



**Department of Health and Human Services
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Applicant: Green Cross Corporation

Product: GCC 10% IGIV (Immune Globulin Intravenous, Human)

Subject: Preclinical Pharm-Tox Review

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Introduction

This BLA from Green Cross Corporation (GCC) seeks approval for 10% human IGIV preparation for primary humoral immunodeficiency. The product is a liquid preparation, purified from human source plasma using a modified Cohn-Onceley fractionation process. The proposed dose is 300 – 900 mg/kg every 21 or 28 days.

Pharmacology and Toxicology

The sponsor conducted nonclinical studies using the GCC preparation (referred to as GC5107 or GCC 10% in the submission and throughout this review) that include a GLP single dose toxicity and toxicokinetic study in rats, a thrombogenicity study in rabbits, three safety pharmacology studies in rats (2 studies) and mice (1 study) and two

proof-of-concept (primary pharmacology) studies; a listing of these studies is shown in Table 1. The studies are reviewed here.

Table 1: Pharmacology and Toxicology Studies

Type of Study	Test System	Testing Facility	Study Number	GLP Compliance
Toxicology				
Single-Dose IV toxicity study in rats with Toxicokinetics	Rats	(b) (4)	15-RA-827	Yes
Thrombogenic risk in rabbits	Rabbits	(b) (4)	32172	Yes
Primary Pharmacology				
The efficacy test of IGIV, 10% Liquid on (b) (4) mouse infected with <i>Streptococcus pneumonia</i>	Mice	(b) (4)	12-ME-090N	No
The efficacy test of IGIV, 10% Liquid on (b) (4) mouse infected with <i>Klebsiella pneumoniae</i>	Mice	(b) (4)	12-ME-598N	No
Safety Pharmacology				
Effects of IGIV, 10% Liquid on the Body Temperature and General Behavior of (b) (4) Mice after a Single Intravenous Dose	Mice	(b) (4)	15-MS-828	Yes
Effects of IGIV, 10% Liquid on the Respiration Rate and Tidal Volume of (b) (4) Rats after a Single	Rats	(b) (4)	16-RS-305	Yes
Effects of IGIV, 10% Liquid on Blood Pressure and Heart Rate of (b) (4) Rats after a Single Intravenous Dose	Rats	(b) (4)	15-RH-830	Yes

Excipient and Impurities

GCC 10% contains 100 mg/mL protein in a liquid preparation formulated at pH 4.5-5.5 with 18.8 mg/mL glycine as a (b) (4). Select release specifications are shown in Table 2. These specifications, including formulation, are common in other approved IGIV preparations and do not raise toxicologic concerns with respect to human exposure.

Potential impurities that could arise from manufacturing process or container closure were assessed using extractable and leachable studies and toxicologic assessments. These assessments were reviewed and found acceptable.

Table 2: GCC 10% IGIV Drug Product Release Specification (Modified from submission)

Category	Test	Acceptance Criteria
Product Characteristics	pH (b) (4)	4.5 – 5.5
	(b) (4)	(b) (4)
	Total Protein	(b) (4)

Excipient	Glycine	15.0 – 22.6 mg/mL
Purity and Impurities	Protein Composition: IgG	≥ 96% of total protein
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	(b) (4)	(b) (4)
	Particulate Matter: Visible	No visible particles

Conclusions

Approval recommended: there are no pharmacology and toxicology issues that would prevent this BLA from being approved.

Complete review

Toxicology

Study Number: 15-RA-827

Study title: A Single-Dose Intravenous Infusion Toxicity and Toxicokinetic Study of GC5107 in (b) (4) Rats

Performing laboratory: (b) (4)

Objective: To assess toxicity of a single intravenous dose of GC5107 administered by infusion pump in (b) (4) rats.

Study Design: This is a GLP study where n=10 sex/dose weighing 277-324 g (M) and 177-211g (F) received 0, 1000, 2000, and 3000 mg/kg test article or saline via IV infusion using a catheter implanted in the tail vein. An additional n=5/sex/dose received the same doses and were used for toxicokinetic (TK) analysis. Dose volume of up to 30 mL/kg was administered at an infusion rate 0.25 mL/min.

Outcome measures: Clinical signs daily; body weight on days 1, 2, 4, 8 and 14; urinalysis in week 2; hematology, coagulation, and clinical chemistry on day 14, gross necropsy, complete histopathology at the end of the study (day 14). For the TK study, blood was collected from the jugular vein on the day before administration (0 hr), 1, 4, 8, 24, 48, 72, 96, 168, 240 and 336 hours after administration. The blood was coagulated, centrifuged at 3000 rpm for 10 min and serum stored at -70 °C till ready to be analyzed by nephelometry. TK analysis was performed by non-compartmental analysis using the (b) (4)

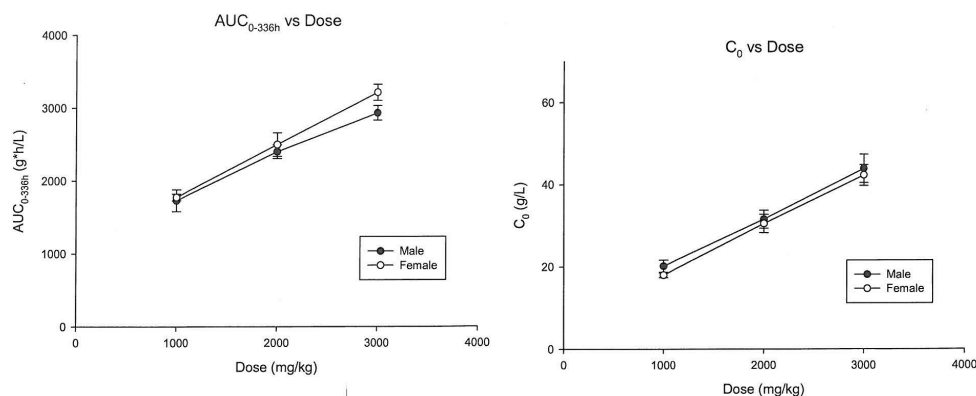
Results: Erythema and edema were observed in both sexes of all test article-treated groups; the clinical signs were resolved by day 2 and not correlated to microscopic findings upon histopathology. Reddish urine was observed in females at ≥ 2000 mg/kg on Day 1; they recovered on Day 2. Hematology changes such as increased mean corpuscular volume (MCV, high dose males), mean cell hemoglobin (MCH) and red cell width (RDW, both sexes at high dose) and hemoglobin distribution width (HDW, high dose females). These changes and reddish urine are likely due to hemolytic anemia that can result in animals treated with high dose of human IGIV and it is not correlated with hemolysis in humans. Other changes included increased total protein (TP) and reduced albumin/globulin (A/G) ratio in females at all doses and increased Cl^- and Na^+ in females and males respectively. The TP and A/G ratio were expected due to infusion of high levels of protein solution. The changes in ion ranges occurred without related findings in kidneys or upon urinalysis, thus are likely not related to test article administration. Upon necropsy and histopathological analysis, only sporadic findings were seen in n=1 male receiving middle dose and were not considered related to test article. One injection site thrombus was found in n=1 male receiving the high dose. No systemic thrombi were found. Thus, the local site thrombus is considered to be related to the high infusion rate and potential catheter damage and not test-article related.

TK analysis showed the expected concentration decline with time, with AUC and C_0 (C_{\max}) values showing dose-relationship (Table 3). The increase in systemic exposure to IgG was linear but less than dose proportional (Figure 1).

Table 3: AUC and C_{\max} (C_0) parameters in rats (from submission)

Group / (Dose)	Gender	AUC _{0-336h} (g*h/L)	Ratio	C_0 (g/L)	Ratio
G5 / 1000 mg/kg	Males	1732	1.0	20.2	1.0
	Females	1776	1.0	18.0	0.89
G6 / 2000 mg/kg	Males	2401	1.0	31.6	1.0
	Females	2499	1.0	30.5	1.0
G7 / 3000 mg/kg	Males	2931	1.0	43.9	1.0
	Females	3213	1.1	42.3	1.0

Figure 1. Dose proportionality of TK parameters in rats (from submission)



Conclusions

In the study, the highest dose, 3000 mg/kg, 3-10 times higher than the sought human dose can be considered NOAEL. The study supports the safety of this preparation in human patients.

Study Number: 32172

Study Title: Examination of GC5107 and Reference Items on Thrombogenic Risk in Rabbits after Intravenous Administration

Study Objective: To assess thrombogenicity of three batches of the test article in a Wessler in vivo thrombogenicity assay.

Performing Laboratory: (b) (4)

Study design: A GLP study where n=3 (b) (4) white rabbits/sex/group weighing 1.6-2.4 kg (M) and 1.6-2 kg (F) received one of three doses (500, 1000 and 2000 mg/kg) of one of three batches of the test article (total of 9 groups/sex), positive control (FEIBA 40 U), negative control (saline) and a marketed 10% IGIV product (KIOVIG) for a total of 12 groups/sex or n=36/sex in the study.

Prior to the injection of the test items, the animals were anesthetized (IV sodium pentobarbital (2.2 - 3.2 mL of a 3% Pentobarbital-Na/mL per animal)). A 1 to 2 cm length of both the external vena jugularis was freed from its surrounding structures and its tributaries were ligated. The test items were administered as a slow bolus injection into a marginal vein of the right ear. Within 25 seconds after completion of the injection, the previously exposed vena jugularis was gently ligated (on a length of approximately 1 - 2 cm). This ligated vein of the right segment remained in situ for 10 minutes. The segment was then removed from the animal, its content was emptied into a Petri dish containing 30 mL of a 5% sodium citrate solution and the contents of the dish were examined. The following scoring table was used (Table 4).

Results and conclusions: No thrombi of any kind were seen in the study (average score 0 ± 0) other than in positive control animals (average score 4 ± 0).

Table 4: Scoring for clots in Wessler test (from submission)

Score	Observation
0	no clot
0.5	a few macroscopic strands of fibrin are barely visible
1.0	a few macroscopic strands of fibrin
1.5	one or several thrombi ≤ 1.5 mm in length or diameter
2.0	one or several thrombi > 1.5 mm in length or diameter
2.5	several thrombi > 2 mm ≤ 3 mm in length or diameter
3.0	one large thrombus > 3 mm in length or diameter
3.5	two or more large thrombi > 3 mm in length or diameter
4.0	a single thrombus forming a cast of the isolated segment

Safety Pharmacology

Study Number: 15-MS-828

Study title: Safety Pharmacology Study: Effects of GC5107 on the Body Temperature and General Behavior of (b) (4) Mice after a Single Intravenous Dose

Performing laboratory: (b) (4)

Study objective: To assess the central nervous system function by measuring body temperature and general behavior in (b) (4) mice after a single intravenous dose of GC5107 (also known as (b) (4) test).

Study Design: A GLP study where n=10/sex/group ^{(b) (4)} mice weighing 32.56-37.20 g (M) and 25.73-30.88 g (F) received test article at doses of 0, 500, 1000, and 2000 mg/kg intravenously via the tail vein with a 26-gauge needle at a rate of 2 mL/min.

Outcome measures: Autonomic CNS measures (body temperature, lacrimation, piloerection, etc.), neuromuscular (posture, body tone, ptosis etc.), sensorimotor (touch response, pinna reflex, righting reflex etc.) and general behavior was observed pre-dose and 30, 60, 120, 240, 360 min and 24 hr post-dose and 7 days post-dose; body weight was measured on day 1, 2, 4 and 8 and overall toxicity of the study drug was assessed at necropsy. A list of the observations is shown in table 5.

Table 5: A listing of outcome measures for the CNS safety pharmacology study (from submission)

Parameters	Methods	Scores
(1) Body temperature	Measuring the body temperature (rectal temperature).	°C
(2) Catalepsy	Observing whether animal hanged down on the meshed cylinder (10 cm in diameter) moves with four feet within 8 seconds.	0: Absent/Normal 1: Present/Abnormal
(3) Loss of Traction	Observing whether animal hanged on the horizontal bar (30 cm high) falls within 5 seconds.	0: Absent/Normal 1: Present/Abnormal
(4) Tremors	Observing the tremors.	0: Absent/Normal 1: Present/Abnormal
(5) Convulsion	Observing the convulsion.	0: Absent/Normal 1: Present/Abnormal
(6) Exophthalmos	Observing the abnormal protrusion of the eyeball in the orbit.	0: Absent/Normal 1: Present/Abnormal
(7) Piloerection	Observing the status of hair erection.	0: Absent/Normal 1: Present/Abnormal
(8) Salivation	Observing the secretion of saliva.	0: Absent/Normal 1: Present/Abnormal
(9) Lacrimation	Observing the shedding tears.	0: Absent/Normal 1: Present/Abnormal
(10) Diarrhea	Observing the diarrhea.	0: Absent/Normal 1: Present/Abnormal
(11) Skin color	Observing the ear and tail color changes.	0: Normal 1: Abnormal
(12) Pinna reflex	Confirming reflex to stimulation on auricle.	0: Present/Normal 1: Absent/Abnormal
(13) Righting reflex	Rolling animal.	0: Instantly correct the posture back 2: Slowly correct the posture back 4: No reaction
(14) Tail elevation (straub tail)	Observing the degree of tail raising.	0: Tail is on the bottom 2: Tail is stiffened and elevated at an angle of 0-90 degrees 4: Tail is stiffened and elevated over an angle of 90 degrees
(15) Ptosis	Observing the drooping degree of the upper eyelid.	0: Normally eyes are open 2: Eyelids slightly lowered 4: Eyelids are closed

(16) Abdominal tone	Massaging the abdominal region of animals.	0: No resistance. Abdominal walls do not get back to normal
		2: Decreased resistance. Abdominal walls slowly get back to normal 4: Normal resistance. Abdominal walls quickly get back to normal 6: Increased resistance. Abdominal walls are very hard to be pressed 8: Extremely increased resistance like wooden board
(17) Locomotion	Observing the animal motion.	0: No movement despite of stimulus 2: Slow or decreased movement 4: Normal movement 6: Increased movement 8: Extremely increased or continuous movement
(18) Respiration rate	Observing the respiration rate and frequency.	0: Distinctly abnormal breathing and long breathing interval 1: Slow and difficult breathing, dyspnea 2: Moderately slow but regular breathing, bradypnea 4: Normal and regular breathing 8: Extremely rapid breathing, polypnea
(19) Death	Occurrence of death.	0: Absent/Normal 1: Present/Abnormal

Results

There were no deaths or overt clinical signs in any of the animals and no drug related toxicities observed in this study.

Study Number: 15-RH-830

Study title: Evaluation of Blood Pressure and Heart Rate after a Single-Intravenous Injection of GC5107 in

(b) (4) Rats

Performing laboratory: (b) (4)

Study objective: To assess the blood pressure and heart rate in rats after a single intravenous dose of GC5107.

Study Design: A GLP study where n=8/sex/group (b) (4) rats weighing 164.50-213.07 g (M) and 131.07-164.39 g (F) received 0, 500, 1000, and 2000 mg/kg test article IV in the tail vein via a catheter and an infusion pump (rate 0.25 ml/min). After dosing, animals were placed in restrainer for measuring the blood pressure and heart rate measured pre-dose and 30, 60, 120, 240, 360 minutes (min) and 24 hr post-dose. Negative control group received saline and underwent the same procedure as the test article groups.

Results: Females in low and middle dose had significantly higher systolic blood pressure (SBP), diastolic blood pressure (DBP) or mean arterial blood pressure (MAP) at some of the time points. There was no dose-response relationship in this sign. Full recovery was observed by 6 hours. Erythema and edema were seen in both males and females, there was no correlation between the high BP animals and those that exhibited erythema and edema.

Conclusions: A transient increase in BP was seen in females of low and middle dose but not those that received the high dose or in males that received any dose. Thus, the finding is considered sporadic and not related to test article administration.

Study Number: 16-RS-305

Study title: Safety Pharmacology Study: Effects of GC5107a on the Respiratory Rate and Tidal Volume of

(b) (4) Rats after a Single Intravenous Dose

Study objective: To assess the respiratory system by measuring respiratory rate and tidal volume in rats after a single intravenous dose of GC5107

Study design: Same design as the previous study, but with the following changes: only 2 animal/group were dosed and measured in one single day, the outcomes measured were respiratory rate and tidal volume at 30, 60, 120, 240, 360 minutes and 24 hours, using whole body plethysmography (b) (4) chamber for 10 minutes. A mean for each parameter were calculated, and the minute volume was computed for each time point.

Results and Conclusions: No differences in outcome measures between the control and test-article treated groups were observed in this study indicating no effects in respiratory parameters are expected following administration of GCC 10%.

Primary Pharmacodynamics

Study Number 12-ME-090N

Study Title: Effect of GC5107 in (b) (4) Mice Infected with *Streptococcus Pneumoniae*

Study Objective: To evaluate the effect of GCC 10% IGIV on (b) (4) mice infected with varying doses of *S. pneumoniae*.

Performing laboratory: (b) (4)

Study Design: 8-10 female (b) (4) mice aged 8 weeks and weighing 17-21 g, received test article GCC 10% IGIV (500 mg/kg), negative control (Human Albumin 20 %, 500 mg/kg), or reference control (Gammagard Liquid 10%, 500 mg/kg) intravenously using lateral tail vein. The following day (18-20 hours post administration), each group was challenged with 0.2×10^6 , 1×10^6 , 5×10^6 , 25×10^6 or 125×10^6 colony forming unit (cfu) *Streptococcus Pneumoniae* bacteria intravenously via tail vein; the groups and the doses are shown in Table 6. The survival rates, the survival times, and the lethal dose 50% (LD50), were calculated. The mice were monitored for a total of 14 days.

Results: Statistically significant increase in mean survival time (MST) was seen in IGIV groups compared to negative control. Specifically, animals receiving albumin had MST of 1.65 ± 0.10 days, those treated with GCC 10 % and Gammagard Liquid had MST of 7.70 ± 0.85 and 7.50 ± 0.87 days, respectively. Survival rates were 0% for animals receiving Albumin, and 100, 100, 30, 0, 0% of animals receiving either GCC 10% or Gammagard liquid, and after infection with concentrations of *Streptococcus pneumonia* 0.2, 1, 5, 25, 125 x 10^6 CFU, respectively.

Conclusions: GCC 10% preparation is effective in preventing *S. pneumoniae* infection in mice. The efficacy in this animal model is comparable to an approved IGIV 10% product.

Study Number 12-ME-598N

Study Title: Effect of GCC 10% IGIV in (b) (4) Mice Infected with *Klebsiella pneumoniae*

Study Objective: To evaluate the protective effect of GCC 10% against *Klebsiella pneumoniae*.

Study Design: The design was similar to the previous study with the challenge dose of the pathogen being 8, 40, 200, 1000 and 5000 x 10^6 CFU/animal.

Results:

Animals treated with Human Albumin 20% exhibited 75%, 38%, 0%, 0%, and 0% survival rate after being challenged with 8, 40, 200, 1000 and 5000 x 10^6 CFU *K. pneumoniae*, respectively. Animals that received GCC 10% had 100%, 80%, 40%, 30% and 0% survival rate and those dosed with Gammagard Liquid 10% had 90%, 70%, 70%, 30% and 0% survival rate against the given challenges. These animals showed a prolonged survival time compared to Human Albumin group. No statistically significant differences between the two IGIV treatments was found.

Conclusions: GCC 10% preparation is effective in preventing *K. pneumoniae* infection in mice. The efficacy in this animal model is comparable to an approved IGIV 10% product.

Table 6: Study Design

Group	<i>Streptococcus pneumoniae</i> conc. (10 ⁶ CFU)	Sex	Number of animal	Identification of animal	Volume (mL/kg)	Dose (%)	Test article
G1	0.2	F	8	1-8	15	3.3	Albumin Inj. 20 % 50 mL
	1	F	8	9-16	15	3.3	
	5	F	8	17-24	15	3.3	
	25	F	8	25-32	15	3.3	
	125	F	8	33-40	15	3.3	
G2	0.2	F	10	41-50	15	3.3	I.V.-Globulin SN Inj. 10 %
	1	F	10	51-60	15	3.3	
	5	F	10	61-70	15	3.3	
	25	F	10	71-80	15	3.3	
	125	F	10	81-90	15	3.3	
G3	0.2	F	10	91-100	15	3.3	10 % Gammagard Liquid
	1	F	10	101-110	15	3.3	
	5	F	10	111-120	15	3.3	
	25	F	10	121-130	15	3.3	
	125	F	10	131-140	15	3.3	

G1: Negative control

G2: Test article

G3: Reference control

Toxicologic Assessments of Impurities

Sponsor defined the analytical evaluation threshold (AET) based on the principles outlined in ICH M7 and PQRI-PODP recommendations, using (b) (4) µg/day as the most conservative safety concern threshold for any impurity with less than a lifetime exposure. Based on the daily maximum dose and the vial sizes, this threshold for GCC 10% was calculated to be (b) (4) µg/vial or (b) (4) µg/mL. After performing extractable-leachable studies, three organic compounds, namely two leachables ((b) (4)) and one extractable ((b) (4)) (Table 8), were identified as being present at levels higher than the AET (Table 7) at 6-month time point (T6). (The leachable study is ongoing to be completed at the 24-month time point.)

(b) (4)

Additional toxicologic assessments of these compounds were performed by a (b) (4) to determine permitted daily exposures (PDE) based on available toxicologic information in the literature. Considering the highest dose for GCC 10% (900 mg/kg) the PDE values were used to derive a maximum allowable concentration (MAC) for these compounds in the final drug product. The calculated PDE and MAC values are shown in Table 8. The MAC is lower than the amounts detected after performing a 6-month leachable study.

Table8: Acceptable Limits of Extractable/Leachable Compounds

(b) (4)

Conclusions: The organic compounds detected at 6 months do not represent a toxicologic concern.