I concur with this review. M. Serabian 3/07/18

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

BLA NUMBER:	STN #125659.000
DATE RECEIVED BY CBER:	August 14, 2017
DATE REVIEW COMPLETED:	March 7, 2018
PRODUCT:	Plasminogen (Human) Lyophilized powder for reconstitution Intravenous [Proprietary name, RYPLAZIM™]
APPLICANT:	ProMetic BioTherapeutics Inc.
PROPOSED INDICATION:	Replacement therapy in pediatrics and adults with congenital plasminogen deficiency
PHARM/TOX REVIEWER:	Cheauyun (Theresa) Chen
PHARM/TOX BRANCH CHIEF:	Mercedes Serabian
PRODUCT (CMC) REVIEWERS:	Alexey Khrenov, Ze Peng
CLINICAL REVIEWER:	Steve Winitsky
PROJECT MANAGER:	Pratibha Rana
DIVISION DIRECTOR:	Tejashri Purohit-Sheth
OFFICE DIRECTOR:	Wilson Bryan

EXECUTIVE SUMMARY:

Ryplazim™ is plasminogen derived from human plasma that is isolated and purified using the (b) (4) The nonclinical testing program for human plasminogen consisted of *in vivo* studies in healthy, immune competent rats: 1) pharmacokinetic (PK) assessment following single intravenous (IV) administration of human plasminogen in lyophilized (b) (4) formulation, and 2) toxicology assessment following daily IV administration of human plasminogen in (b) (4) for five consecutive days. No adverse findings were observed at the highest dose level administered (21.8 mg/kg/day). The plasma half-life in rats ranged from 3.7 to 7.1 hours, which is significantly different from the plasma half-life in humans, of approximately (b) (4). Although plasminogen knockout mice exist, *in vivo* pharmacology studies were not performed by the applicant due to the potential for development of cross-species anti-human antibodies. The applicant instead provided data from the scientific literature review to support the physiological role of human plasminogen in the treatment of Type I Plasminogen deficiency. Safety pharmacology, developmental and reproductive toxicology (DART), and genotoxicity studies with human plasminogen were not

conducted. In addition, the sponsor conducted limited nonclinical studies evaluating the toxicity of various resins and processing reagents used in the manufacturing process for Ryplazim™. Data generated from toxicity studies in rats, as well as *in vitro* genotoxicity assays, did not reveal any safety concerns.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:
Based on review of the pharmacology and toxicology data presented in STN #125659/0, there were no major nonclinical deficiencies identified in this submission. Following review of the submitted data, there are no requests for further nonclinical testing of Ryplazim™ at this time.

Formulation and Chemistry:
Plasminogen is a naturally occurring protein that is synthesized by the liver and circulates in blood. Ryplazim™ is derived from human plasma using a (b) (4) . Per the submission originally the (b) (4) . The lyophilized plasminogen is formulated in (b) (4) NaCl, (b) (4) Sodium Citrate, (b) (4) Sucrose, and (b) (4) Glycine at pH (b) (4), and is (b) (4) to generate a lyophilized drug product, that is supplied as a sterile solution for IV administration.

Abbreviations

A/G	Albumin/Globulin ratio (calculated)
ALB	Albumin
ALK or ALP	Alkaline phosphatase
ALT	Alanine Aminotransferase
APTT	Activated Partial Thromboplastin time
AST	Aspartate Aminotransferase
AUC	Area under the curve
AUC_{last}	Area under the curve from time zero to the last quantifiable concentration
$AUC_{0\text{-}inf}$	Area under the curve from time zero to infinity
B	Basophils
BUN	Blood Urea Nitrogen
BW	Body weight
C_0	Apparent concentration at time zero
CA	Calcium
CHOL	Cholesterol
C_{last}	Last quantifiable concentration
CL	Chloride
Cl	Apparent plasma clearance
C_{max}	Maximum observed concentration
CREAT	Creatinine
DMSO	Dimethyl sulfoxide

DS	Drug substance
E	Eosinophils
EACA	ε-amino caproic acid
(b) (4)	
g	Gram
(b) (4)	
GLOB	Globulin (calculated)
GLU	Glucose
HCT	Hematocrit
HDW	Hemoglobin Distribution Width
GLP	Good Laboratory Practice
HGB	Hemoglobin
hr	Hour
(b) (4)	
IP	Intraperitoneal
ISFs	Impurity safety factors
IV	Intravenous
K	Potassium
kg	Kilogram
L	Lymphocytes
(b) (4)	
Lyo	Lyophilized
LUC	Leukocytes
M	Monocytes
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Cell Volume
mg	Milligram
ml	Milliliter
MPV	Mean Platelet Volume
N	Neutrophils
NA	Sodium
(b) (4)	
(b) (4)	
PCV	Packed cell volume (calculated)
pI	Isoelectric point
PK	Pharmacokinetics
PHOS	Phosphorus
Pg	Plasminogen
PLT	Platelets
(b) (4)	
PT	Prothrombin Time
RDW	Red cell distribution width
Retic	Reticulocyte count

RET ABS	Absolute Reticulocytes (calculated)
RBCs	Red Blood Cells
ROA	Route of administration
SC	Subcutaneous
SD	Standard deviation
$T_{1/2,e}$	Elimination half-life
T-BIL	Total Bilirubin
TK	Toxicokinetic
T_{last}	Time of last quantifiable concentration
T_{max}	Time when the maximum concentration is observed
(b) (4)	
TP	Total Protein
TRIG	Triglycerides
Tris	Tromethamine
V_z	Apparent volume of distribution
WBCs	White Blood Cells

Related Files:
IND #16186 - Plasminogen (Human); for treatment of Type I Plasminogen deficiency or hypoplasminogenemia held by ProMetic BioTherapeutics Inc; Phase 2; ACTIVE

CBER MF (b) (4) **– MASTER FILE TYPE V -** (b) (4) Final Product Testing; Sponsor - (b) (4)

CBER MF (b) (4) **- MASTER FILE TYPE V-** (b) (4) , Facility and process for preparing (b) (4) stoppers for the pharmaceutical industry; Sponsor - (b) (4) .

CDER MF (b) (4) **- MASTER FILE TYPE III** - glass type (b) (4) (b) (4) (b) (4) borosilicate (b) (4) vial; Sponsor - (b) (4)

CDER MF (b) (4) **- MASTER FILE TYPE III -** (b) (4) for Human drugs and biologics packaging; Sponsor - (b) (4)

CDER MF #(b) (4) **MASTER FILE TYPE III -** (b) (4) for Human drugs and biologics packaging; Sponsor - (b) (4)

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INTRODUCTION

Hypoplasminogenemia is a rare autosomal recessive disease. The prevalence is estimated at 1.6/1,000,000[1]. The disease manifests clinically with fibrous depositions on mucous membranes throughout the body, resulting in a variety of conditions affecting the ears, sinuses, tracheobronchial tree, genitourinary tract, and gingiva. One of the well-described and most common symptoms is ligneous conjunctivitis, consisting of thick, woody growths on the conjunctiva of the eye triggered by local irritation, injury, infection, or eye surgery, which can result in blindness. Other symptoms include hydrocephalus; malformations of the gastrointestinal tract, renal tract, and cerebellar vermis; and impaired wound healing. The prognosis for plasminogen-deficient individuals depends on the site and extent of the lesions. Currently, there is no approved therapy in the US for the treatment of hypoplasminogenemia.

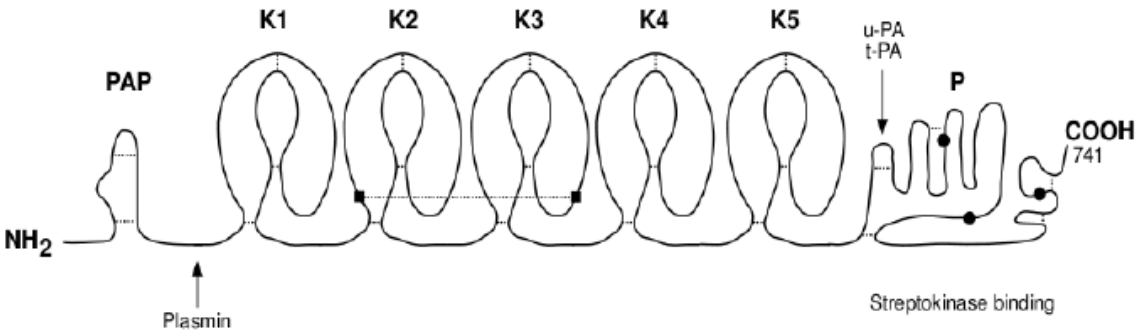
Native plasminogen is produced in two main forms: Glu- and Lys-Plasminogen, which are named for the N-terminal amino acid of either glutamic acid or lysine, respectively. Glu-plasminogen has a circulating half-life of 2.25 days, while Lys-plasminogen has a half-life of 0.8 days[2]. Ryplazim™ is lyophilized form of Glu-plasminogen that is purified using (b) (4) The longer half-life of Glu-plasminogen provides the advantage of a longer half-life, thus intervals between dosing, compared to Lys-plasminogen.

Plasminogen is a zymogen of plasmin (Figure #1). This protein contains 791 amino acids with a molecular weight of approximately 90 kD and an isoelectric point (pI) of approximately 7.0, although differential glycosylation and/or removal of the N-terminal activation peptide can result in a pI range of 6.2 to 8.0. It is a single-chain protein with 24 intra-chain disulfide bridges, 5 kringle domains (involved in the binding to fibrin and to the inhibitor α2-antiplasmin), a serine protease domain (P), and an activation peptide (AP) consisting of the first 77 amino acids. There is one N-linked glycosylation site and one O-linked site, although a second O-linked site has

[1] Tefs K, et al. (2006) Molecular and clinical spectrum of type I plasminogen deficiency: a series of 50 patients. Blood 108:3021-3026.
[2] Mehta R and Shapiro AD (2008) Plasminogen deficiency. Haemophila 14, 1261-1268.

been identified[3]. Approximately 70% of the plasminogen in circulation is only O-linked glycosylated, while the remaining 30% contains both N- and O-linked sugars.

Figure #1: Schematic of the Kringle Domains of Plasminogen

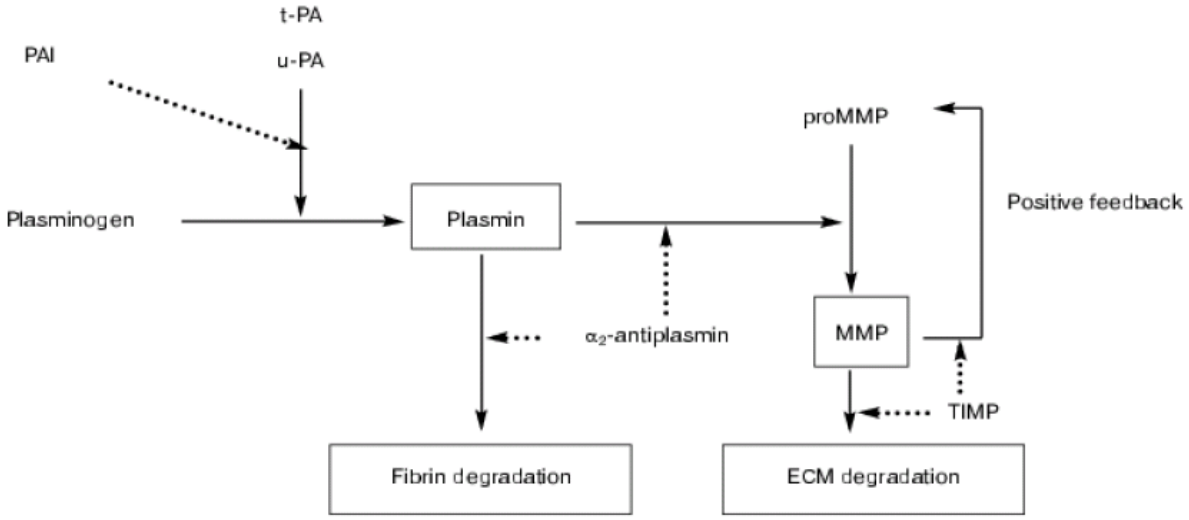


Source: Module 2.4 in the BLA

Ryplazim™ is plasma-derived human plasminogen. Following synthesis in the liver, this proenzyme circulates in the blood, where it is converted to plasmin by tissue-type plasminogen activator (t-PA) or urokinase plasminogen activator (u-PA). Plasmin then degrades fibrin and converts latent matrix metalloproteinases (pro-MMPs) into active MMPs, which in turn further degrade extracellular matrix (ECM) as part of the tissue healing/remodeling process[4] (Figure #2). Plasmin is involved in the lysis of clots and clearance of extravasated fibrin[5]. Activated plasminogen is also involved in wound healing, cell migration, tissue remodeling, angiogenesis, and embryogenesis[6].

[3] Goldberg HJ, et al. (2006) Posttranslational, reversible O-glycosylation is stimulated by high glucose and mediates plasminogen activator inhibitor-1 gene expression and Sp1 transcriptional activity in glomerular mesangial cells. Endocrinology 147(1):222-31.
[4] Lijnen HR and Collen D (1999) Matrix metalloproteinase system deficiencies and matrix degration. Thromb Haemost. 82:837-45.
[5] Collen D, et al. (1975) Human Plasminogen: in vitro and in vivo evidence for the biological integrity of NH2-terminal glutamic acid plasminogen. Thromb Res 7:515-529.
[6] Castellino FJ and Ploplis VA (2005) Structure and function of plasminogen/plasmin system. Thromb Haemost 93:647-54.

Figure #2: Fibrin Degradation via Plasminogen



Source: Module 2.4 in the BLA

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

In Vitro Studies
No *in vitro* pharmacology studies were conducted.

In Vivo Studies
Although plasminogen knockout (Plasminogen$^{-/-}$) mice exist, no *in vivo* pharmacology studies were conducted. Per the applicant, systemic administration of human plasminogen in animals will result in the development of cross-species anti-human antibodies, thus translation of the resulting animal data to humans will not be relevant, therefore making use of this mouse model impractical. The applicant instead cited several scientific publications using this murine model to support the physiological role of plasminogen in the treatment of plasminogen-deficiency in humans. These publications are summarized below:

Review of selected publications that support proof-of-concept

1) Bugge et al (1995)[7] developed a plasminogen-deficient homozygous (Plasminogen$^{-/-}$) knockout mouse model (generated by deletion of 9-kb DNA within the plasminogen gene), and used this model to study the physiological role of plasminogen in development, hemostasis, and reproduction. The results showed that the mice survived to

[7] Bugge TH, et al. (1995) Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. Genes and Development 9:794-807.

adulthood and were fertile. However, the mice were predisposed to severe thrombosis and exhibited thrombotic lesions in the liver, stomach, rectum, lungs, pancreas, and other tissues beginning at a young age (5 weeks old). Histological examinations revealed fibrin deposits in the liver and ulcerated lesions in the gastrointestinal tract between 5 and 21 weeks of age. The publication suggests that plasminogen plays a critical role in fibrinolysis and hemostasis.

2) Ploplis et al (1995)[8] studied the effects of plasminogen disruption on thrombosis, growth, viability, and fertility in a plasminogen-deficient mouse model (generated by replacing the murine plasminogen gene with a neomycin phosphotransferase expression cassette). The results showed that the mice developed spontaneous fibrin deposition due to impairment of thrombolysis, suffered retarded growth, and had reduced fertility and survival. In addition, approximately 20% of the mice developed rectal prolapse by 7.4 ±0.6 weeks of age, while healthy controls and heterozygous littermates did not display these findings. Histological examinations revealed fibrin deposits in the liver, lungs, and stomach (linked to gastric ulcers) at 6-12 weeks of age. The publication suggests that the plasminogen system plays an important role in fibrinolysis and hemostasis.

3) Bugge et al. (1996)[9] studied the effects of homozygous plasminogen and fibrinogen deficiency in plasminogen/fibrinogen double-deficient (plasminogen$^{-/-}$ and fibrin$^{-/-}$) mice. Plasminogen deficient mice displayed abnormalities (severe body weight loss, life span reduction, fibrin deposit in liver, ulceration in gastrointestinal tract, etc.). In contrast, the double-deficient mice displayed a phenotype similar to that of healthy control animals. The publication supports the important physiological role of plasminogen in fibrinolysis.

4) Lijnen et al (1996)[10] studied single bolus IV administration of purified mouse plasminogen (1 mg/mouse) in the 10- to 17-week-old plasminogen knockout mice. The results showed significant plasminogen-mediated effects including, restoration of thrombolytic potential and reduction of endogenous fibrin deposition in the liver at 24 hours (hrs) post-dose.

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies with human plasminogen were conducted.

[8] Ploplis VA, et al. (1995) Effects of disruption of plasminogen gene on thrombosis, growth, and health in mice. Circulation 92:2585-93.
[9] Bugge TH, et al. (1996) Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. Cell 87:709-719.
[10] Lijnen HR, et al. (1996) Restoration of thrombolytic potential in plasminogen-deficient mice by bolus administration of plasminogen. Blood 88:870-876.

PHARMACOKINETIC STUDIES

Summary List of Pharmacokinetic (PK) Studies

One PK study was conducted.

In Vivo Study

Study Number	Study Title/Publication Citation	Report Number
1	Pharmacokinetic Studies of Plasminogen in Rats: Assessment of Different Routes of Administration (b) (4) Lyophilized Formulations	PDR-5026.035

Comment:

- As previously noted, the human plasminogen (b) (4) However, (b) (4) was never administered to humans; the (b) (4) lyophilized formulation due to (b) (4) issues.

Study #1

Report Number		PDR-5026-035
Date Report Signed		August 2, 2017
Title		Pharmacokinetic Studies of Plasminogen in Rats: Assessment of Different Routes of Administration (b) (4) Lyophilized Formulations
GLP Status		No
Testing Facility		Not provided
Objectives		• (Part 1) To determine the PK profile of multiple doses of Plasminogen drug product (DP) in rats when given a single dose by the IV, intraperitoneal (IP) or subcutaneous (SC) routes of administration (ROAs). • (Part 2) To compare the PK profiles of a (b) (4) lyophilized formulation
Study Animals	**Strain/Breed**	(b) (4)
	Species	Rat
	Age	Not provided
	Body Weight	Not provided
	Gender	Males only
	#/group	3/group
	Total #	33
Test Articles		• (human) Plasminogen Lot (b) (4) in a (b) (4) • (human) Plasminogen Lot #2388-101 in a lyophilized formulation; reconstituted in (b) (4) NaCl, (b) (4) Na Citrate, (b) (4) sucrose, (b) (4) glycine (b) (4) **Note:** This is the product administered in the Phase 1 clinical trial
Control Article		Not included
Route of Administration (ROA)		IV, IP, or SC injection
Study Groups and Dose Levels		**Study 1** – Dose levels and ROA ((b) (4) formulation)

Group	Dose level (mg/kg)	ROA
1	1	IV
2	6	IV

		3	18	IV
		4	18	IP
		5	18	SC
		Study 2 – PK comparison between the (b) (4) lyophilized formulation - single IV injection		
		Group	**Dose level (mg/kg)**	**Formulation**
		1	2	(b) (4)
		2	6	(b) (4)
		3	18	(b) (4)
		4	2	Lyophilized
		5	6	Lyophilized
		6	18	Lyophilized
Dosing Regimen		Single		
Randomization		Not provided		
Description of Masking		Not provided		
Scheduled Sacrifice Time Points		Not provided		
Study Parameter	**PK assessment**	Blood samples were collected from the saphenous vein at 0, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 hrs post-dose. The concentrations of human plasminogen were determined by a fully validated (b) (4)		

Note:

- The (b) (4) used was validated in study Report No. MC14I-0015 (provided in Module 4.2.2.1).

Key Results:

- Part 1 –
  - The PK profile of plasminogen following IV administration was dose-dependent. The plasminogen mean half-life was comparable at 6 and 18 mg/kg, but approximately two-fold lower at 1 mg/kg. The clearance of plasminogen at 1 mg/kg was also about two-fold less compared to the profile following administration of 6 and 18 mg/kg (Table #1).

Table #1: Summary of plasminogen PK parameters following IV administration in rats

Dose (mg/kg)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng/mL)	$T_{1/2}$ (h)	CL (mL/h/kg)
1	42.2±5.9	675.2±156.5	10±1.2	25.7±6.8
6	253.1±8.9	1642.0±216.1	4.7±0.2	61.6±8.3
18	702.1±76.9	5380.0±464.7	5.3±0.6	56.0±5.0

Source: Report No. PDR-5026-035 in the BLA

  - IP injection of plasminogen resulted in mean C_{max} and AUC levels that were higher than the levels following IV and SC administration. The mean half-life was lower compared to values obtained following IV and SC administration (Table #2).

Table #2: Comparison of PK parameters following 18 mg/kg administration of plasminogen by IV, IP, or SC injection

Route	C_{max} (ng/mL)	AUC $_{0-\infty}$ (ng/mL*h)	T ½ (h)
IV	702.1±76.9	5380.0±464.7	5.3 ± 0.6
IP	845.0 ± 93.0	13788.7 ± 761.0	6.7 ± 0.6
SC	94.5 ± 15.1	2680.6 ± 395.1	7.5 ± 0.8

Source: Report No. PDR-5026-035 in the BLA

- Part 2 – Human plasminogen in (b) (4) lyophilized formulation demonstrated similar PK parameters in rats following single IV administration (Table #3).

Table #3: Comparison of PK parameters of lyophilized (b) (4) formulation of human plasminogen in rats following single IV administration

	Pg Lyo (mg/kg)			Pg (b) (4) (mg/kg)		
Dose	**2**	**6**	**18**	**2**	**6**	**18**
AUC (ng x hr/mL)	263.1	1340.2	3615.6	215.4	1168.3	3102.2
T ½ (h)	5.6	5.4	5.7	4.9	5.7	4.9
Cmax (ng/mL)	32.7	171.1	441.5	30.8	142.5	451.9

Source: Report No. PDR-5026-035 in the BLA

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

One toxicology study with human plasminogen was conducted.

Toxicology Study:

Study Number	Study Title/Publication Citation	Report Number
2	A 5-Day Repeat-Dose Toxicity Study of Human Plasminogen in Rats with a 2-Week Recovery	0440RP78.002

Study #2

Report Number		0440RP78.002
Date Report Signed		June 29, 2015
Title		A 5-Day Repeat-Dose Toxicity Study of Human Plasminogen in Rats with a 2-Week Recovery
GLP Status		Yes
Testing Facility		(b) (4)
Objective		To evaluate the toxicity and toxicokinetic (TK) profiles of human plasminogen when administered by IV injection to (b) (4) rats once daily for a minimum of 5 consecutive days, followed by a 2-week recovery
Study Animals	**Strain/Breed**	(b) (4)
	Species	Rat

<table>
<tr><td rowspan="4"></td><td>Age</td><td colspan="3">8-9 weeks old</td></tr>
<tr><td>Body Weight</td><td colspan="3">Males (242 to 271 g); females (170 to 203 g)</td></tr>
<tr><td>#/sex/group</td><td colspan="3">15/sex/group (toxicology groups); 3 or 6/sex/group (TK groups)</td></tr>
<tr><td>Total #</td><td colspan="3">81/sex</td></tr>
<tr><td colspan="2">Test Article</td><td colspan="3">Human Plasminogen (Lot/Batch No (b) (4) in (b) (4)</td></tr>
<tr><td colspan="2">Control Article</td><td colspan="3">Vehicle ((b) (4) NaCl in (b) (4) Citrate buffer (b) (4) (Lot/Batch No. (b) (4)</td></tr>
<tr><td colspan="2">Route of Administration</td><td colspan="3">IV injection via the lateral tail vein (3.46 mL/kg)</td></tr>
<tr><td colspan="2" rowspan="14">Study Groups and Dose levels</td><td colspan="3"><u>Toxicology Groups</u></td></tr>
<tr><td>Group</td><td>Dose level (mg/kg/day)</td><td>No/group</td></tr>
<tr><td>1</td><td>0</td><td>15/sex</td></tr>
<tr><td>2</td><td>1.2</td><td>15/sex</td></tr>
<tr><td>3</td><td>7.3</td><td>15/sex</td></tr>
<tr><td>4</td><td>21.8*</td><td>15/sex</td></tr>
<tr><td colspan="3"><u>TK Groups</u></td></tr>
<tr><td>Group</td><td>Dose level (mg/kg/day)</td><td>No/group</td></tr>
<tr><td>5</td><td>0</td><td>3/sex</td></tr>
<tr><td>6</td><td>1.2</td><td>6/sex</td></tr>
<tr><td>7</td><td>7.3</td><td>6/sex</td></tr>
<tr><td>8</td><td>21.8*</td><td>6/sex</td></tr>
<tr><td colspan="3">Note: Per the applicant, 21.8 mg/kg/day is the maximum feasible dose based on the concentration and injection volume of the test article.</td></tr>
<tr><td colspan="2">Dosing Regimen</td><td colspan="3">Repeat administration once daily for 5 consecutive days, beginning at Day 1</td></tr>
<tr><td colspan="2">Randomization</td><td colspan="3">Based on body weights (BWs)</td></tr>
<tr><td colspan="2">Description of Masking</td><td colspan="3">Not provided</td></tr>
<tr><td colspan="2">Scheduled Sacrifice Time Points</td><td colspan="3">Days 6 (n=10/sex/group) and 20 (n=5/sex/group) post-dose</td></tr>
<tr><td rowspan="2">Study Parameters</td><td>Mortality</td><td colspan="3">Twice daily</td></tr>
<tr><td>Clinical Observations</td><td colspan="3">Prior to dosing, immediately following dosing, approximately 1-2 hours post-dose, and daily thereafter until sacrifice.</td></tr>
</table>

	Body Weights (BWs)	At randomization and daily prior to each administration (Days 1-5), and weekly thereafter (Days 12 and 19).
	Food Consumption	Daily during Days 1-5 and weekly thereafter (Days 13 and 19) (quantitative)
	Clinical Pathology	• Hematology[11], erythrocyte morphology, coagulation[12], chemistry[13]. Blood was collected after overnight fasting and before sacrifice (n=10/sex/group at Day 6 and 5/sex/group at Day 20) • Urinalysis[14] (Samples were collected overnight prior to sacrifice)
	Postmortem Analyses	• Necropsy (gross pathology) • Organ weights[15] • Histopathology[16]
	TK	0.5, 1, 4, 6, 8, and 24 hrs post-dose at Days 1 and 5

Key Results:

- There were no unscheduled deaths.

- There were no test article-related abnormal findings for clinical observations, BWs, food consumption, clinical pathology, organ weights, gross pathology or histopathology parameters.

- TK - A dose-dependent TK profile was observed. The exposure and elimination profiles of the human plasminogen were similar after both single and repeat IV administration. The mean plasma half-life ranged from 3.7 to 7.1 hrs and the clearance ranged from 3.6 to 6.9 ml/hr/kg. No significant differences between the sexes were observed with regards to exposure or elimination, although male rats had a slightly higher plasminogen exposure based on C_{max} and AUC values, and slightly lower clearance, compared to female rats (Table #4).

[11] Hematology parameters: RBCs, WBCs, MCH, MCHC, MCV, PLT, HCT, HGB, and Retic.
[12] Coagulation parameters: APTT and PT.
[13] Chemistry parameters: ALT, ALB, A/G, ALP, AST, CA, CL, CHOL, CREAT, GLOB, GLU, PHOS, K, NA, T-BIL, TP, TRIG, and BUN.
[14] Urinalysis parameters: Specific gravity, pH, protein, glucose, ketone, urobilinogen, appearance/color, bilirubin, blood, leukocytes, nitrites, and microscopic examination of formed elements.
[15] Organ weights for the following tissues: Adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, uterus with cervix, and thyroids/parathyroids.
[16] Histopathology for the following tissues: Aorta, heart, salivary gland(s), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, liver, trachea, larynx, lung with mainstem bronchus, sternum with bone marrow, thymus, spleen, lymph node- mandibular, mesenteric lymph nodes, gut-associated lymphoid tissue, kidneys, urinary bladder, ovaries, uterus, cervix, vagina, testes, epididymides, prostate, seminal vesicles, adrenals, pituitary, thyroid/parathyroid, skin, mammary gland, skeletal muscle (thigh), femur with articular surface, eye with optic nerve, sciatic nerve, brain, spinal cord (cervical, midthoracic, lumbar), lacrimal glands, injection site(tail), and gross findings.

Table #4: Toxicokinetic parameters for plasminogen in rat plasma following single intravenous dose of plasminogen (Day 1) and following five consecutive daily intravenous doses of plasminogen (Day 5) to male and female (b) (4) rats

Group #	Day	Sex	Dose Level (mg/kg/day)	C_0 (µg/ml)	C_{max} (µg/ml)	T_{max} (hr)	C_{last} (µg/ml)	T_{last} (hr)	AUC_{last} (µg*hr/ml)	AUC_{0-inf} (µg*hr/ml)	$T_{1/2,e}$ (hr)	Cl (ml/hr/kg)	V_z (ml/kg)
6	1	F	1.2	18.5	26.0	1	0.337	24	172	174	3.8	6.9	37.8
7	1	F	7.3	161	162	1	4.59	24	1166	1198	4.8	6.1	41.9
8	1	F	21.8	667	564	0.5	12.4	24	3931	4008	4.4	5.4	34.3
6	1	M	1.2	37.2	31.2	0.5	1.41	24	246	257	5.6	4.7	37.5
7	1	M	7.3	159	170	1	7.07	24	1459	1510	5.1	4.8	35.3
8	1	M	21.8	623	657	1	23.1	24	5237	5404	5.0	4.0	29.0
6	5	F	1.2	37.2	32.4	0.5	7.80	8	140	179	3.7	6.7	35.5
7	5	F	7.3	221	189	0.5	6.52	24	1305	1354	5.3	5.4	41.3
8	5	F	21.8	699	607	0.5	9.86	24	4103	4161	4.0	5.2	30.5
6	5	M	1.2	31.5	33.2	1	1.41	24	244	256	5.7	4.7	38.6
7	5	M	7.3	219	193	0.5	8.05	24	1550	1610	5.2	4.5	34.3
8	5	M	21.8	782	706	0.5	52.2	24	5492	6010	7.1	3.6	37.2

Source: Report No. 0440RP78.002 in the BLA

Developmental and Reproductive Toxicology Studies:

No developmental and reproductive toxicology studies were conducted.

Genotoxicity Studies:

No genotoxicity studies were conducted with Ryplazim™. However, several genotoxicity studies were conducted on some of the manufacturing resin components. Summaries of these studies are presented later in this review memo.

Carcinogenicity/Tumorigenicity Studies:

No carcinogenicity/tumorigenicity studies were conducted.

Other Safety/Toxicology Studies:

Nonclinical Evaluation of Manufacturing (b) (4) and Processing Reagents - Summary List of Toxicology Studies

Study Number	Study Title/Publication Citation	Report Number
3	Analysis of Extractables from Various (b) (4)	104-000-001
4	Analysis of Extractables from Various (b) (4)	104-000-002.v2
5	(b) (4) : Single Dose Subcutaneous Toxicity Study in the Rat	1683-15-D614
6	(b) (4) : 28 Day Subcutaneous Toxicity Study in the Rat	1683/017-D6154

Study Number	Study Title/Publication Citation	Report Number
7	(b) (4) Maximum Tolerated Dosage Study by Intravenous (bolus) Administration to (b) (4) Rats **Note:** (b) (4)) is no longer used in the (b) (4) manufacturing process for Ryplazim™. Instead, (b) (4) is used. **Therefore, this study report was not reviewed.**	AFY-010-024555
8	(b) (4) Toxicity at Maximum Tolerated Repeated Intravenous (bolus) Administration in Rats	116-005
9	(b) (4) : Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma (b) (4) Cells (MLA) using the (b) (4)	1683-12-D6183
10	(b) (4)	1683-13-D6171
11	(b) (4)	AFY-016-052992
12	Toxicological Studies - QSAR Summary (Issue 1) **Note:** This study report is identical to pages 10-36 of Report Number 'PBL/R22/PC3880 PC3890 PC3906 PC3125 and PC3850'. **Therefore, this study report was reviewed under Study #13.**	AEA-R-ED 59924
13	Summary Report for Structural Relationship Analysis of (b) (4) Studies	PBL/R22/PC3880 PC3890 PC3906 PC3125 and PC3850

Human plasminogen is purified from human source plasma using the (b) (4) depicted in Figure 3. (b) (4)

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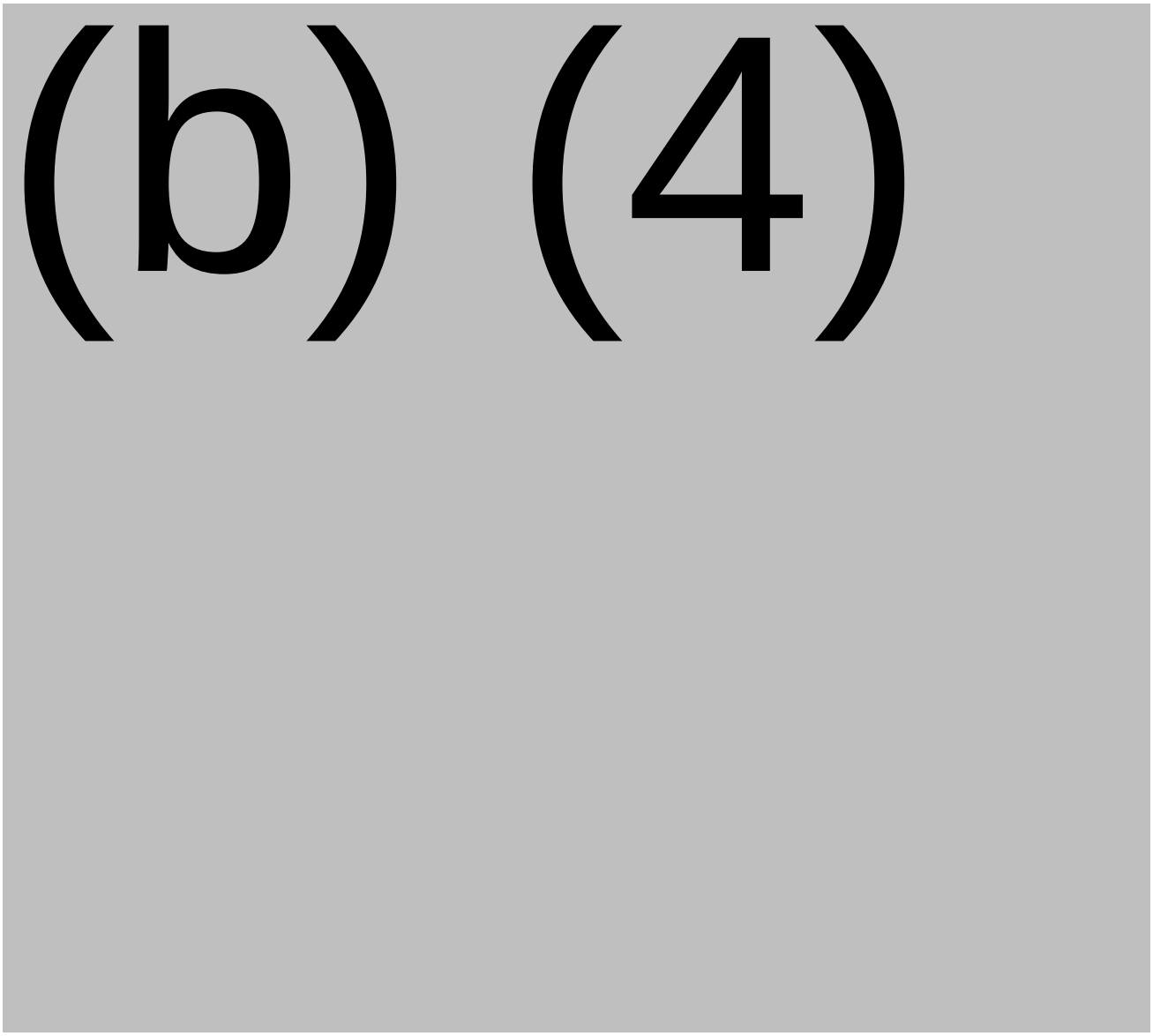
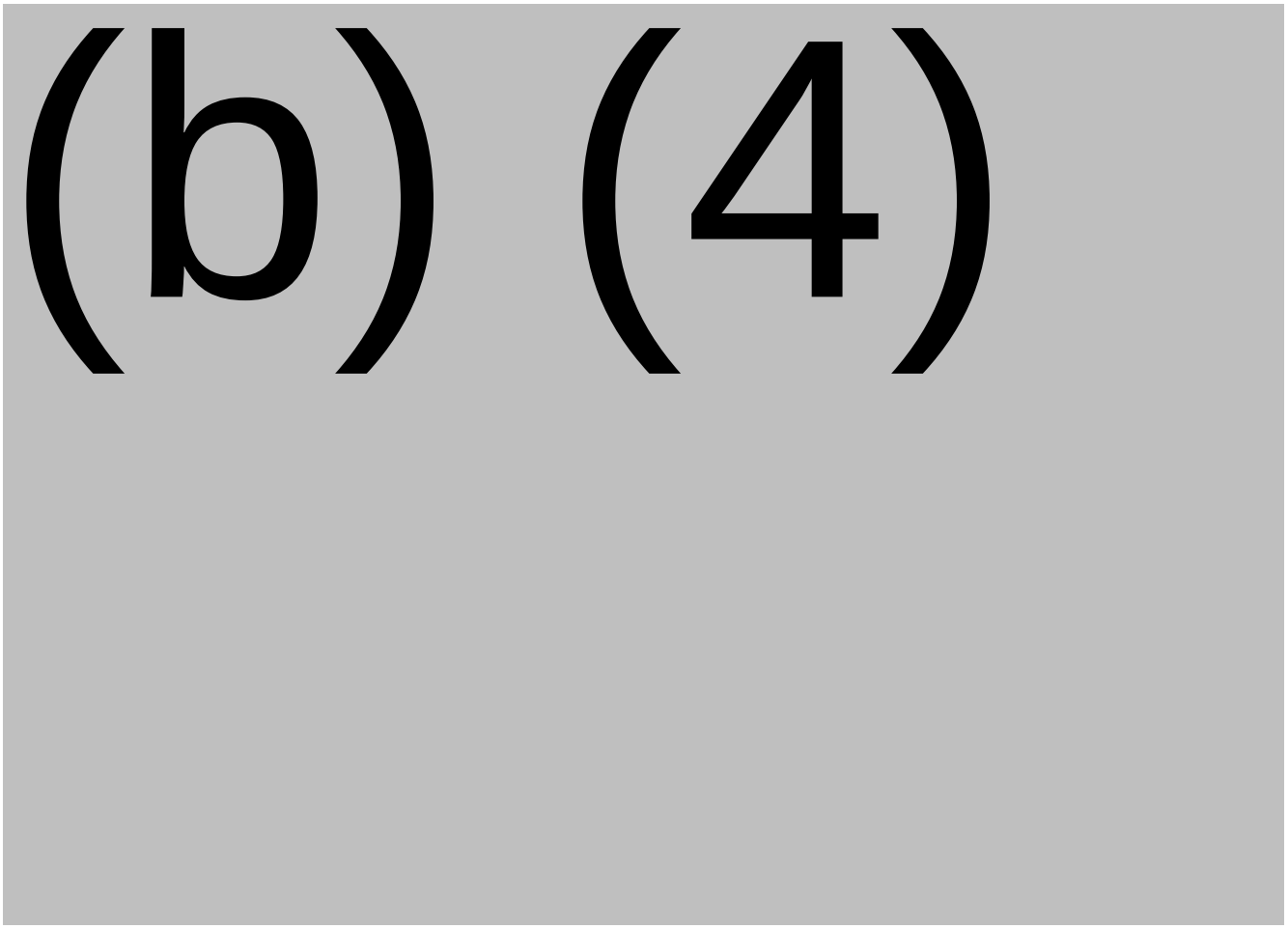


Comment:

- The chemical structure of the (b) (4) (b) (4) was inconsistently presented in: 1) Report 117 R20-270814 (Figure #4), 2) Report PG_R-0027.01 (Figure #5), and 3) Report PBL/R22/PC3880 (Figure #6). In response to FDA's information request sent via email communication on 03/01/18, the applicant stated that the (b) (4) structure depicted in Figure #5 was the material tested in nonclinical Studies #8 and #11 (Amendment #014, 03/06/18). This is the (b) (4) used in the manufacturing process for the plasminogen drug substance.

13 pages have been determined to be not releasable: (b)(4)



APPLICANT'S PROPOSED LABEL

Section 13 ('Nonclinical Toxicology') should be revised with the appropriate wording to accurately reflect the available nonclinical data.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be adequately addressed in the labeling. The nonclinical data support approval of the license application

KEY WORDS/TERMS

Plasminogen, Ryplazim™, (b) (4) rats, pharmacokinetics, toxicity, genotoxicity