



**Department of Health and Human Services
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Applicant: Cangene Corporation (Emergent Biosolutions)

Product: Anthrax Immune Globulin Intravenous (Human);
Proposed proprietary Name: ANTHRASIL

Subject: Preclinical Pharm-Tox Review

Introduction

Anthrax Immune Globulin Intravenous (Human) (herein referred to as AIGIV or NP-015) is a human IGIV preparation manufactured from pooled, fractionated plasma collected from healthy donors vaccinated with anthrax vaccine, BioThrax®. The sponsor is seeking approval for the treatment of toxemia associated with inhalational anthrax. The potency is expressed in units (U) based on the toxin neutralization ability of AIGIV in the toxin neutralization assay. The final preparation contains (b) (4) to 70 mg/mL IgG in (b) (4) mL solution, has a potency ≥ 60 U/vial and is formulated with 10% maltose and 0.03% polysorbate 80 (PS80).

No separate toxicology studies were conducted for AIGIV or its components. To support licensure of the preparation, sponsor submitted two Good Laboratory Practice (GLP) toxicity studies performed with an IGIV product manufactured by Cangene by a similar manufacturing process. These studies are reviewed here.

The efficacy of AIGIV was studied in two animal species: rabbit and cynomolgus macaque. The efficacy studies are not the subject of this review. However, supporting data from toxicologic endpoints in these studies are reviewed here.

Published literature for manufacturing related impurities present in AIGIV as well as excipients used in formulation are also reviewed here.

Conclusion

There are no toxicology issues that would prevent this application from being approved.

1. Toxicological assessment of the impurities and excipients

Dose

AIGIV is proposed to be administered at doses and infusion rates shown in Table 1 (from submission).

Table 1 Dosing for AIGIV (From the proposed PI, draft)

| Patient Group | Dose | Starting Infusion Rate (first 30 minutes) | Incremental Infusion Rate if Tolerated (every 30 minutes) | Maximum Infusion Rate |
|-------------------------------------|-----------|--|---|--|
| Adults (≥17 years) | 7 vials | 0.5 mL/min | 1 mL/min | 2 mL/min |
| Pediatric 1 year to <17 years | 1–7 vials | 0.01 mL/kg/min (do not exceed the adult rate) | 0.01 mL/kg/min | 0.04 mL/kg/min (do not exceed the adult rate) |
| <1 year | 1 vial | 0.01 mL/kg/min | 0.01 mL/kg/min | 0.04 mL/kg/min |

Sponsor submitted a toxicological assessment of the impurities and excipients found in AIGIV which is summarized in Table 2 (modified from submission to add safety margins for adult and pediatric populations). Table 3 summarizes highest exposure to excipients and impurities in AIGIV from this preparation and other approved IGIV products. The highest exposure to the product in a per kg basis would be when 2 vials of AIGIV are administered to a 10 kg child. Even at this dose, both safety margins and exposure comparison with other approved IGIV products, do not suggest safety concerns regarding the excipients and impurities when AIGIV is used according to the label.

Table 2: Excipients and Impurities, Highest Exposure Levels from AIGIV Administration

| Excipient/ Residue | Product Highest Acceptance Limit | Maximum Exposure from Administration of AIGIV (per kg) | Dose of Biological Relevance in Animal Toxicity Studies | Safety Margin, Adults | Safety Margin, Pediatric |
|--------------------------|--|--|---|-----------------------------|--------------------------------|
| Total protein | (b) (4) | (b) (4) | 5000 mg/kg, NOAEL in single dose IV study in rats | 14 | 8 |
| Maltose | (b) (4) | (b) (4) | 10 g/kg, NOAEL in repeated dose IV study in rabbits (1 month duration) | 16 | 9 |
| Polysorbate 80 (PS80) | (b) (4) | (b) (4) | 62.5 mg/kg, NOAEL in developmental study in rabbits | 30 | 17 |

| | | | | | |
|------------------------------|---------|---------|--|--------|--------|
| Tri-n-butyl Phosphate (TnBP) | (b) (4) | (b) (4) | 80 mg/kg, LOAEL in single dose IV study in rats | 15,608 | 9,181 |
| Triton X-100 (TX-100) | (b) (4) | (b) (4) | 1200 mg/kg, LD ₅₀ in IV study in mice | 45,714 | 26,891 |

Table 3: Excipients and impurities in AIGIV compared to other approved IGIV products

| Excipient, worst case scenario exposure with AIGIV | Excipient, worst case scenario exposure with other IGIV products |
|--|--|
|--|--|

(b) (4)

Toxicology studies

Study Number: 1914-002

Title: An acute single dose toxicity study of immune globulin intravenous (IGIV), NP-021 in rats; GLP

Testing facility: (b) (4)

Aim: To evaluate the potential acute toxicity of IGIV product, NP-021 in rats following single intravenous administration and a 14-day recovery period.

Study Design: 5 animals/sex/group received either control (0.9% Sodium Chloride for Injection, USP) or the test article (NP-021) at a dose of 1000, 2000, or 5000 mg/kg via IV infusion, through a surgically implanted femoral catheter, at an infusion rate of 2.5 mL/kg/hour.

A satellite group of 12 animals/sex were used to evaluate toxicokinetics (TK) of IGIV at a dose of 2000 mg/kg. The study design is summarized in Table 4.

Table 4: Study Design (from submission)

| | Treatment | Number of Animals |
|--|-----------|-------------------|
|--|-----------|-------------------|

| Group | Product and dose in mg/kg | (mg/animal) ^b | (mL/animal) ^c | Duration of Infusion (hrs) ^d | Toxicology (Main Study) | | Toxicokinetics | |
|---|----------------------------|--------------------------|--------------------------|---|-------------------------|--------|----------------|---------|
| | | | | | Male | Female | Male | Females |
| 1 | Normal saline ^a | N/A | 15.0 | 20 | 5 | 5 | - | - |
| 2 | NP-021-1000 | 300 | 3.0 | 4 | 5 | 5 | - | - |
| 3 | NP-021- 2000 | 600 | 6.0 | 8 | 5 | 5 | 12 | 12 |
| 4 | NP-021- 5000 | 1500 | 15.0 | 20 | 5 | 5 | - | - |
| ^a Commercially available 0.9% Sodium Chloride for Injection, USP (saline) is used as control article and is given at the volume equivalent to the highest dose volume of NP-021 ^b Based on 300 g body weight of animal ^c NP-021 is 10% IGIV product containing 100mg/mL protein ^d Based on flow rate of 2.5 mL/kg/hr NA: Not Applicable | | | | | | | | |

Outcome measures: cage-side observations twice daily, body weights on Day -1, 7 and 15; food consumption recorded daily; hematology and clinical pathology evaluations on day 14; necropsy and histopathology on day 14. Time points for TK study were: predose, 1, 8, 24, 48, 72, 96, 168, 240, and 336 hours postdose; 4 animals/collection time points were used.

Results: Red material in pan/bedding noted for 3 of 5 males and 1 of 5 females at high dose on Day 2. Red discolored urine is the likely cause for this finding (hemolysis due to the very high dose of IGIV). Mean food consumption was decreased in males at middle and high dose and in females at dose on day 1 or 2.

The serum concentrations and toxicokinetic parameters calculated for NP-021 were similar in male and female rats. Combined mean AUC_{0-∞}, AUC₀₋₃₄₄, C_{max}, T_{max}, CL, t_{1/2} and V_{ss} were 2640 hr•mg/mL, 2000 hr•mg/mL, 26.8 mg/mL, 1.00 hour (first sampled time point), 0.0127 mL/min/kg, 179 hours and 172 mL/kg, respectively.

Conclusion: 5 g/kg IGIV in rats can be considered NOAEL under the conditions of this study.

Study Number: 1914-003

Title: Local Tolerance Study Of Immune Globulin Intravenous (IGIV), NP-021 In Rabbits; GLP

Test Article: NP-021

Testing Facility: (b) (4)

Aim: To evaluate the local tolerance of the test article (NP-021) in Albino rabbits when administered as a bolus injection through the intravenous, intra-arterial, perivascular, subcutaneous and the intra-muscular routes and to determine if the product was tolerated at the potential sites of application and misapplication.

Study Design: 9 New Zealand White rabbits per sex per group (weighing 3.41 to 3.71 for males kg and 3.07 to 3.56 kg for females) received the test article at doses and routes of administration shown in Table 5. For each dose route, saline (0.9% Sodium Chloride for Injection, USP) was

administered to the contralateral side for comparison. The locations were: for intra-venous administration marginal ear vein via a temporary catheter, for intramuscular administration the leg, for intra-arterial administration the auricular artery, for the perivascular administration the tissue surrounding the marginal vein and for the subcutaneous administration the the subscapular region lateral to the midline on the back of the animal.

Outcome measures: Cage-site observations daily, detailed clinical observations and dermal irritation scoring twice daily using Draize scoring shown in Tables 6 and 7; body weights on day -1, 7, and 14. Three animals /sex/ group were necropsied on Day 2, 5, and 15 and macroscopic and microscopic examinations were performed on the ears and other injection sites.

Table 5: Group Assignments

| Group Number | Dose Level ^a | Dose Route | Number of Animals ^b | |
|---|-------------------------|----------------|--------------------------------|--------|
| | | | Male | Female |
| 1 | 200 mg/kg | intravenous | 9 | 9 |
| | 50 mg/animal | intramuscular | | |
| 2 | 50 mg/animal | intra-arterial | 9 | 9 |
| | 20 mg/animal | perivascular | | |
| | 50 mg/animal | subcutaneous | | |
| ^a Saline was administered to the contralateral side for each dose route. | | | | |
| ^b Three animals/sex/group were necropsied on Days 2, 5, and 15. | | | | |

Table 6: Erythema and Eschar Scoring

| Score | Observation |
|-------|---|
| 0 | No erythema |
| 1 | Very slight erythema (barely perceptible) |
| 2 | Well-defined erythema |
| 3 | Moderate to severe erythema |
| 4 | Severe erythema (beet redness) to slight eschar formation (injuries in depth) |
| | Maximum possible score = 4 |

Table 7: Edema Scoring

| Score | Observation |
|-------|--|
| 0 | No edema |
| 1 | Very slight edema (barely perceptible) |
| 2 | Slight edema (edges of area well-defined by definite raising) |
| 3 | Moderate edema (raised approximately 1 mm) |
| 4 | Severe edema (raised more than 1 mm and extending beyond area of exposure) |

| | |
|--|----------------------------|
| | |
| | Maximum possible score = 4 |

Results: There were no test article-related clinical findings, effects on body weight, or macroscopic observations. No animals showed moderate to severe erythema or higher scoring or slight edema or higher scoring. There were no differences between the IGIV treatment and saline controls in macro- or microscopic evaluations of the injection sites.

Other Studies

Single dose pharmacokinetic (PK) studies were conducted with AIGIV in rabbit and cynomolgus macaque models. Severe toxicity and mortality were observed in the rabbit, but not in cynomolgus monkeys. This is a brief review of the rabbit study where the toxicity was exhibited and the analysis of available data regarding its cause and potential risk in the clinic.

Study Number: (b) (4) 658-G005681

Aim: PK study in New Zealand White Rabbit

GLP study

Study design is summarized in Table 8.

Results: A severe hemolysis reaction, anemia and subsequent hypoxia and mortality occurred in 10 out of 10 rabbits receiving the highest amount of IGIV. The hemolysis did not occur when a lower dose of the same lot (group 2) or a second lot of AIGIV preparation with a higher specific activity (groups 3-5 in Table 8) were administered. The dose-effect relationship to the amount of human IGG was shown *in vitro* by a modified Coombs agglutination test using rabbit RBCs. Thus it is concluded that hemolysis is not due to anti - anthrax toxin antibodies, but rather due to non-toxin specific antibodies present in IGIV.

Table 8 Experimental Design (modified from submission to include mortality and specific activity)

| Treatment Group | No. of Animals | Dose (U/kg) | Specific Activity (U/g) | Administered IgG amount at Dose (mg/kg) | Mortality |
|-----------------|----------------|-------------|-------------------------|---|-----------|
| 1 | 10 | 10 | 23.5 | 426 | 1/10* |
| 2 | 10 | 40 | 23.5 | 1705 | 10/10 |
| 3 | 2 | 20 | 46.3 | 432 | 0/2 |
| 4 | 2 | 30 | 46.3 | 648 | 0/2 |
| 5 | 2 | 40 | 46.3 | 864 | 0/2 |

^a Lot 24405011 (1.29 U TNA/mL and 55 mg protein/mL)

^b Lot 10602912 with a higher specific activity of AIGIV (2.73 U TNA/mL and 59 mg protein/mL)

*The animal may have suffered a thromboembolic event, perhaps due to rapid infusion or damage during blood collection

Reviewer Comments: Rabbits tissues (including RBCs), as all other non-primates and new world primate species, contain α -gal epitope, whereas humans and old world primates do not. Additionally, 1% of human antibodies are anti-gal (Galili, 2013) so it is likely the hemolytic reaction in rabbits is due to these antibodies (Macher & Galili, 2008). Cynomolgus monkeys, being old-world monkeys, like humans do not have 1,3 galactosyl transferase and thus do not contain the agal epitope. This animal model does not show toxicity following AIGIV administration.

References

- Galili, U. (2013). Discovery of the natural anti-Gal antibody and its past and future relevance to medicine. *Xenotransplantation*. 20, 138-147, doi:10.1111/xen.12034 [doi].
- Macher, B. A. & Galili, U. (2008). The Gal α 1,3Gal β 1,4GlcNAc-R (alpha-Gal) epitope: a carbohydrate of unique evolution and clinical relevance. *Biochim. Biophys Acta* 1780, 75-88, doi:S0304-4165(07)00268-1 [pii];10.1016/j.bbagen.2007.11.003 [doi].