protective antigen, PA) on the pharmacokinetics of Anthrax Immune Globulin Intravenous (Human AIGIV) in monkey. In this study, 64 cynomolgus macaques randomized to four groups of 16 animals each were exposed to a target aerosol spore dose of 200 x LD50. Upon detection of toxemia by the PA$^{(b)\ (4)}$ assay, animals were treated intravenously with one of three doses (7.5, 15 or 30 U/kg) of AIGIV or a single dose of placebo (IGIV) administered at an equivalent dose volume to that of the highest dose of AIGIV (units based on potency assessed by TNA). Animals were monitored for bacteremia, toxemia, clinical signs and hematology for 28 days followed by an additional observation period of 62 days for a total of 90 days post-exposure. The primary endpoint was survival on Day 28 post anthrax exposure. The study design is summarized in Table 1.

Table 1: Study design for the impact of Anthrax on the PK of AIGIV in monkey

Group	No. of Animals	LD$_{50}$ Equivalents (SD)	Treatment at the Onset of Toxemia	Treatment Dose (TNA U/kg)	Dose Volume (mL/kg)	Protein Given[a] (g/kg)	Approximate Duration of Infusion
1	16	154 (46)	IGIV	N/A[b]	11	0.6	4–5 hr[c]
2	16	164 (48)	AIGIV	7.5	2.74	0.16	0.5–1hr[d]
3	16	151 (44)	AIGIV	15	5.5	0.32	2–3 hr[c]
4	16	146 (18)	AIGIV	30	11	0.65	4–5 hr[c]

[a] Each vial of AIGIV contains approximately 2.73 U/mL of TNA IgG and 59 mg/mL of total protein.

[b] IGIV is also approximately (b) (4) of total protein) and was used at the same volume as that of the highest dose of AIGIV product.

[c] Infusion started at 1.5 mL/kg/hr, increasing by 0.5 ml/kg/hr at hourly intervals to a maximum of 3 mL/kg/hr.

[d] Product infused at a constant flow rate of 1.5 mL/kg/hr.

All animals that received a full infusion of AIGIV were included in the PK analysis with the following exceptions: Two animals (at 30 U/kg) were erroneously treated when still not toxemic and were excluded from all analyses. Four animals (2 at 7.5 U/kg, 1 at 15 U/kg and 1 at 30 U/kg) had no evaluable anti-PA TNA results and were excluded from the PK analysis.

Due to the nature of the study (exposure to aerosol spore), animals died prior to their scheduled termination time. For assessment of C_{max} and T_{max} values, all animals that survived post infusion were included in the analysis. For assessment of AUC (both AUC_{0-7} and AUC_{0-14}), all animals that survived to seven or fourteen days post-infusion were included in the analysis.

There were 21 animals that died prior to Day 7 and were excluded from the AUC analysis but included in C_{max} and T_{max} analysis.

Blood samples for PK analysis were taken at 24, 30, 36, 42, 48, 54, 60, 66, 72 hours, 7, 10, 14, 21 and 28 days relative to anthrax challenge. Blood samples were collected from surviving animals through day 28 post-challenge. However, many of the animals that survived past day 14 (336 hours) post challenge exhibited a rebound of anti-PA levels. Therefore, the PK analysis of AIGIV was only performed through day 14 post challenge. The AUC values are summarized in Table 2. Mean concentration-time plot for 3 doses are shown in Figure 1.

Table 2: The AUC (mu x hrs/mL) values of different doses of AIGIV in monkey

Parameter		7.5 U/kg	15 U/kg	30 U/kg
	N	5	7	9
$AUC_{(0-7)}$	Mean ±SD	7918 ± 2039	13634 ± 3647	35030 ± 10606
$AUC_{(0-14)}$	Mean ±SD	14379 ± 1830	23957 ±7919	55595 ± 15959

C_{max} and T_{max} values are not presented here due to unreliability of the assessment since the first blood sample was taken at 24 hours.

Figure 1: Mean TNA concentration-time profile in monkey
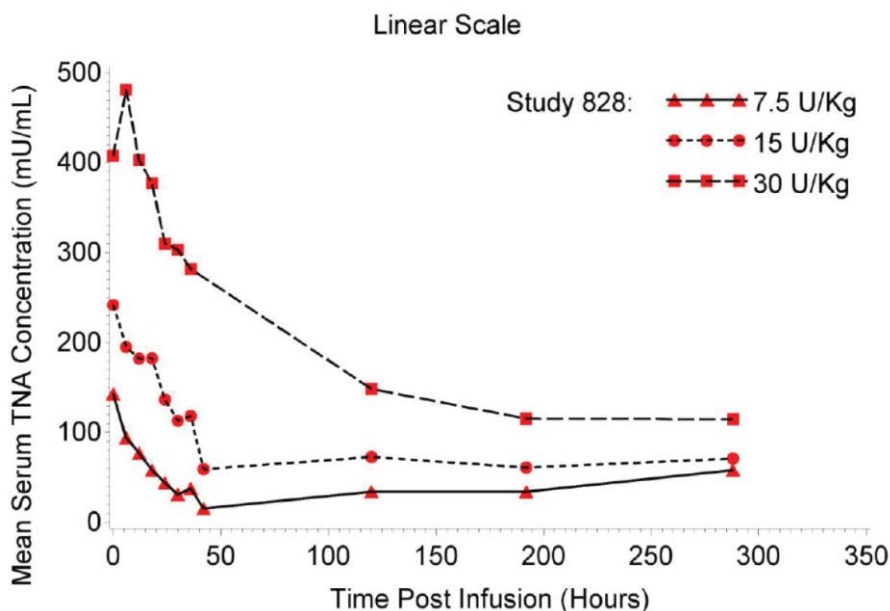
The PK results observed in this study should be interpreted with caution. The lack of early blood sample collection impacted the reliable determination of AIGIV C_{max} and T_{max}. The variation of AIGIV treatment start time and duration for each individual animal further complicated the PK analysis. Many animals that survived past day 14 (336 hours) post challenge exhibited a rebound of anti-PA levels, indicating a humoral immune effect in response to B.

anthracis infection. There was limited number of animals with calculable AUC because only half of the study animals (21/48) had available data points beyond seven days post infusion.

Comments: PK parameters (with the exception of AUC) could not be assessed in this study. Although not needed, too many blood samples were taken from day 1 to day 3. The clearance of NP-015 was not different in the group of monkeys with aerosolized anthrax spores than the group of monkeys who were not given the toxin. The clearance of NP-015 in normal healthy monkey based on $AUC_{(0-7)}$ and $AUC_{(0-14)}$ was 18.5 and 13.9 mL/day/kg (30 U/kg), respectively. The clearance of NP-015 in the exposed monkey, based on $AUC_{(0-7)}$ and $AUC_{(0-14)}$ was 21 and 13 mL/day/kg, respectively.

STUDY #7

Study Title: Therapeutic Efficacy of Anthrax Immune Globulin Intravenous (AIGIV), NP-015 in Rabbit Model of Inhalation Anthrax: (GLP Study No. 1207-100005104)

The primary objective of this study was to determine the efficacy of Anthrax Immune Globulin Intravenous (NP-015) in comparison to normal human Immune Globulin Intravenous (IGIV) (placebo) when treatments were administered after the first detection of protective antigen (PA) in serum. The secondary objective was to characterize the pharmacokinetics of NP-015 in rabbits. The report below only deals with the PK of AIGIV in exposed (inhalational anthrax) rabbits. The efficacy review can be found in study #10.

New Zealand white rabbits (n =110, 55 male and 55 female) were challenged with *Bacillus anthracis* (Ames strain) spores via aerosol exposure. The average challenge dose received by the animals was 194 ± 33 x LD50. The exposed animals were treated with either a single intravenous infusion of 15 U/kg of NP-015 or an equivalent volume of IGIV (50 rabbits per treatment group) at the onset of toxemia (PA detection). The remaining 10 animals served as process controls. The average time to treatment was 32.4 h post-challenge.

A pre-formulated solution of NP-015 in frozen form at a concentration of approximately 3.19 U/mL of NP-015 (potency confirmed by Toxin Neutralization Assay [TNA]) was administered to the animals. The test article was in 50mL glass containers with an extractable drug volume of 23.85mL (actual fill volume is (b) (4) . The formulation was stored between -15°C and (b) (4) . All aerosol exposures occurred with a well characterized, single lot of *B. anthracis* (Ames strain; Lot B-37) spores. One aerosol LD50 in New Zealand White rabbits was 1.05×10^5 spores. The target aerosol exposure in the study was 200 x LD50. Serum antihuman IG levels were measured by (b) (4) in animals treated with NP-015. The analyzed specimens included those collected at baseline, days 7, 14, 21 and 28 post-infusion. Following exposure to anthrax spores, serum specimens from treatment groups were analyzed by (b) (4) assay in order to identify the onset of toxemia for initiation of treatment. Survival was the primary endpoint used to determine the efficacy of NP-015 over placebo.

NP-015 concentrations for pharmacokinetic study were measured by TNA and (b) (4) after intravenous (IV) infusion at a dose of 15 U/kg. Twelve out of the 16 animals that survived past day 7 exhibited a humoral immune response to PA. This resulted in large increase in concentrations of anti-PA antibodies for both the TNA and (b) (4). Therefore, PK analysis was only performed up to day 7. Blood samples were collected at 1 h, 12 h, 24 h, 48 h; days 3, 5, 7, 10, 14, 21, 28 post-infusion and day 36 post-challenge (end of the study). Pharmacokinetic parameters were analyzed by non-compartmental analysis and are shown in Table 1. The concentration-time profiles of NP-015 are shown in Figures 1-2. Gender had no impact on the PK of NP-015 in rabbits.

Immunogenicity was analyzed at the baseline, prior to infusion and days 7, 14, 21 and 28 post-infusion serum specimens for anti-human IgG levels by (b) (4) in animals treated with NP-

015. Eight of the 50 animals in the NP-015 group showed positive titers for anti-human IG suggesting that the rabbits exhibited a humoral response to human IG. Four of the eight animals with positive titers were only from specimens collected on days 7 through 28 post-infusion. The other four animals had positive titers at prior to infusion, three of these four animals died during study. The surviving animal positive at prior to infusion was also positive on days 14 and 28 post-infusion. Animals in the IGIV groups were not tested for antihuman IgG titers.

Table 1: Pharmacokinetic parameters of NP-015 in rabbits

Parameters	(b) (4)	TNA
C_{max} (µg/mL)	395 ± 57 (n =50)	**364** ± 68* (n =50)
$AUC_{(0-7)}$ (ugxday/mL)	690 ± 256 (n =36)	**743** ±283* (n = 28)
CL (mL/day/kg)	26.8 ± 19.0 (n =30)	26.3 ± 21.9 (n =28)
Half-life (days)	0.96 ± 0.62 (n = 30)	1.19 ± 0.74 (n =22)

Dose = 15 U/kg; the units for C_{max} and $AUC_{(0-inf)}$ are mU/mL and dayxmu/mL, respectively.

Impact of immunogenicity on the PK of NP-015 in treated rabbits:

For (b) (4) data, none of the observed anti-human IgG responses exhibited any significant effect on AIGIV PK parameters when comparing animals by immunogenicity status on any study day. For TNA data, only when comparing animals by immunogenicity status at day 21 was the mean half-life significantly reduced by 32% (clearance increased by 10-15%) for animals with anti-human IgG antibodies compared to the levels observed in animals without anti-human IgG antibodies. Of note, this comparison involves a small number (n=4) of animals. The reduction could also be due to differences in the rate of clearance of AIGIV in the diseased animals.

Conclusions:

The PK parameters are different in the group of rabbits with aerosolized anthrax spores than the group of rabbits who were not given the toxin. The clearance of NP-015 is more than 2-fold higher in the rabbits who received the toxin than those rabbits who were not administered the toxin. The clearance of NP-015 in normal healthy rabbits based on $AUC_{(0-7)}$ and $AUC_{(0-14)}$ was 12.6 and 9.7 mL/day/kg (15 U/kg), respectively. The clearance of NP-015 in the exposed rabbit, based on $AUC_{(0-7)}$ and $AUC_{(0-14)}$ was 26.3 and 23.1 mL/day/kg, respectively.

Figure 1: TNA concentration-time profile of NP-015 in rabbits
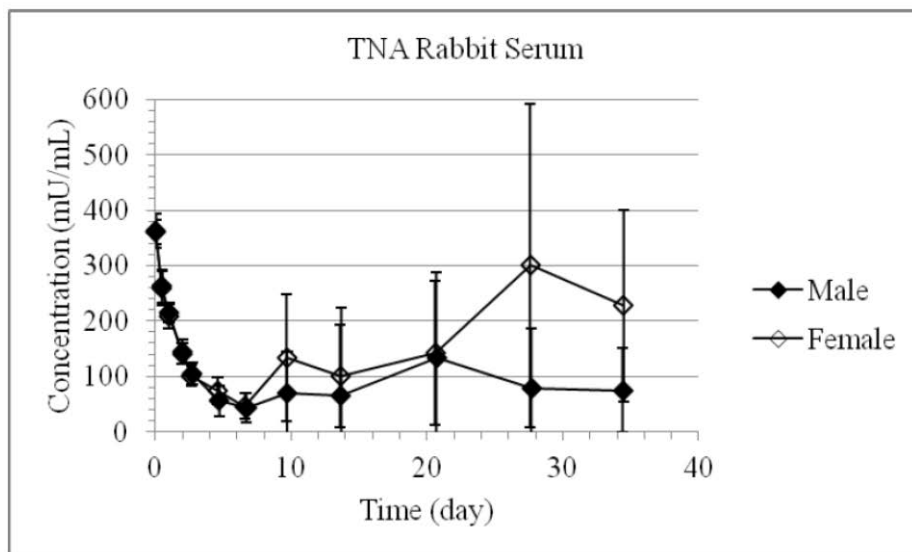
Figure 2: Anti-PA IgG (b) (4) concentration-time profile of NP-015 in rabbits
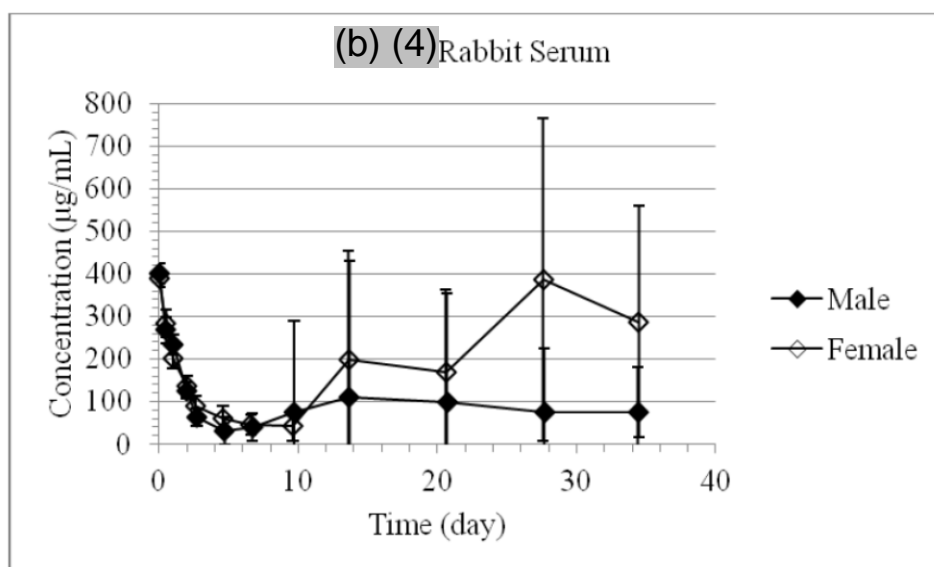
Efficacy Studies in Monkeys and Rabbits

**Study #8**

Study Title: Determination of Dose Range Efficacy of Anthrax Immune Globulin (AIG), NP-015 in Cynomolgus Monkeys Exposed to Inhalation Anthrax: GLP Study (b) (4) 828-G005780).

In this study, 64 cynomolgus macaques randomized to four groups of 16 animals each were exposed to a target aerosol spore dose of 200 x LD$_{50}$. Upon detection of toxemia by the PA (b) (4) assay, animals were treated intravenously with one of three doses (7.5, 15 or 30 U/kg) of AIGIV or a single dose of placebo administered at an equivalent dose volume to that of the highest dose of AIGIV (units based on potency assessed by TNA). Animals were monitored for bacteremia, toxemia, clinical signs and hematology for 28 days followed by an additional observation period of 62 days for a total of 90 days post-exposure. The primary endpoint was survival on day 28 post anthrax exposure.

Only 6% (1/16) of placebo-treated animals survived the lethal anthrax exposure. Survival rates of 27% (4/15), 44% (7/16) and 71% (10/14) were observed with 7.5, 15 and 30 U/kg doses of AIGIV, respectively. Both 15 and 30 U/kg doses of AIGIV had significantly higher survival rates than the placebo treated group (Figure 1). There was no statistically significant difference in the efficacy between the 15 and 30 U/kg doses of AIGIV (p=0.1235).

Figure 1: Survival Rates Comparing AIGIV and IGIV Treated Groups in Toxemic-treated Cynomolgus Macaques

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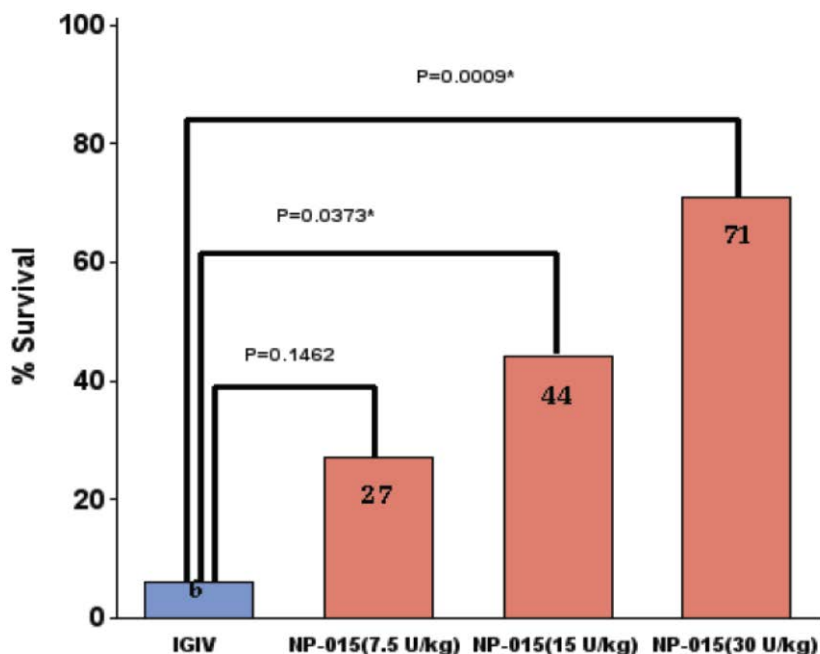
Rebound toxicity was observed in the two lower dose groups for AIGIV (Table 1), while the 30 U/kg group, with a higher exposure level to AIGIV, exhibited no rebound toxicity and higher survival rates.

Table1: Relationship between Toxin Recurrence and Mortality

AIGIV Dose	Total Mortality	Frequency of Toxin Recurrence	Mortality among Animals with Toxemia Recurrence
IGIV	15/16 (94%)	N/A	N/A
AIGIV 7.5 U/kg	11/15 (73%)	8/15 (53%)	8/8 (100%)
AIGIV 15 U/kg	9/16 (56%)	2/15[a] (13%)	2/2 (100%)
AIGIV 30 U/kg	4/14 (29%)	0/14 (0%)	N/A

[a] One animal (ID 2864) excluded from recurrence due to persistence of toxemia following treatment

Conclusions: A single dose of AIGIV administered at the onset of clinical disease was efficacious in improving the survival rates in symptomatic cynomolgus macaques exposed to a lethal dose of *B. anthracis* spores via the inhalation route. While the 30 U/kg dose trended higher in survival rate versus the 15 U/kg dose (71 vs. 44%, respectively), there was no statistically significant difference in the efficacy between the 15 and 30 U/kg doses of AIGIV (p=0.1235); therefore, the 15 U/kg dose was chosen as an equivalent human dose in the NHPs.

Comments: Although, 30 U/kg dose in terms of efficacy was not statistically significant than 15 U/kg, it appears that 30 U/kg dose was superior in producing efficacy than 15 U/kg dose. Not only total mortality rate was higher in 15 U/kg dose group (56% vs 29%) but recurrence of toxin was also noted in this dose group.
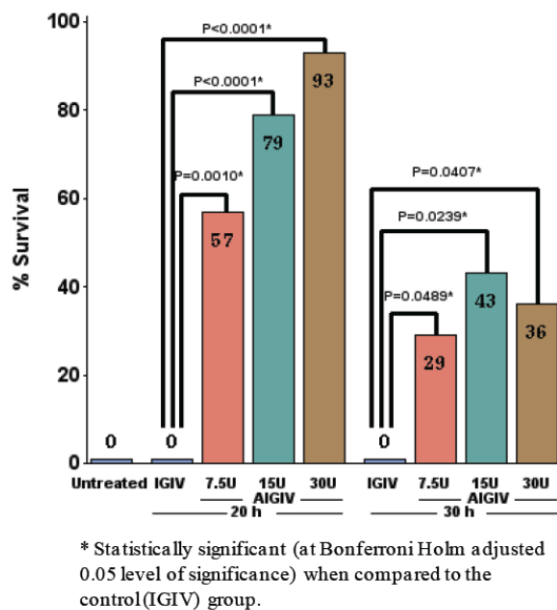
Study Title: Determination of Dose and Time Range Efficacy of Anthrax Immune Globulin (AIG), NP 015 In Rabbits Exposed to Inhalation Anthrax: GLP Study (b) (4) 677-G005681).

The objective of this study was to assess the efficacy of AIGIV at three dose levels given either of at 20 or 30 hours post-exposure in rabbits exposed to lethal doses of *B. anthracis* (Ames strain) spores by the aerosol route. In this study, 122 rabbits were randomized into eight treatment groups and one untreated control group. Following exposure, rabbits received either IGIV or one of three doses of AIGIV at 20 or 30 hours post-exposure. The doses of AIGIV tested were 7.5, 15 and 30 U/kg (units based on potency assessed by TNA assay). Animals were monitored for survival and clinical manifestations of disease including abnormal body temperature, clinical signs, hematological abnormalities, bacteremia, and circulating levels of *B. anthracis* PA over a 28-day period. The primary efficacy endpoint for this study was survival at day 28 post-exposure.

All untreated and placebo (IGIV) control animals died. A statistically significant improvement in survival was observed with all three doses of AIGIV given at 20 hours post-exposure compared to placebo treatment. Survival of 57, 79 and 93% was attained for the 7.5, 15 and 30 U/kg dose levels, respectively (Figure 1). A summary of survival rates is presented in Table 1.

**Figure 1 Survival Rates Comparing AIGIV and IGIV Groups Treated
at 20 or 30 Hours Post-exposure**

* Statistically significant (at Bonferroni Holm adjusted 0.05 level of significance) when compared to the control (IGIV) group.

Although the 30 U/kg dose trended higher in survival rate versus the 15 U/kg dose (93 vs. 79%, respectively), there was no statistically significant difference in survival between the two dose levels. When the treatments were delayed to 30 hours post-exposure, the survival rates decreased to 29, 43 and 36%, respectively but were still significantly higher than the 0% survival observed in the placebo control group.

Table 1: Summary of Survival Rates and Comparison of Treatment Group to the Control Group

Group Treatment (Group, Dose, Time)	No. Survived/ Total	Survival Rate (95% CI)	One-sided Fisher's Exact p-Value Compared to Control Groups					
			Group 1		Group 2		Group 3	
			Unadjusted	Bonferroni-Holm	Unadjusted	Bonferroni-Holm	Unadjusted	Bonferroni-Holm
Group 1 No Treatment	0/10 (0%)	0.00 (0.00, 0.31)	–	–	–	–	–	–
Group 2 IGIV (20 h)	0/14 (0%)	0.00 (0.00, 0.23)	–	–	–	–	–	–
Group 3 IGIV (30 h)	0/14 (0%)	0.00 (0.00, 0.23)	–	–	–	–	–	–
Group 4 AIGIV (7.5 U/kg; 20 h)	8/14 (57%)	0.57 (0.29, 0.82)	0.0041[a]	0.0163[b]	0.0010[a]	0 0010[b]	N/A	N/A
Group 5 AIGIV (7.5 U/kg; 30 h)	4/14 (29%)	0.29 (0.08, 0.58)	0.0942	0.0942	N/A	N/A	0.0489[a]	0.0489[b]
Group 6 AIGIV (15 U/kg; 20 h)	11/14 (79%)	0.79 (0.49, 0.95)	0.0001[a]	0.0007[b]	<0.0001[a]	<0.0001[b]	N/A	N/A
Group 7 AIGIV (15 U/kg; 30 h)	6/14 (43%)	0.43 (0.18, 0.71)	0.0223[a]	0.0669	N/A	N/A	0.0080[a]	0.0239[b]
Group 8 AIGIV (30 U/kg; 20 h)	13/14 (93%)	0.93 (0.66, 1.00)	<0.0001[a]	<0.0001[b]	<0.0001[a]	<0.0001[b]	N/A	N/A
Group 9 AIGIV (30 U/kg; 30 h)	5/14 (36%)	0.36 (0.13, 0.65)	0.0471[a]	0.0942	N/A	N/A	0.0204[a]	0.0407[b]

[a] Survival rate in the treatment group was significantly greater than the rate in the control group at the 0.05% level of significance

[b] Survival rate in the treatment group was significantly greater than the rate in the control group at the Bonferroni-Holm adjusted 0.05% level of significance

The data indicate that AIGIV treatment provided protection over that of the placebo (IGIV) treatment in the rabbit model of inhalational anthrax. AIGIV given early (20 hours) after the anthrax exposure was more efficacious than when given later (30 hours) in the disease progression, irrespective of the dose. No statistically significant difference in survival was observed between different doses of AIGIV given at either 20 or 30 hours post-exposure. The dose of 15 U/kg of AIGIV was selected as a minimum efficacious dose (MED) based on survival rate.

STUDY #10

Study Title: Therapeutic Efficacy of Anthrax Immune Globulin Intravenous (AIGIV), NP-015 in Rabbit Model of Inhalation Anthrax: (GLP Study No. 1207-100005104).

The primary objective of this study was to determine the efficacy of Anthrax Immune Globulin Intravenous (NP-015) in comparison to normal human Immune Globulin Intravenous (IGIV) (placebo) when treatments were administered after the first detection of protective antigen (PA) in serum.

New Zealand white rabbits (n =110, 55 male, 55 female) were challenged with *Bacillus anthracis* (Ames strain) spores via aerosol exposure. The average challenge dose received by the animals was 194 ± 33 x LD_{50}. The exposed animals were treated with either a single intravenous infusion of 15 U/kg of NP-015 or an equivalent volume of IGIV (50 rabbits per treatment group) at the onset of toxemia (PA detection). The remaining 10 animals served as process controls. The average time to treatment was 32.4 h post-challenge.

All aerosol exposures occurred with a well characterized, single lot of *B. anthracis* (Ames strain; Lot B-37) spores. One aerosol LD_{50} in New Zealand White rabbits was 1.05 x 10^5 spores. The target aerosol exposure in the study was 200 x LD_{50}. Serum antihuman IgG levels were measured by (b) (4) in animals treated with NP-015. The analyzed specimens included those collected at baseline, days 7, 14, 21 and 28 post-infusion. Following exposure to anthrax spores, serum specimens from treatment groups were analyzed by (b) (4) assay in order to identify the onset of toxemia for initiation of treatment. Survival was the primary endpoint used to determine the efficacy of NP-015 over placebo.

All rabbits (50/50) in the NP-015 group and 96% (48/50) in the IGIV group were bacteremic prior to treatment. Of the animals that were bacteremic prior to treatment, 26% (13/50) that received NP-015 survived to the end of the study while only 2% (1/48) rabbits that received IGIV survived. The median times to death were 75.8 and 148.5 h post-challenge for IGIV and NP-015-treated animals, respectively. The overall survival and time to death were significantly greater in the NP-015 group.

Survival was the primary endpoint used to determine the efficacy of NP-015 over placebo. In Table 1 descriptive statistics and test results for survival and time to death are shown. The descriptive statistics include the proportions of surviving animals with 95 percent confidence intervals and Kaplan-Meier median time to death with 95 percent confidence intervals for each treatment group (IGIV and NP-015). Fisher's exact test indicated that the proportion of survivors in the NP-015 group was significantly greater than that in the IGIV group. Furthermore, the Log-Rank test indicated that time to death in the NP-015 group was significantly greater than that in the IGIV group (Table 1).

A logistic regression model was fitted to the survival data with effects for treatment group (IGIV=0, NP-015=1) and the base-10 log-transformed toxin level prior to infusion. After adjusting for toxin levels prior to infusion, the odds of survival in the NP-015 group were

significantly greater than those in the IGIV group. For animals having the same toxin levels prior to infusion, the odds of survival in the NP-015 group were estimated to be approximately 57 times those in the IGIV group (Table 2).

Table 1: Proportions of Surviving Animals with Exact 95% Confidence Intervals and Kaplan-Meier Median Time to Death with 95% Confidence Intervals by Group for Treated Animals That Received a Full Dose of Either IGIV or NP-015 and Were Bacteremic at Least Once Prior to Treatment, along with Results of the Two-sided Fisher's Exact Test and the Two-sided Log-Rank Test

Group	Number Survived/N	Proportion of Survivors (Exact 95% Confidence Interval)	Fisher's Exact Test P-value	Kaplan-Meier Median Time to Death in Hours Post-Challenge (95% Confidence Interval)	Log-Rank Test P-value
IGIV	1/48	0.02 (0.00, 0.11)	0.0009*	75.8 (70.7, 83.8)	<0.0001*
NP-015	13/50	0.26 (0.15, 0.40)		148.5 (113.8, 175.8)	

* = Significant at the 0.05 level.

Table 2: Parameter estimates of logistic regression

Effect	Parameter Estimate	Likelihood Ratio Test P-value	Odds Ratio (95% Confidence Interval)
Log_{10} Toxin Level PTI	-2.28	0.0014*	0.10 (0.03, 0.41)
NP-015	4.04	0.0031*	57.09 (3.92, 832.05)

PTI = Prior to infusion
* = Significant at the 0.05 level

All animals that died during study had gross and/or microscopic evidence of anthrax, including the presence of rod-shaped bacteria consistent with *B. anthracis* in one or more organs. There were no *B. anthracis*-related findings in study survivors. No microscopic findings were found in organs (including brain) from study survivors examined, with the exception of two female animals treated with NP-015 which were recorded as having focal dermal necroses. The lesions were negative for *B. anthracis* and this finding was considered incidental with no relation to the *B. anthracis* challenge or treatment.

There was no significant difference in the median time from challenge until toxemia (first instance of PA detected in the serum post-challenge; 24.1 h for IGIV group and 24.3 h for NP-015 group) or from challenge until bacteremia (24.3 h for IGIV group and 24.7 h for NP-015 group) between the treatment groups. There was also no significant difference in the PA levels just prior to treatment (23.02 ng/mL for IGIV group and 26.29 for NP-015 group; geometric means).

Comments: NP-015, when administered therapeutically at the onset of toxemia, significantly increased survival and the clearance of circulating PA and bacteremia compared to the placebo in rabbits exposed to aerosolized anthrax spores. However, it should be recognized that only 26% (13/50) rabbits that received NP-015 survived to the end of the study while only 2% (1/48) rabbits that received IGIV survived. Compared with IGIV, the survival rate was higher in the rabbits

who received NP-015 yet, 74% of the total number of rabbits did not survive. This indicates a lack of appropriate dose or the product is not efficacious.

AIGIV Combination Therapy in the Rabbit

STUDY #11

Study Title: Determination of Delayed Time-course Efficacy of Anthrax Immune Globulin (AIG), NP-015 given in Combination with Levofloxacin in Rabbits Exposed To Inhalation Anthrax: Non-GLP Study (b) (4)898-G005681)

In this study, therapeutic efficacy of AIGIV given in combination with levofloxacin was assessed in comparison to placebo control (IGIV) given in combination with levofloxacin when treatment was administered at up to 60 hours after exposure to lethal doses of *B. anthracis* (Ames strain) spores.

This was a non-GLP, randomized, placebo-controlled, open-label and parallel-group comparative study. Seventy-two rabbits were divided into nine groups of eight animals. All animals were aerosol challenged with a target of spore dose of 200 x LD50 *B. anthracis* (Ames strain) in three cohorts with animals from each group. Four groups were administered with 15 U/kg of AIGIV via slow intravenous (IV) infusion in combination with 50 mg/kg levofloxacin (given orally, once a day for three days) starting at 30, 36, 48, or 60 hours post-exposure. Four other groups were administered placebo (IGIV volume equivalent to AIGIV dose) via slow IV infusion in combination with the same regimen of levofloxacin at the same time points post-exposure. AIGIV or placebo infusion was initiated within 30 minutes of the first levofloxacin dose. One group of eight animals remained untreated following exposure. Animals were observed to 30 days post-challenge for survival, body temperature, clinical observations, hematology, bacteremia and toxemia.

All of the untreated control animals died following anthrax exposure. A survival rate of 100% (8/8) was observed in the IGIV plus levofloxacin groups that received treatments starting at 30, 36 and 48 hours post-exposure. A survival rate of 87.5% (7/8) was observed for the group that received treatment at 60 hours post exposure.

Treatment with AIGIV plus levofloxacin resulted in similar survival rates of 87.5, 100, 100 and 75% in the 30, 36, 48 and 60 hour treatment groups, respectively. The data demonstrated the efficacy of levofloxacin when given in combination with IGIV or AIGIV as 94% (59/63) of treated animals from all groups survived, even when treatment was significantly delayed. The difference in the survival rates between the IGIV (placebo) plus levofloxacin and AIGIV plus levofloxacin was not statistically significant at any of the time points tested.

While added benefit over antibiotics could not be observed for survival, this study did demonstrate effect of AIGIV on toxemia. In the AIGIV treated groups, all animals cleared PA toxemia post-AIGIV administration and only 4/31 (13%) of AIGIV treated animals exhibited a single transient positive PA result for toxemia at the 12 or 18 hour time point post-dosing. In comparison, IGIV treated placebo control animals exhibited more persistent toxemia, with 26/32

(81%) having positive PA results for 18 to 90 hours post-treatment. All untreated animals that died were toxemic and bacteremic at the time of death.

Comments: The study indicates the efficacy of levofloxacin alone in toxin treated rabbits and AIGIV does not appear to be more efficacious than levofloxacin or provided added benefit to levofloxacin treatment.

Study Title: Determination of Added Benefit of NP-015 over Levofloxacin by Delayed Treatment in New Zealand White Rabbits Exposed to Inhalational Anthrax: Non-GLP (1079-G005681)

The objective of this study was to evaluate the therapeutic efficacy of AIGIV over placebo (IGIV) when either was administered with levofloxacin at different time points post-challenge with 200 x LD_{50} aerosolized spores of *B. anthracis*. Treatment groups received 15 U/kg AIGIV or IGIV with levofloxacin starting 60, 72, 84, or 96 hours post-challenge. Animals treated at 60 hours had 10 animals per group, the 72 and 96 hour treatment groups had 36 animals per group and the 84 hour treatment group had 32 animals per group. In addition, an untreated control group had 18 animals. Animals were observed for 32 days post-anthrax exposure.

Pre-treatment mortality (i.e., animals that died before completing the infusion) ranged from 10 to 70%, and increased with lag time when the treatments were delayed to 60, 72, 84 and 96 hours post exposure (Table 1).

Table 1: Survival Data from Rabbit Combination Treatment Study

Treatment Time	No. Animals Exposed	Mortality Prior to Treatment	Treatment[a]	Survival among Bacteremic Animals Treated
Control	18	N/A	None	NA
60 hours	20	2 (10%)	IGIV + Levofloxacin	9/10 (90%)
			AIGIV + Levofloxacin	8/8 (100%)
72 hours	72	29 (40%)	IGIV + Levofloxacin	9/18 (50%)
			AIGIV + Levofloxacin	15/23 (65.2%)
84 hours	64	45 (70%)	IGIV + Levofloxacin	2/8 (25%)
			AIGIV + Levofloxacin	4/10 (40%)
96 hours	72	57 (79%)	IGIV + Levofloxacin	2/8 (25%)
			AIGIV + Levofloxacin	5/7 (71.4%)
Total	246	133 (54%)		54/92

[a] AIGIV was dosed at 15 U/kg for all groups. IGIV was administered at a comparable dose volume

Survival rates observed for the IGIV plus levofloxacin treated groups were 90%, 50%, 25% and 25% when treatments were initiated at 60, 72, 84 and 96 hours post exposure, respectively. At these same time points, survival rates among the AIGIV plus levofloxacin groups were 100, 65.2, 40 and 71.4%, respectively. Even with significantly delayed treatment, antibiotics alone were highly effective. Differences in the survival rates and time to death observed between IGIV plus levofloxacin and AIGIV plus levofloxacin treatments were not statistically significant at any of the time points tested.

The majority of AIGIV treated animals became negative for PA (toxemia) within one hour post-infusion of AIGIV and remained negative, even with the delayed treatment from 60 to 96

hours post-anthrax challenge and high levels of toxemia pretreatment. In contrast, IGIV placebo treated animals remained toxemic up to three days after initiating antibiotic treatment.

STUDY #13

Study Title: Efficacy Evaluation of NP-015 (AIGIV) in Combination with Levofloxacin when administered at 96 h Post exposure in the Rabbit Model of Inhalational Anthrax: GLP Study (1182-100011472).

In this study, the therapeutic efficacy of the combination treatment of AIGIV and levofloxacin was evaluated over that of IGIV (placebo control) and levofloxacin when either treatment was initiated 96 hours after aerosol exposure. It was a GLP, randomized, blinded, placebo controlled, single center study with 336 rabbits. All animals were aerosol challenged with a target of spore dose of 200 x LD50 *B. anthracis*. Animals surviving to 96 hours post-challenge (n = 81) were randomized to treatment with IGIV and 50 mg/kg levofloxacin or 15 U/kg AIGIV and 50 mg/kg levofloxacin. Three doses of levofloxacin were given once daily for three days starting at infusion. Animals were observed over a 36 day period. In addition to assessments for survival, toxemia and bactermia were also assessed. Survival was the primary endpoint used to assess added benefit of AIGIV over levofloxacin when levofloxacin was administered in combination with AIGIV or IGIV at 96 hours post-exposure.

Of 64 modified intent-to-treat (MITT) animals that were positive for toxemia and bacteremia prior to treatment, 31 of them received AIGIV and 33 of the rabbits received IGIV. Of these 33 animals in the IGIV group, 13 animals survived through 36 days post-exposure (13/33, 39% survival). Thirty-one animals in the MITT analysis set received AIGIV and 18 animals survived through day 36 (58% survival). The 19% absolute difference in survival rate between the AIGIV and IGIV groups did not reach statistical significance (p=0.1353; Z-test); however, that difference is clinically meaningful.

Due to the delay in the treatment time of 96 hours post exposure in this study, there was a significant pre-treatment mortality, suggesting that animals were in their late stages of the systemic disease at the time of treatment. Although protected antigen (PA) was undetectable following treatment, the mortality continued in treated animals, suggesting that toxin levels at the time of treatment may play a role in the outcome. A post hoc statistical analysis was conducted to explore the relationship between survival and pre-treatment toxemia levels.

• When animals had prior to treatment (PTT) PA levels of <200 ng/mL, most animals survived irrespective of the treatment. These data suggest that at lower PTT PA levels, levofloxacin alone can rescue >90% of exposed animals (IGIV plus levofloxacin 91.7%, AIGIV plus levofloxacin 88.9%).

• When PTT PA levels were >800 ng/mL, death was uniform (mortality of 10/10 in IGIV plus levofloxacin and 8/8 in AIGIV plus levofloxacin) irrespective of treatment, indicating an upper limit for PA levels at which exposed animals reach a point of no return.

The applicant states that "This suggests a possibility for the added benefit with AIGIV treatment in a subpopulation with toxin levels from 200 to 800 ng/mL. Indeed, rabbits with pre-treatment PA levels between 200 and 800 ng/mL that received IGIV plus levofloxacin had a survival rate of 18.2% (Figure 1). In contrast, animals from this subpopulation that received AIGIV plus levofloxacin had a survival rate of 71.4%".

Figure 1: Rabbits Treated with Levofloxacin Based on PTT PA Level
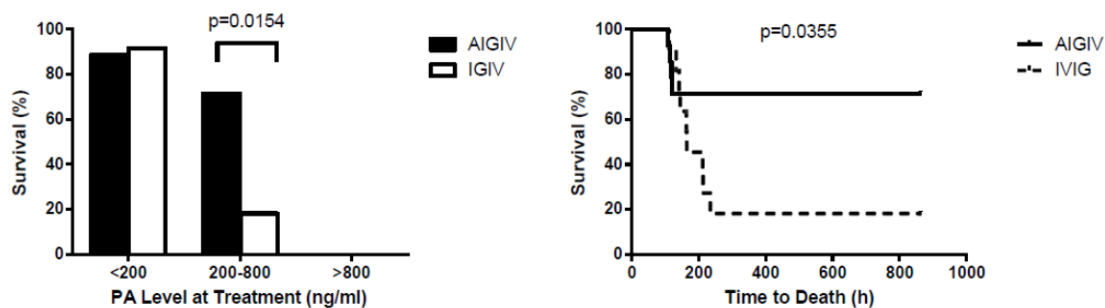
STUDY #14

Study Title: Therapeutic Efficacy of NP-015 given in Combination with Ciprofloxacin against Inhalation Anthrax Challenge in Cynomolgus Monkeys: Non-GLP (**987-G005780).**
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    A total of 72 cynomolgus macaques were divided into four groups; 12 animals were untreated controls and the remaining animals were randomized to three treatment groups of 20 animals each treated at 64 hours post-exposure with ciprofloxacin and either IGIV placebo or 15 or 30 U/kg AIGIV. All animals were aerosol challenged with a target spore dose of 200 x LD50 *B. anthracis* (Ames strain). Sixty-four hours post-challenge, animals received a loading dose of 32 mg/kg ciprofloxacin followed by nine maintenance doses (16 mg/kg) every 12 hours. Animals also received either a placebo infusion of IGIV, 15 or 30 U/kg AIGIV. Placebo IGIV was administered at the same volume as 30 U/kg AIGIV. For the first seven days, animals were observed for clinical signs every six hours and twice daily from day 8 to day 73, which was the end of the observation period. The primary endpoint was survival. The results of the study indicated that antibiotic treatment alone successfully treated anthrax infection (Table 1) and the survival rate was comparable between IGIV + ciprofloxacin and AIGIV + ciprofloxacin treated groups.

Table 1: Survival in Cynomolgus Macaques after Treatment with Combination Therapy 64 Hours Post-exposure

Group	Survival	
	All Animals	**Bacteremic Animals**
Untreated	1/12 (8%)	1/12 (8%)
IGIV + Ciprofloxacin	12/19 (63%)	9/12 (75%)
AIGIV (15 U/kg) + Ciprofloxacin	11/18 (61%)	10/12 (83%)
AIGIV (30 U/kg) + Ciprofloxacin	12/19 (63%)	11/14 (79%)

    The relationship between the pre-treatment PA levels and survival were explored in cynomolgus macaque combination therapy studies. No survival was observed regardless of treatment (IGIV plus ciprofloxacin, 15 U/kg AIGIV plus ciprofloxacin, or 30 U/kg AIGIV plus ciprofloxacin) when PTT PA level was >1000 ng/mL, establishing a point-of-no return where animals could not recover even with treatment. At PTT PA ≤1000 ng/mL, there was 75% survival in the IGIV plus ciprofloxacin group and 78% survival in the AIGIV plus ciprofloxacin group. No significant survival difference between IGIV and AIGIV treatments was established in the primary efficacy endpoint analysis for the study.