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

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| **To**: | BLA STN 125335/0 |
| **Cross Reference:** | IND (b)(4) |
| **From:** | Evi Struble, Ph.D., Pharmacologist, LPD, DH, OBRR |
| **Through:** | Dorothy E. Scott, M.D., Laboratory Chief, LPD, DH, OBRR |
| **CC:** | Debra Cordaro, RPM, HFM-370 |
| **Applicant:** | Rare Disease Therapeutics, Inc. |
| **Product:** | Anascorp®/Centruroides (Scorpion) Immune $F(ab')_2$ (Equine) Injection |
| **Subject:** | Final Memo, Nonclinical Pharmacology/Toxicology |

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Brief Description of BLA Submission ..... 2
*Proposed indication* ..... 2
*Dose* ..... 2
Conclusions and Recommendation ..... 2
Complete Review ..... 3
*Review of animal studies submitted with the BLA* ..... 3
Results ..... 3
Reviewer Conclusions ..... 3
*Review of the excipients and impurities contained in the formulation* ..... 5
Glycine ..... 5
Sucrose ..... 5
Cresol ..... 6
(b)(4) ..... **Error! Bookmark not defined.**
Borates and Boron ..... 7
*Label* ..... 7
*References* ..... 8

**Brief Description of BLA Submission**

Anascorp® is a lyophilized $F(ab')_2$ fragment of immunoglobulin G (IgG) made from the blood of horses immunized with the venom of four different Centruroides scorpions. The BLA submission contains data from one GLP animal study conducted in rats and five clinical trials.

*Proposed indication*

For "treatment of scorpion envenomation."

*Dose*

3 vials, each vial reconstituted with 5 mL sterile WFI (b)(4); then that volume diluted in 50 mL of 0.9% NaCl (b)(4) and delivered in a 10 min IV infusion. Further vials, up to a total of 5 can be administered at 30-60 min intervals.

**Conclusions and Recommendation**

There are no preclinical issues to prevent this application from being approved.

The GLP animal study submitted (#1299-001, Acute Toxicology Study in Rats) cannot be used to derive a NOAEL of Anascorp® in animals for label use as the sponsor failed to demonstrate that intended systemic exposure with the biologic was achieved in this study.

Based on the potential for adverse reactions it is recommended that an warning for cresol be included in the PI for Anascorp® such as: "Localized reactions and generalized myalgias have been reported with the use of cresol as an injectable excipient". It is also recommended that sponsor be requested to remove cresol from the product to improve its safety profile.

The other impurities and excipients present in Anascorp® final product are safe when used according to PI.

**Complete Review**

*Review of animal studies submitted with the BLA*

Title: Crotaline (Pit Viper) Equine Immune F(AB)2: an Acute Intravenous Toxicity Study in Rats
Study Number: 1299-001
Aim: Acute toxicology Study in Rats
Performing Lab: -------(b)(4)--------
Test item: Anavip Crotaline (pit viper) equine immune $F(ab')_2$
Stability data not available
GLP study
Test Model: n=20 M and 20 F Crl:CD rats, 6 weeks old
Design: block randomized 5M, 5F/ group receiving either vehicle or test article at 500, 2000 and 5000 mg/kg IV for up to 60 minutes via the tail vein. The rats were observed for 14 days and then sacrificed and necropsied.
Outcome measurements: Cageside observations, clinical observations at 1, 2, 4 hrs post infusion and on day 1 and daily thereafter, body weight, urinalysis, (b)(4) of blood collected at 1 hr pos-dose, clinical pathology on all animals, necropsy.
**No histopathology was performed**.

**Results**

The local and systemic toxicities of Anavip are as follows:

4/20 deaths 2 M in the middle and 2 M in the high dose
Major irritation at the injection sites at all doses
Dose dependent injection site tissue necrosis, including tail shedding at all doses, especially the high dose.

These toxicities could be related to the very high concentration of the test article injected, namely 180 mg/ml, resulting to a highly viscous liquid.

The NOAEL for systemic toxicity was determined at 500 mg/kg. There was no safe dose for local toxicity.

**Reviewer Conclusions**

1. Due to the very high concentration as well as the high viscosity of the administered test item the intended systemic exposure was not achieved. The table below shows the discrepancy between Anavip injected dose (last column) and the plasma amount (column shaded gray) as determined by (b)(4) at 1 hr post injection.
2. Protein concentration analysis presented in Appendix B shows differences in protein concentration in administered samples with one sample having no protein present.

Table 1

| Group | Anavip Plasma Concentration (μg/ml)[a] | Anavip Plasma Amount (mg/kg) [b] | Anavip Dose (mg/kg) |
|---|---|---|---|
| 2m | 38 | 2.432 | 500 |
| 2m | 94 | 6.016 | 500 |
| 2m | 3205 | 205.12 | 500 |
| 2m | 791 | 50.624 | 500 |
| 2m | 1 | 0.064 | 500 |
| 2f | 928 | 59.392 | 500 |
| 2f | 1954 | 125.056 | 500 |
| 2f | 1331 | 85.184 | 500 |
| 2f | 3244 | 207.616 | 500 |
| 3m | 109 | 6.976 | 2000 |
| 3m | 3558 | 227.712 | 2000 |
| 3m | 216 | 13.824 | 2000 |
| 3m | 316 | 20.224 | 2000 |
| 3m | 7383 | 472.512 | 2000 |
| 3f | 283 | 18.112 | 2000 |
| 3f | 13154 | 841.856 | 2000 |
| 3f | 2226 | 142.464 | 2000 |
| 3f | 176 | 11.264 | 2000 |
| 3f | 4182 | 267.648 | 2000 |
| 4m | 201 | 12.864 | 5000 |
| 4m | 49 | 3.136 | 5000 |
| 4m | 8778 | 561.792 | 5000 |
| 4m | 20211 | 1293.504 | 5000 |
| 4m | 21261 | 1360.704 | 5000 |
| 4f | 2813 | 180.032 | 5000 |
| 4f | 197 | 12.608 | 5000 |
| 4f | 29869 | 1911.616 | 5000 |
| 4f | 121 | 7.744 | 5000 |
| 4f | 9730 | 622.72 | 5000 |

[a] Study Report, Appendix 1

[b] Calculated by the reviewer using 64 ml/kg as the rat blood volume[3]

In conclusion, due to the lack of consistent exposure in each group no conclusions can be drawn as to the safety and NOAEL of Anavip.

*Review of the excipients and impurities contained in the formulation*

Table 2 shows the excipients and impurities in Anascorp® (based on the submission); the maximum exposure is calculated from the maximum dose of 10 vials used in the clinical trials.

Table 2 Excipients and Impurities in Anascorp

| Compound | Amount | Maximum Exposure After 10 Vials |
|---|---|---|
| Glycine | ---(b)(4)--- mg/vial | (b)(4) mg |
| Sucrose | ---(b)(4)--- mg/vial | (b)(4) mg |
| Sodium Chloride | 45 - 80 mg/vial | 800 mg |
| Borates | NMT 1.0 mg/vial | 10 mg |
| Sulfate | ---(b)(4)--- | (b)(4) mg |
| Cresol | ---(b)(4)--- | (b)(4) mg |

With the exception of cresol and borates, all the other compounds are commonly found in IGIV products. Sodium chloride and sulfate are inorganic salts commonly used in pharmaceutical formulations, including IgIV products, thus represent no safety risk to patients. An analysis of the safety of all the other excipients in Anascorp® is presented below.

**Glycine**

Final specification of Glycine (FW 75.07 g/mol) is set at ---(b)(4)--- mg/vial. This corresponds to a maximum exposure to glycine of (b)(4) mg following a dose of 10 vials of Anascorp®. Table 3 shows concentration of glycine in different approved IGIV products. Given the volumes of these products used during IGIV therapy for PIDD i.e. several hundred mL, the amount of glycine in Anascorp® will result in exposures that are smaller than those routinely obtained in clinical practice with approved IGIV therapies. Thus, glycine in Anascorp® formulation does not represent a safety risk to patients.

Table 3 Glycine in IGIV Approved Products and Anascorp®

| Product Name/Concentration (Sponsor) | Glycine Concentration According to Label | Glycine Dose[a] |
|---|---|---|
| Gamunex (Talecris) | 0.24 M (18 mg/mL) | 8,100 mg |
| Gammagard Liquid/10% (Baxter) | 0.25 M (18.7 mg/mL) | 8,415 mg |
| Gammagard S/D/5% (Baxter) | 0.30 M (22.5 mg/mL) | 20,250 mg |
| Anascorp®/ (Rare Disease Therapeutics, Inc) | | (b)(4) mg |

[a] Calculated assuming an IGIV dose of 600 mg/kg and a 75 kg patient

**Sucrose**

Sucrose is present in the final formulation of Anascorp® at a total amount of --(b)(4)-- mg/vial. Sucrose from IGIV products has been associated with Acute Renal Failure (ARF) when resulting in sucrose exposure of 1 g/kg[5], perhaps smaller for susceptible populations. If receiving a dose of 10 vials of Anascorp®, a patient's exposure to sucrose would be (b)(4) mg (Table 2). This, for a 75 kg patient, corresponds to (b)(4) mg/kg ((b)(4) g/kg), and for a 2.5 kg neonatal patient would be

(b)(4) mg/kg. As such, taking ten vials of Anascorp® would result in an exposure to sucrose ------(b)(4)------ smaller than the dose associated with kidney damage in adults and newborns, respectively. Thus, the amount of sucrose present in the final formulation of Anascorp® is unlikely to cause kidney damage when the product is used according to PI.

**Cresol**

Cresol is used as ------(b)(4)----- during manufacturing steps and is present in final Anascorp® product at a specification ----(b)(4)-----. As such, for each vial filled with (b)(4) bulk product the maximum amount of cresol would be (b)(4) mg resulting in a total patient exposure of (b)(4) mg cresol from 10 vials.

Cresol is present in many insulin and insulin analogs, as well as growth hormone preparations where it is used ------(b)(4)-----. For example, Apidra (insulin glulisine [rDNA origin] injection) contains 3.15 mg/ml cresol or 3.15 mg for each 100 U of insulin. Taking 100 U of insulin would result in similar exposure to cresol as that coming from 10 vials of Anascorp.

Cresol at these doses when used as an injectable has been associated with with myalgia and elevated creatine kinase activity[1], and malignant hyperthermia[2]. For this reason, the following warning is included in both classes of the injectables mentioned: "Localized reactions and generalized myalgias have been reported with the use of cresol as an injectable excipient".

National Toxicology Program (NTP) has evaluated the potential genetic toxicology of cresols in cell culture and *in vitro* as well as potential carcinogenicity *in vivo* in 2 year studies in rats and mice. All *in vitro* tests did not show a signal whereas in 2 year studies cresol showed "equivocal evidence" of carcinogenetic activity in male F344/N rats (marginally increased incidence of renal tubule adenoma at a dose of 15,000 ppm or at least 750 mg/kg) and "some evidence" of carcinogenic activity in female mice (increased incidence of forestomach squamous cell papilloma at 10,000 ppm or at least 1,429 mg/kg) [4]. Additional nonneoplasmic lesions were also seen at all doses used, starting at 230 mg/kg in rats and 300 mg/kg in mice, or ~5,000x or more the exposure from Anascorp® use.

Based on the indication of the product, i.e. not intended for chronic use, the lack of mutagenic signal during *in vitro* tests, and the regulatory precedent i.e. its presence in insulin and growth hormone products, the use of cresol in Anascorp® is not likely to pose a carcinogenicity risk to patients.

In conclusion, this reviewer recommends a label warning for cresol be included in the labeling of Anascorp®. It is also recommended that sponsor be required in a PMC to remove cresol from the product to improve its safety profile.

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---(b)(4)---

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----------(b)(4)----------
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**Borates and Boron**

Borates are an impurity in the final formulation of Anascorp® with a specification set at NMT 1 mg/vial, resulting in a patient exposure of NMT 10 mg after taking 10 vials of the product. Borates are found at small amounts in vaccines such as gardasil and recombinant HAV, in ophthalmic solutions, topical irrigant solutions, in OTC vitamin preparations and dietary supplements, and are part of a normal diet.

Borates are rapidly absorbed from oral intake and readily available systemically ([6] and references within). They are not metabolized by humans or animals and are excreted unchanged[7]. For example, ~ 99% of the administered dose was excreted in urine over a 120-hr period following intravenous administration of doses of 570 to 620 mg boric acid to healthy adult human volunteers[6].

EPA has determined an RfD for boron (a dose likely to be safe for chronic exposure) [8] equal to 0.16 mg/kg/day when taken orally, i.e. ~12 mg/day for a 75 kg human. This dose was calculated from data obtained in dietary studies in pregnant rats where the adverse effect observed at higher doses was a decrease of fetal body weight. Taking into account the formula weight of boron (B, 11 g/mol) and borate ($BO_3^{3-}$, 59 g/mol), ~12 mg/day boron corresponds to ~12x59/11 = 64 mg/day borate.

The exposure to borate following administration of 10 vials of Anascorp® would be 10 mg, i.e. more than 6x smaller than RfD. As such, borates do not represent a safety risk for patients when Anascorp® is used according to PI.

*Label*

This reviewer recommends the following changes to the label printed in red typeface. Alternatively section 13 can be omitted from the label.

WARNINGS AND PRECAUTIONS

- Anascorp® should not be administered to patients with a known history of hypersensitivity to horse proteins unless the benefits outweigh the risks. (4)
- Localized reactions and generalized myalgias have been reported with the use of cresol as an injectable excipient. (Possible reference suggestions 1, 2)

13 NONCLINICAL TOXICOLOGY

13. 1 Carcinogenesis, mutagenesis, impairment of fertility

Animal carcinogenicity and reproduction studies have not been conducted with Anascorp.

13.2 Animal Toxicology and/or Pharmacology

There were no GLP animal studies performed with Anascorp.

~~A non-GLP acute toxicity study was conducted in mice receiving Anascorp® by intravenous and intraperitoneal injection at doses of 1 to 5 g/kg. No deaths were observed at any dose or route. The $LD_{50}$ for both routes was greater than 5 g/kg. However, a GLP acute toxicity study in rats was conducted with Antivipmyn (Crotalidae (Pit~~

~~Viper) Equine Immune F(ab)$_2$), an antisnake F(ab)$_2$ that has the same physicochemical characteristics as Anascorp. Under the conditions of this study, where rats received a single IV dose of, the no-observed-adverse-effect-level (NOAEL) for systemic toxicity was determined to be 500 mg/kg.~~

Rationale for the changes: Due to the lack of verifiable standards in performing the study, a non-GLP study cannot be used to make label claims. Due to deficiencies in design and lack of interpretable data, the study conducted with Anavip cannot be used to make label claims.

*References*

[1]Bach MA, Blum DM, Rose SR, Charnas LR, J Pediatr. 1992 Oct; 121(4):650-651 "Myalgia and elevated creatine kinase activity associated with subcutaneous injections of diluent"

[2] Wappler F, Roewer N, Köchling A, Braune H, Reissinger T, Schulte am Esch J, Intensive Care Med. 1996 Aug; 22(8):809-12 "Fulminant malignant hyperthermia associated with ketoacidotic diabetic coma"

[3]Karl-Heinz Diehl *et al*, J. Appl. Toxicol. 21, 15–23 (2001) "A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes"

[4]Natl Toxicol Program Tech Rep Ser. 2008, Jul(550):1-120. [National Toxicology Program technical report series] Toxicology and Carcinogenesis Studies of Cresols (CAS No. 1319-77-3) in Male F344/N Rats and Female B6C3F1 Mice (Feed Studies)

[5]Rubin Zhang and Harold M. Szerlip, Southern Medical Journal, 93 (9), 901-904 (2000) "Reemergence of Sucrose Nephropathy: Acute Renal Failure Caused by High-Dose Intravenous Immune Globulin Therapy"

[6]Jansen, J. A., Anderson, J., and Schou, J. S. Arch. ToxicoL 55, 64-67 (1984); "Boric acid single dose pharmacokinetics after intravenous administration to man"

[7]Murray, F. J. *ReguL Toxicol Pharmacol* **22,** 221-230 (1995); "A human health risk assessment of boron (boric acid and borax) in drinking water"

[8]"Reference Dose (RfD) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime", IRIS, EPA.