

MEMORANDUM



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

Pharmacology / Toxicology Review Memorandum

Date: 9-18-2007
From: Paul W. Buehler
Through: Abdu Alayash, Basil Golding and Susan Abbondanzo
Supervisor Concurrence:

Note: I approve this review but not as an expert in the field

To: Mark Shields and Roman Drews
Subject: BLA 125248 Thrombin (Recombinant)
Sponsor: ZymoGenetics
Receipt Date: 12-28-2006
Final Review : 9-18-2007

Recommendation: Recombinant human thrombin (5000-IU vials rhThrombin) for topical use to control surgical bleeding in conjunction with an absorbable gelatin sponge is approvable from a pharmacology and toxicology perspective.

Proposed Indication: Recombinant human thrombin (rhThrombin) is being proposed for use as an adjunct to hemostasis when control of bleeding by conventional surgical techniques is not sufficient or practical. rhThrombin is intended for topical use in conjunction with an absorbable gelatin sponge for hemostasis during surgery.

Proposed Dose and Administration: Dosing will depend on the size and number of bleeding sites. All doses are prepared from 5000 IU vials (number determined by clinician) reconstituted with 5 mL sterile saline (1000 IU/mL).

The amounts of study drug used in the clinical trials supporting this submission are listed in the table below.

Volume of rhThrombin used in clinical trials:

Surgery	Volume ¹ (mL)	bThrombin (N=206)	rhThrombin (N=205)	Total (N=411)
Overall	n	205	205	410
	Mean (SD)	11.7 (8.3)	11.6 (8.4)	11.7 (8.3)
	Median	10.0	10.0	10.0
	Min, Max	1, 40	1, 48	1, 48
SPINE	n	61	61	122
	Mean (SD)	7.7 (7.2)	8.6 (9.1)	8.2 (8.2)
	Median	5.0	5.0	5.0
	Min, Max	1, 30	1, 48	1, 48
LIVER	n	63	62	125
	Mean (SD)	18.0 (9.7)	17.2 (9.2)	17.6 (9.4)
	Median	20.0	16.5	19.0
	Min, Max	9, 40	5, 40	5, 40
PAB	n	43	44	87
	Mean (SD)	10.4 (5.1)	10.3 (4.7)	10.3 (4.9)
	Median	10.0	10.0	10.0
	Min, Max	1, 28	4, 28	1, 28
AV GRAFT	n	38	38	76
	Mean (SD)	9.0 (2.6)	8.8 (3.5)	8.9 (3.1)
	Median	10.0	9.0	9.0
	Min, Max	4, 14	3, 20	3, 20

*Spine=Spinal Surgery; Liver=Hepatic Resection Surgery; PAB=Peripheral Arterial Bypass; AV Graft=Arteriovenous Graft

Note: Column header counts are the number of randomized subjects who received blinded study drug at one of six bleeding site types.

1 Volume = Amount provided - Amount Remaining

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Toxicology studies supporting these doses can be derived from study **RES-10783**

“Pharmacokinetics of [¹²⁵I]-rhThrombin after application of an [¹²⁵I]-rhThrombin-saturated Gelfoam-100 sponge in a liver wound model in female New Zealand white rabbits”. In this study the % absorption from a severe wound model was determined to be 0.37%. In combination with study **ZGI 1499-005** “A 6-week immunogenicity study of rhThrombin administered by SC injection to cynomolgus monkeys” was safe at a dose of 346 U/kg. The highest exposure level in animal studies was determined to be approximately 9.25 fold higher than the estimated maximum clinical exposure following topical wound site application.

Drug Product: The composition of each 5000 IU vial of rhThrombin drug product is as follows:

Material	Grade	Quantity Delivered	Overage	Function
α -thrombin				Active pharmaceutical ingredient
histidine				Buffering agent
mannitol				Lyophilization bulking agent
Sucrose				Stabilizer
Sodium chloride				Stabilizer
Polyethylene glycol 3350				Stabilizer
Calcium chloride (dihydrate)				Stabilizer

IU=International unit

List of Studies (Pharmacology and Toxicology):

Pharmacology:

Type of Study/Description	GLP ¹	Test System	Method of Administration	Testing Facility	Study Number (Report)	Location: (See Pharmacology Written Summary) Section
In Vitro Pharmacodynamics						
Plasma clot formation		Rat, rabbit, cynomolgus monkey and human sodium citrated plasma	Ex vivo	ZymoGenetics	NA (RES-10537)	1.2.1.1
Inhibitor complex formation		Purified human ATIII; human and cynomolgus monkey EDTA plasma	In vitro and ex vivo	ZymoGenetics	NA (MTD-10188; RES-10261)	1.2.1.1
In Vivo Pharmacodynamics						
Rat hand-repnection model using gauze and Gelfoam		Springue-Dawley rat	Topical	ZymoGenetics	NA (RES-10242)	1.2.1.1
Rabbit hepatic injury model using gauze and Gelfoam		New Zealand White rabbit	Topical	ZymoGenetics	NA (RES-10296)	1.2.1.1
Porcine splir thickness model		_____ pig	Topical (spray)	_____	NA (RES-10656)	1.2.1.1
Process Comparison Pharmacodynamics						
Inhibitor complex formation		Purified human ATIII and alpha-2-macroglobulin; synthetic human hirudin	In vitro	ZymoGenetics	NA (RES-10577)	1.6.1.1
Plasma clot formation		Human plasma	Ex vivo	ZymoGenetics	NA (SAR 10222)	1.6.1.1

Type of Study/Description	GLP ¹	Test System	Method of Administration	Testing Facility	Study Number (Report)	Location: (See Pharmacology Written Summary) Section
Rat Lami-caphrectomy model using Gelfoam		Sprague-Dawley rat	Topical	ZymoGenetics	N/A (RES-10570)	1.6.1.2
Rabbit hepatic injury model using Gelfoam		New Zealand White rabbit	Topical	ZymoGenetics	N/A (RES-10566)	1.6.1.1
Safety Pharmacology						
No specific studies performed (see Toxicology Written Summary, Sections 1.2 and 1.3)						
Pharmacodynamic Drug Interactions						
No studies performed						

¹ An entry of "Yes" indicates that the study includes a GLP compliance statement.

Toxicology:

Study Type and Duration Study Number	GLP Compliance	Route of Administration	Species
Single-dose toxicity			
Surgical Implant Study (see ZGI 1499-007)	GLP	Abdominal implantation to hepatic wound	Cynomolgus Monkey
Surgical Implant Study (see ZGI 1499-009)	GLP	Abdominal implantation to hepatic wound	Cynomolgus Monkey
Repeated-dose toxicity			
Tolerability Study (see RES-10236)	Non-GLP	IV, SC	Sprague-Dawley Rat
Repeated-dose Study (see ZGI 1499-005)	GLP	SC	Cynomolgus Monkey
Local Tolerance			
Dermal (see ZGI 1499-003)	GLP	Topical	New Zealand White Rabbit
Ocular (see ZGI 1499-004)	GLP	Topical	New Zealand White Rabbit
Other Studies			
Cytotoxicity (see ZGI 1499-002)	GLP	In Vitro	Mouse L929 fibroblasts
Follow-up Cytotoxicity (see RES-10527)	Non-GLP	In Vitro	Mouse L929 fibroblasts
PTA Impurity (see ZGI 1499-008)	Non-GLP	IV, IM, IP, SC	Mouse
PTA Impurity (see ZGI 1499-006)	GLP	IV, SC	Mouse

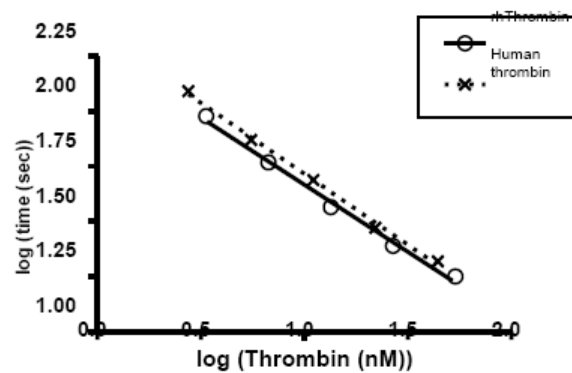
Note: Our use of the term "topical" includes the application of rhThrombin to the wound/organ surface with an absorbable gelatin sponge. In some surgical settings, the rhThrombin-treated gelatin sponge is left in place to maintain hemostasis.

1: Summary of Pharmacology Studies

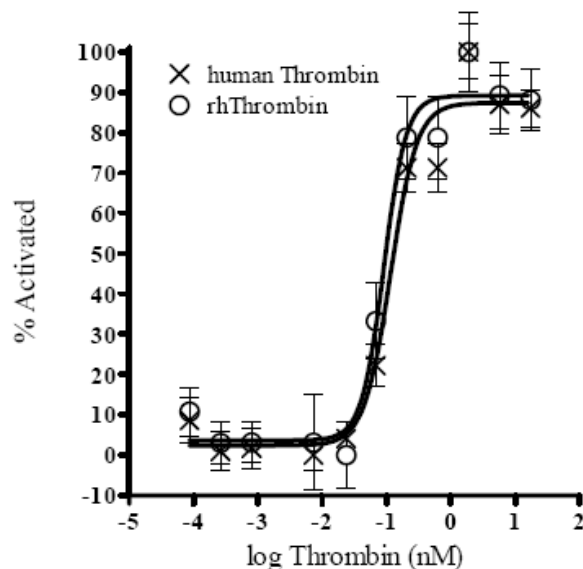
1.1 *In vitro* Pharmacology

Activity of rhThrombin in the conversion of fibrinogen to fibrin was measured by clot formation and activation of platelets and FXIII in thrombin clot activity assays studies # MTD-10034 and # QC-038 and RES - 10337. Figure (A) below from indicates similar response in the fibrinogen cleavage assay for rhThrombin (open circles) versus human Thrombin (crosses). Figure (B) shows the activation of platelets by rhThrombin (open circles) versus human Thrombin (crosses).

A



B



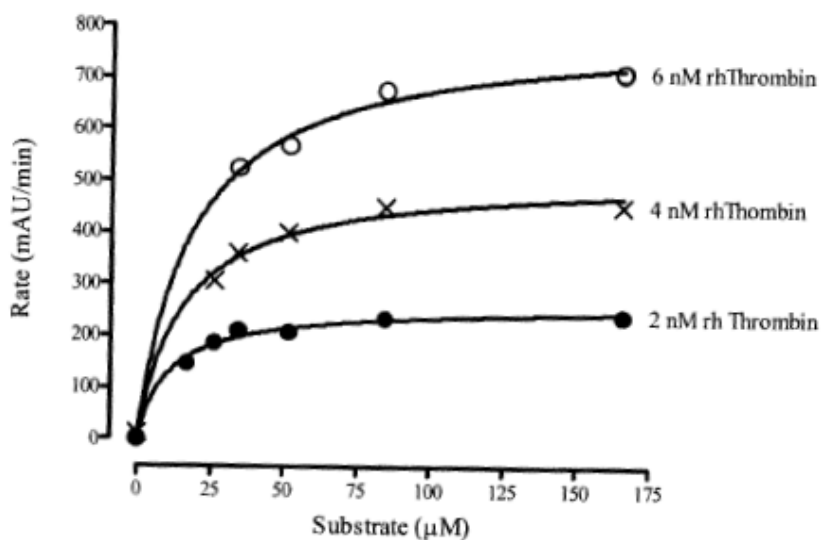
RES-10577 (00) –Comparison of the kinetic constants and inhibitor binding properties of rhThrombin produced by the pilot scale and commercial scale processes using the chromogenic activity assay and SE-HPLC.

Objective:

This study was performed the interaction of rhThrombin batches to inhibitors was measured with the chromogenic activity assay and with SE-HPLC. Evaluation of the enzymatic activity of rhThrombin in the presence of ATIII and hirudin was performed ($\alpha 2$ – macroglobulin ($\alpha 2M$) was not specifically evaluated since the presence of the $\alpha 2M$ -thrombin complex.

Results:

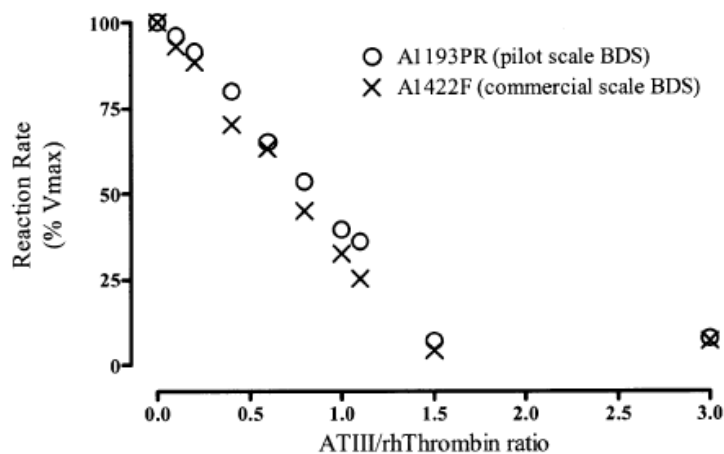
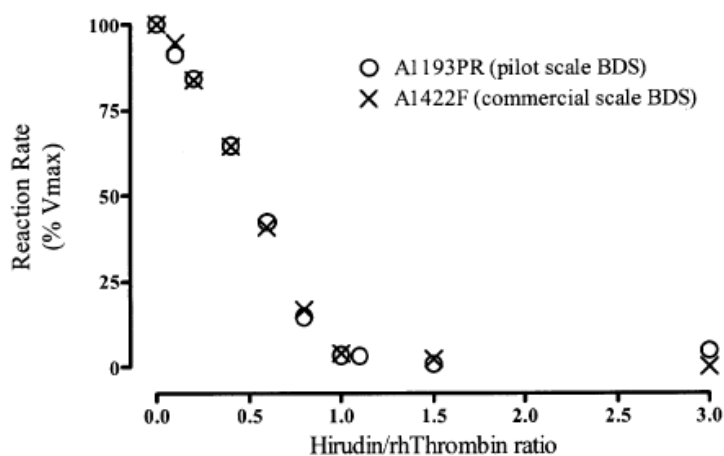
Representative curves of the reaction rate (mAU/min) as a function of substrate concentration.



Calculation of rate constants for pilot scale and commercial lots of rhThrombin are shown in tables below.

A1193PR (pilot scale)			A1422F (commercial scale)		
K_m (μ M)	V_{max} (mAU/min)	k_{cat} (sec-1)	K_m (μ M)	V_{max} (mAU/min)	k_{cat} (sec-1)
16.33	490.4	274.6	18.09	504.6	282.6
19.32	512.1	286.8	21.28	548.2	307.0
18.31	487.9	273.2	16.01	505.2	282.9
20.84	509.8	285.5	17.77	505.2	282.9
16.69	510.2	285.7	18.21	527.5	295.4
18.73	546.6	306.1	17.6	526.8	295.0
16.36	531.3	297.5	16.82	522.5	292.6
20.34	527.3	295.3	20.84	536.7	300.6
21.5	555.5	311.1	20.48	540.4	302.6
mean	18.7	519	18.6	524.1	293.5
stdev	2	23.2	1.9	16.3	9.1

The inhibition of rhThrombin enzymatic activity with hirudin and antithrombin III are shown below.

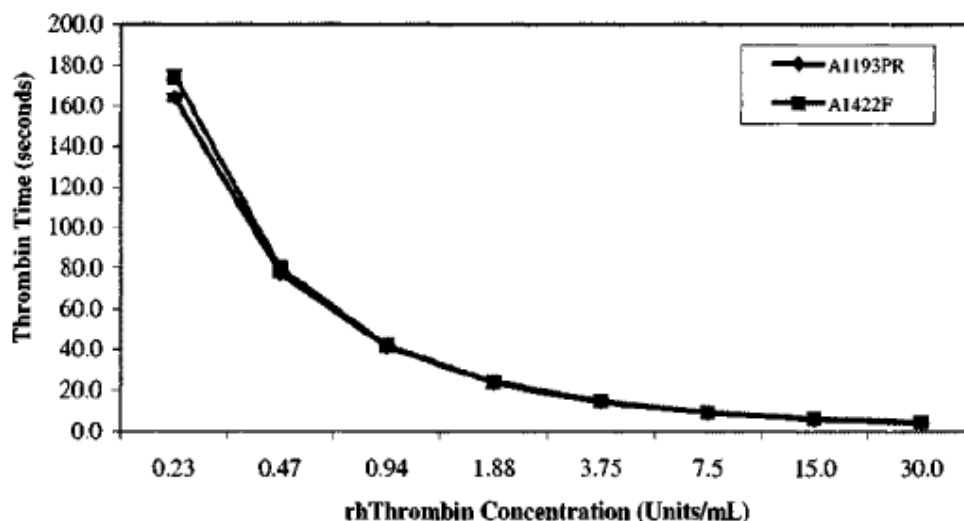


Conclusions:

Comparable kinetic constants (K_m and V_{max}) were measured for the activity of rhThrombin (pilot scale and commercial batches). Both hirudin and antithrombin blocked the activity of rhThrombin batches comparably and comparable rhThrombin –inhibitor complexes formed.

SAR-10222 – Comparison of thrombin time: Lot A1193PR and Lot A1422F

This particular study evaluates manufacturing changes occurring at the time of Phase II clinical trials. Thus the primary objective of the study was to compare batch changes in an in vitro clot formation assay. Clotting rates for the two batches as determined by thrombin time demonstrated batch comparability.



1.2 In vivo Pharmacology Study Summaries

RES-10296 A00 – Effect of rhThrombin on blood loss and time to hemostasis in a hepatic injury bleeding model in the rabbit.

This study was preceded by a pilot study CVB006-K, **RES-10566** “rhThrombin bridging study comparing two lots in a rabbit liver wound model”.

Objective:

This study was performed to establish a dose response and further to evaluate efficacy based knowledge gained from the initial dose response study. Both studies were performed in model of liver resection of the left medial and lateral liver lobes.

End points

- Amount of blood lost
- Time to hemostasis

Study groups and dosing:

Table 1. Study groups for dose-response study.

Group	Number of animals	Test Article	Dose Applied to Gauze Sponge
1	5	0.9% sodium chloride	0 U/mL x 0.6 mL = 0 U/pad
2	5	rhThrombin	100 U/mL x 0.6 mL = 60 U/pad
3	5	rhThrombin	500 U/mL x 0.6 mL = 300 U/pad
4	5	rhThrombin	750 U/mL x 0.6 mL = 450 U/pad
5	5	rhThrombin	1000 U/mL x 0.6 mL = 600 U/pad
6	5	rhThrombin	2000 U/mL x 0.6 mL = 1200 U/pad
7	4	bovine thrombin	1930 U/mL x 0.6 mL = 1158 U/pad

Table 2. Study Groups for efficacy study.

Group	Number of animals	Test Article	Dose Applied to Gelfoam Sponge
1	5	0.9% sodium chloride	0 U/mL x 2.0 mL = 0 U/pad
2	5	rhThrombin	1000 U/mL x 2.0 mL = 2000 U/pad
3	5	rhThrombin	500 U/mL x 2.0 mL = 1000 U/pad
4	5	bovine thrombin	1930 U/mL x 2.0 mL = 3860 U/pad
5	4	bovine thrombin	500 U/mL x 2.0 mL = 1000 U/pad

Results:

Blood loss data from dose response study (left lateral and medial liver lobe resection).

Table 3. Left medial total blood loss for dose-response study across treatment groups

Treatment group	Mean	Standard Deviation	Minimum value	Maximum value
1	9.08	3.03	4.20	12.50
2	15.46	16.20	3.10	42.70
3	4.35	2.25	2.30	7.50
4	5.73	0.61	5.10	6.70
5	2.95	1.44	1.80	5.00
6	8.08	6.74	2.10	16.60
7	2.75	0.77	1.90	3.50

Table 4. Left lateral total blood loss for dose-response study across treatment groups

Treatment group	Mean	Standard Deviation	Minimum value	Maximum value
1	9.76	3.24	5.90	14.70
2	11.16	6.20	4.90	19.10
3	4.28	2.54	1.40	6.70
4	6.21	3.43	2.30	10.40
5	2.50	1.73	1.10	5.00
6	7.68	5.18	3.40	15.10
7	2.38	0.59	1.50	2.70

Time to cessation of bleeding from dose response study for the left medial (LM) and left lateral (LL) lobe

Table 5. Statistics for time to cessation of bleeding for each liver lobe for dose-response study.

Treatment group	Liver lobe	Median time to cessation (sec)	Mean time to cessation (sec)	Standard Deviation (sec)	Min time to cessation (sec)	Max time to cessation (sec)
Saline	LM	450.00	466.00	80.81	380.00	600.00
	LL	480.00	485.80	62.60	409.00	580.00
100 U/ml rhThrombin	LM	510.00	530.00	71.76	430.00	600.00
	LL	450.00	410.00	73.14	300.00	470.00
500 U/ml rhThrombin	LM	284.50	278.50	72.89	185.00	360.00
	LL	246.50	243.25	49.42	180.00	300.00
750 U/ml rhThrombin	LM	250.00	242.17	68.29	160.00	310.00
	LL	219.50	209.83	67.32	109.00	300.00
1000 U/ml rhThrombin	LM	187.50	201.25	61.42	150.00	280.00
	LL	170.50	172.50	50.65	120.00	229.00
2000 U/ml rhThrombin	LM	155.00	197.50	124.47	100.00	380.00
	LL	172.50	193.75	73.87	130.00	300.00
1930 U/ml bovine Thrombin	LM	222.50	221.25	17.50	200.00	240.00
	LL	195.00	192.50	58.09	135.00	245.00

Blood loss data from efficacy study

Table 6 Left medial total blood loss for efficacy study across treatment groups

Treatment group	Mean	Standard Deviation	Minimum value	Maximum value
1	2.58	1.77	0.90	5.60
2	0.72	0.52	0.10	1.30
3	0.43	0.35	0.06	1.00
4	0.34	0.15	0.20	0.60
5	0.60	0.29	0.30	1.00
Overall	0.95	1.17	0.06	5.60

Table7 Left lateral total blood loss for efficacy study across treatment groups

Treatment group	Mean	Standard Deviation	Minimum value	Maximum value
1	3.00	2.00	0.80	6.00
2	0.52	0.23	0.20	0.80
3	0.82	0.40	0.20	1.30
4	0.38	0.25	0.01	0.70
5	0.53	0.36	0.20	1.00
Overall	1.07	1.34	0.01	6.00

Conclusion:

Based on these studies rhThrombin is efficacious as a topical hemostatic agent in the rabbit liver injury model.

RES-10656 A00 – Evaluation of efficacy between rhThrombin and bovine thrombin as a sprayable hemostat on a porcine graft/wound model.

Objective:

The objective of the study was to compare the time to hemostasis with sprayable bovine thrombin versus rhThrombin in a porcine skin-graft donor site bleed model. The test articles were rhThrombin, Bovine Thrombin-JMI and saline control.

Methods:

The study was performed in male and female ----- pigs having undergone partial thickness skin wounds. End point measurements were time to hemostasis, body weight, mean arterial pressure, body temperature, and hematology and coagulation panel.

Group assignments:

Pig No.	Body Weight (kg)	Total Number Of Wounds	Wound Size (cm x cm)
5089	67 kg	14 (2 of 14 untreated) ^a	4 x 5.0 to 4 x 5.5
5093	65 kg	12 (3 of 12 untreated)	4 x 5.0 to 4 x 6.0
5094	66 kg	13 (4 of 13 untreated)	4 x 5.0 to 4 x 6.0
5111	56 kg	12	4 x 5.0 to 4 x 6.0
5112	56 kg	12	4 x 5.0 to 4 x 5.5
5151	54 kg	12	4 x 5.0 to 4 x 5.5

Results:

Table 1: Mean \pm S.D. Time to Hemostasis (TTH) for Each Animal in Seconds (n=4)

Pig#	Saline	Bovine Thrombin®- JMI	rhThrombin
5089	218.0 \pm 14.5	115.3 \pm 21.3	96.7 \pm 26.5
5093 [^]	80.3 \pm 21.1	60.0 \pm 11.8	60.0 \pm 6.2
5094 [^]	89.0 \pm 20.7	53.0 \pm 1.0	60.7 \pm 7.4
5111	79.3 \pm 8.2	66.0 \pm 6.7	56.3 \pm 9.3
5151	212.3 \pm 60.0	84.5 \pm 11.6	91.5 \pm 5.5
5112	130.3 \pm 14.5	86.7 \pm 7.6	76.7 \pm 8.0
Overall Mean	139.41 \pm 67.3	79.5 \pm 23.7	74.86 \pm 20.2

[^] Only three wounds per treatment were administered in these animals

Table 2: Statistical Comparison Between Treatment Groups (TTH)

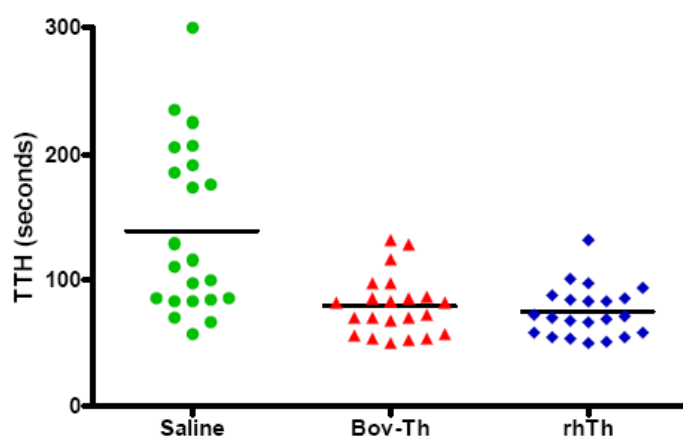
Comparison Groups	Mean (Lower CI, Upper CI)	p Value ^a
rhThrombin – Saline	-61.19 (-111.4, -11.04)	*p = 0.0258
Bovine Thrombin-JMI®-Saline	-57.39 (-106.7, -8.057)	*p = 0.0304
RhThrombin-Bovine Thrombin JMI®	-3.806 (-14.62, 7.0095)	p = 0.4072

^a Paired t-test between groups, *p \leq 0.05 significant
(CI = confidence interval)

Table 3: Individual Animal Hematology and Coagulation Parameter Values

Pig No.	% HCT Baseline	% HCT Terminal	PLT Baseline (K/uL)	PLT Terminal (K/uL)	PT Baseline (seconds)	PT Terminal (seconds)	APTT (seconds)	APTT (seconds)
5089	30.6	28.7	371	360	14.6	13.7	22.0	20.7
5093	30.8	27.8	395	424	11.3	12.5	12.1	15.6
5094	34.6	33.0	295	340	13.4	12.2	13.7	17.6
5111	30.8	28.3	412	375	12.6	10.7	16.6	16.3
5151	28.5	28.2	355	347	11.0	8.6	16.3	16.1
5112	31.2	28.7	253	173	8.5	8.8	14.5	14.3

Time to hemostasis

**Figure 1: Time to Hemostasis (TTH) Dot Plot**

The above dot-plot is a comparison of TTH between wounds sprayed with saline (control), 1000 U/mL Bovine Thrombin-JMI® (Bov-Th) or 1000 U/mL rhThrombin (rhTh). Test article was sprayed at t= 0 seconds, the wound was then blotted and sprayed 4 times with test article. Test article was then sprayed 2 times every 15 seconds until hemostasis was achieved.

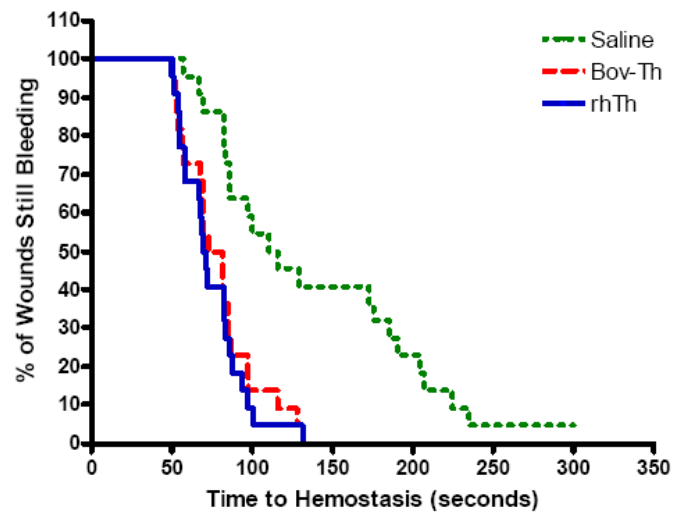
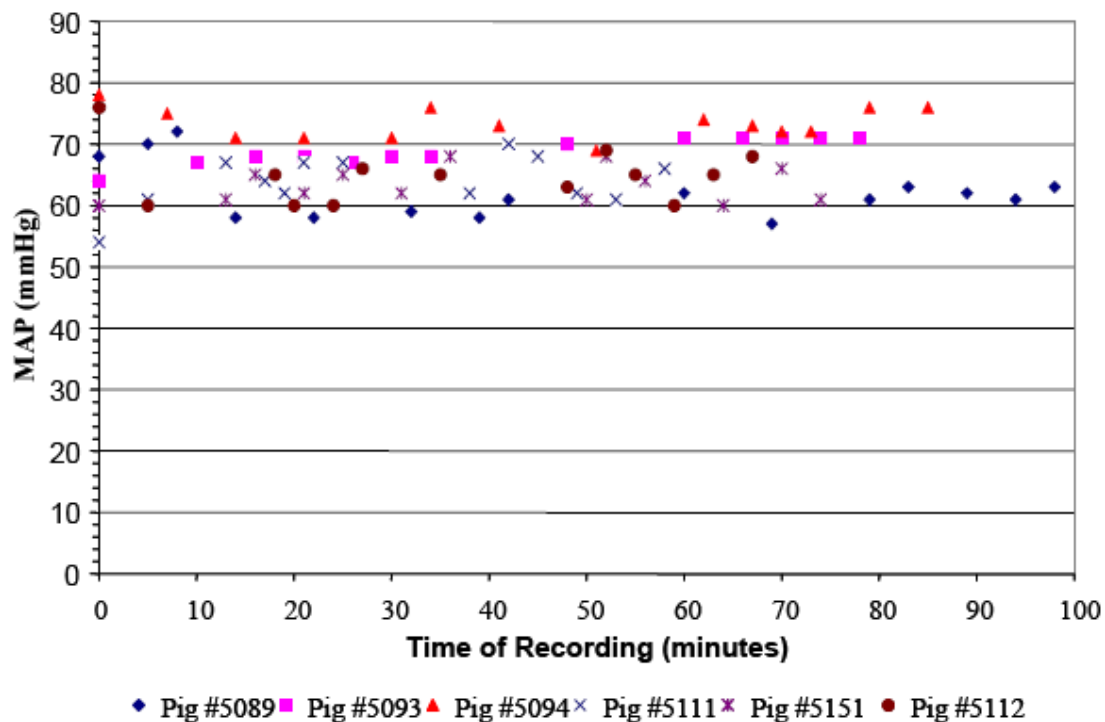


Figure 2: Time to Hemostasis (TTH) Kaplan Meier Plot

The above Kaplan Meier-plot is a comparison of TTH between wounds sprayed with saline (control), 1000 U/mL Bovine Thrombin-JMI® (Bov-Th) or rhThrombin (rhTh). The above dot-plot is a comparison of TTH between wounds sprayed with saline (control), 1000 U/mL Bovine Thrombin-JMI® (Bov-Th) or 1000 U/mL rhThrombin (rhTh). Test article was sprayed at $t = 0$ seconds, the wound was then blotted and sprayed 4 times with test article. Test article was then sprayed 2 times every 15 seconds until hemostasis was achieved. Recombinant human Thrombin showed comparable efficacy in reducing bleeding as compared to Bovine Thrombin-JMI® and both showed superiority to saline (control).

Mean arterial pressure



Conclusion: rhThrombin and bovine thrombin JMI as a sprayable hemostat demonstrated similar efficacy in achieving hemostasis in a porcine-skin graft donor-site bleed model.

RES-10242 A00 – Comparative efficacy of bovine thrombin and ZymoGenetics rhThrombin in a rat kidney bleed model.

This study was preceded by the pilot study **RES-10570** “Comparative efficacy of commercial scale and pilot scale rhThrombin in a rat kidney bleed model.”

Objective:

To evaluate the lowest efficacious dose of rhThrombin needed to achieve homeostasis when applied to gauze or gelfoam and compare the efficacy of bovine thrombin in the rat hemi-nephrectomy model.

End points

- Amount of blood lost
- Time to hemostasis
- Efficacy of gauze versus gelfoam sponge

Study groups and dosing:

Table 1: Study Groups For Test Article + Gauze Sponge

Groups	Number of Rats	Test Article (0.4 mL/patch)	Dose
A	11	Vehicle/Saline	-----
B	5	rhThrombin	100 U/mL
C	5	rhThrombin	500 U/mL
D	5	rhThrombin	1000 U/mL
E	5	rhThrombin	2000 U/mL
F	9	Bovine Thrombin	1000 U/mL

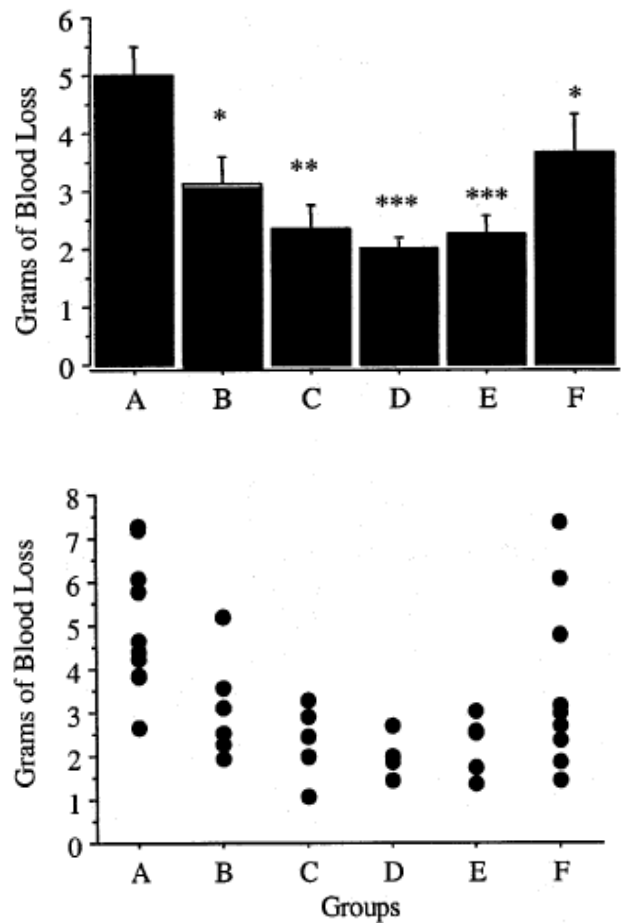
Table 2: Study Groups For Test Article + Gelfoam Sponge

Groups	Number of Rats	Test Article (0.5mL/patch)	Dose
G	7	Vehicle/Saline	-----
H	6	rhThrombin	500 U/mL
I	6	rhThrombin	1000 U/mL
J	5	Bovine Thrombin	1000 U/mL

Results:

Figure 1: Grams of Blood Loss, Gauze Patch + Test Article

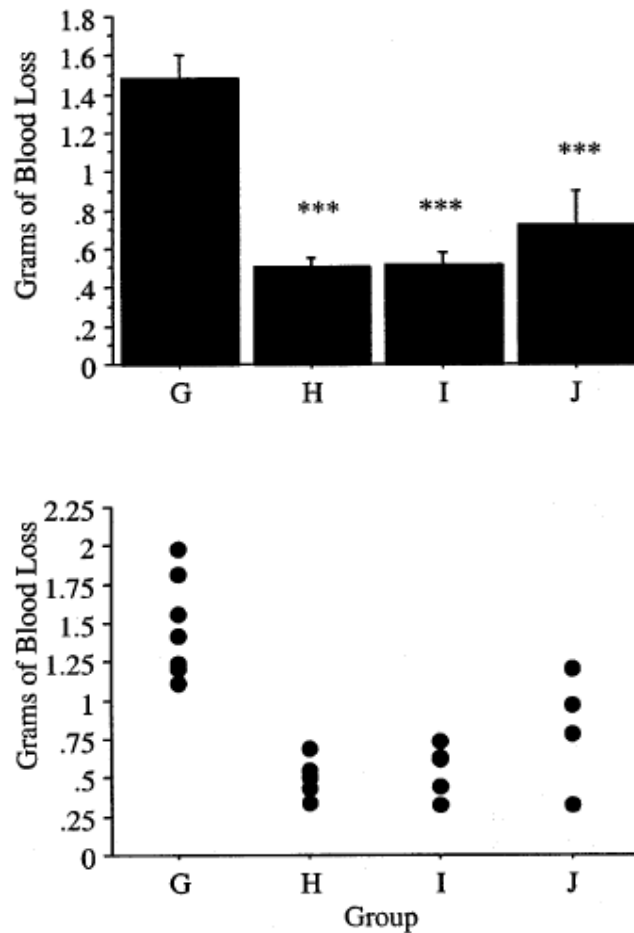
A-Saline, B-rhThrombin 100 U/mL, C- rhThrombin 500 U/mL, D- rhThrombin 1000 U/mL, rhThrombin 2000 U/mL and F-bovine Thrombin 1000 U/mL (Dose 0.1 mL).



Blood loss was significantly reduced in groups B-F as compared to group A and group D was significantly reduced compared to group F. Data is presented as mean value with SEM (One way ANOVA, Fisher PLSD test: *, $p<0.05$, **, $p<0.01$, *** $p<0.001$)

Figure 2: Grams of Blood loss, Gelfoam Patch + Test Article

G-Saline, H-rhThrombin 500 U/mL, I- rhThrombin 1000 U/mL and J-bovine Thrombin 1000 U/mL



Conclusion:

Based on these studies rhThrombin is efficacious as a topical hemostatic agent in the rat partial heminephrectomy bleed model.

2.0 Summary Pharmacokinetic Studies:

PT-1499-001 – Bioavailability and relative tissue distribution of [125 I]-rhThrombin following intravenous and subcutaneous administration to naïve cynomolgus monkeys (Non-GLP)

Objectives:

- (1) to determine the bioavailability of rhThrombin-associated radioactivity following subcutaneous injection of [125 I]-rhThrombin to male cynomolgus monkeys;
- (2) to determine the fate (including complexation to endogenous inhibitors) of circulating [125 I]-rhThrombin following intravenous and subcutaneous injection of [125 I]-rhThrombin;

- (3) to determine the relative tissue distribution of rhThrombin-associated radioactivity following intravenous and subcutaneous injection of [125 I]-rhThrombin to male cynomolgus monkeys using gamma scintigraphy.

Study groups and dosing:

Group 1: Single IV dose target dose level 3.5 U rhThrombin (1.12 ug/kg, 67 uCi/kg)

Group 2: Single IV dose target dose level 350 U rhThrombin (112 ug/kg, 100 uCi/kg)

Results:

PK/bioavailability data from monkeys dosed IV versus SC

Figure 1 Plasma concentration curves for rhThrombin equivalents following IV and SC dosing

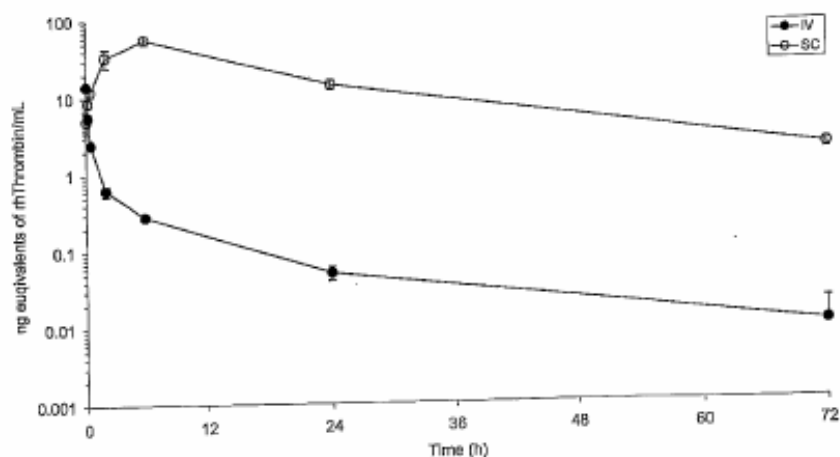


Table 1 PK parameters for rhThrombin equivalents

Parameter	Units*	IV (1.12 ug/kg)	SC (112 ug/kg)
C_0 (IV) or C_{max} (SC)	ng/mL	21.7 (1.8)	55.7 (7.3)
T_{max} (SC)	h	N/A	6.00 (0.00)
$t_{1/2, initial}$ (IV)	h	0.170 (0.003)	N/A
$t_{1/2, \beta}$	h	16.4 (2.9)	14.3 (0.5)
AUC_{0-4}	h•ng/mL	13.0 (1.3)	1222 (142)
AUC_{0-72}	h•ng/mL	13.3 (1.3)	1260 (146)
CL (IV) or CL/F (SC)	mL/h/kg	85.1 (9.2)	89.8 (10.4)
V_d (IV) or V_d/F (SC)	mL/kg	766 (205)	1848 (216)
AUC_{0-72}/D	(h•ng•kg)/(mL•ug)	11.8 (1.2)	11.2 (1.3)
Bioavailability	%	N/A	94.9

* rhThrombin equivalents concentration data were originally expressed as ng/g of plasma, but were converted to ng/mL (assuming a density of plasma of 1g/mL) for pharmacokinetic analysis.

Table 2 rhThrombin/Anti-thrombin III complexes determined by SE-HPLC

Time (hr)	ng/mL	
	IV	SC
Pre-dose	ND	ND
0.083	8.78 (0.40)	ND
0.25	3.19 (0.09)	ND
0.5	1.52 (0.20)	ND
2	BLQ	BLQ
6	BLQ	BLQ
24	ND	BLQ
72	ND	ND

ND (not determined): total radioactivity concentration of sample was less than the cut off of 100,000 cpm/mL (see Methods section for details)

BLQ: below limit of quantification

Table 3 Non Anti-thrombin III complexed rhThrombin determined by SE-HPLC

Time (hr)	ng/mL	
	IV	SC
Pre-dose	ND	ND
0.083	0.530 (0.295)*	ND
0.25	BLQ	ND
0.5	BLQ	ND
2	BLQ	BLQ
6	BLQ	BLQ
24	ND	BLQ
72	ND	ND

ND (not determined): total radioactivity concentration of sample was less than the cut off of 100,000 cpm/mL (see Methods section for details)

BLQ: below limit of quantification

* Value determined from an n=3 out of 4 animals (1 animal was BLQ)

Conclusion:

Based on this study performed in cynomolgus monkeys SC dosing of rhThrombin is approximately 95% bioavailable from the SC route, rhThrombin rapidly complexes to AT III and the liver appears to be the major site of accumulation [¹²⁵I]-rhThrombin.

RES-10783 Pharmacokinetics of [¹²⁵I]-rhThrombin after application of an [¹²⁵I]-rhThrombin-saturated Gelfoam-100 sponge in a liver wound model in female new Zealand white rabbits

Objective:

To determine the amount of [¹²⁵I]-rhThrombin is absorbed from the wound site following application of escalating doses.

Study groups and dosing:

Table 1: Actual Dose of ^{125}I -rhThrombin Administered to Each Rabbit

Rabbit #	Radioactivity Remaining in Sponge Prep Dish (μCi)	Radioactive Dose Admin. (μCi)	Total rhThrombin Dose Admin.	
			(μg)	(Units)
J9794	3.62	297	351	1211
J9795	3.85	297	351	1210
J9797	4.12	297	350	1209
J9799	3.06	298	352	1213

Results:

Figure 1: Plasma concentrations of rhThrombin following application of 350 ug applications to the liver injury site by gel foam application

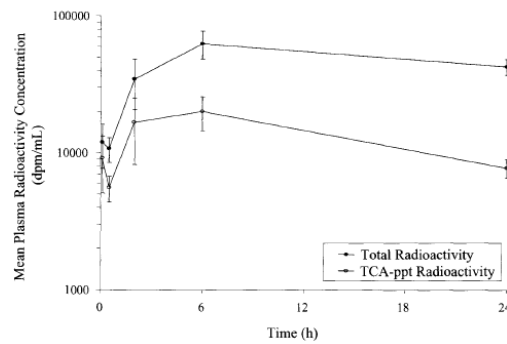


Table 2:

Mean (S.D.) PK Parameters Based on rhThrombin-equiv. Versus Time Data After ^{125}I -rhThrombin/Gelfoam Sponge Application in a Rabbit Liver Wound Model (n=4)

Parameter	Units	rhThrombin-equiv.
C_{\max}	ng-equiv./mL ^a	11.6 (3.7)
T_{\max}	h	2-6 ^b
AUC_{0-4}	h*ng-equiv./mL ^a	182 (35)

^a units for rhThrombin-equiv. concentration versus time data (ng-equiv./mL)

^b range of T_{\max} values

Table 3:

Individual Total Radioactivity Concentrations and Percent Total Dose in Liver Samples Following ¹²⁵I-rhThrombin/Gelfoam Sponge Application in a Rabbit Liver Wound Model

Data	Rabbit #	Plasma	Liver: Adjacent to Sponge	Liver: Distal to Sponge
Total Radioactivity (dpm/mL or dpm/g)	J9794	38200	90300	21000
	J9795	37700	272000	28100
	J9797	44200	506000	30500
	J9799	49200	603000	24200
Tissue:Plasma Ratio	J9794	---	2.37	0.551
	J9795	---	7.22	0.747
	J9797	---	11.5	0.692
	J9799	---	12.3	0.492

Table 4:

Individual PK Parameters Following ¹²⁵I-rhThrombin/Gelfoam Sponge Application in a Rabbit Liver Wound Model

Data	Rabbit #	C _{max} (ng-equiv./mL)	T _{max} (h)	AUC _{0-t} (h*ng-equiv./mL)
rhThrombin-equiv.	J9794	7.15	2	135
	J9795	15.4	2	208
	J9797	13.6	6	209
	J9799	10.2	6	177

Conclusion:

Minimal rhThrombin was detected in plasma from 5 minutes to 24 hours post application. Less than 0.37% of the applied dose was absorbed from the site of liver injury/application. Of the percentage nearly the entire amount of administered radioactivity was found in the liver after 24 hours.

3.0 Summary Toxicology Studies:

3.1 Single dose toxicology

RES-10236 A pilot study of rhThrombin in normal rats

Objective:

To determine the dose-response relationship and overt toxicity of rhThrombin after a single IV injection or 2 SC injections, bovine thrombin was used as a control.

Groups and Dosing:

Table 1. Dosing schedule/amounts delivered

Rat	Test article	Dose day(s)	Dose route	Dose volume	Nominal Units/kg
1	rhThrombin	1, 10	SC	0.12 mL	496 ^a
2	rhThrombin	3, 10	SC	0.14 mL	496
3	rhThrombin	3, 10	SC	0.27 mL	992
4	rhThrombin	3, 10	SC	0.26 mL	992
5	Bovine thrombin	10	IV	0.097 mL	341 ^b
6	Bovine thrombin	10	IV	0.050 mL	178
7	rhThrombin	10	IV	0.050 mL	158

^a The intended SC dose for a subsequent monkey immunogenicity study

^b Nominal reconstituted vial concentrations were 1,000 Units/mL. However, ZGI testing has shown that the bovine thrombin vials are overfilled by approximately 90%, and that the actual vial concentration was 1,920 Units/mL.

Results:

SC Doses – All doses of rhThrombin and bovine Thrombin were tolerated with no clinical or gross toxicity (No maximum tolerated dose was achieved)

IV Doses Bovine Thrombin–rat 6 died with a nominal does of bovine thrombin (178 Units/kg), consitent wit an anaphylactic reaction (shortness of breath and pale eye color) or thromboses. Rat 5 (341 Units/kg) died within 30seconds of dosing.

IV Dose rhThrombin – Rat 7 tolerated a single 158 Units/kg of rhThrombin (14 days).

These doses were found to be mislabeled as 2-fold less than the anticipated dose based on the potency assay.

As a result it is the reviewer's opinion that exposure of rhThrombin generated by 158 Units/kg would be the NOAEL in this pilot study.

1499-005 A 6-week immunogenicity study of rhThrombin in administered by SC injection to cynomolgus monkeys.

Objective: To evaluate the immunogenic and toxicologic response to rhTrombin in cynomolgus monkeys. This study serves as the pivotal toxicology study in the BLA submission.

Groups and Doses:

Group No.	Number of M/F	Dose Material	Dose Level ^a (Units/m ²)	Dose Level ^b (Units/kg)	Dose Volume (mL/kg)	Nominal Conc. (Units/mL)
1	3/3	vehicle	0 (control)	0 (control)	0.346	0 (control)
2	3/3	bovine thrombin	5405	346	0.346	1000 ^c
3	3/3	rhThrombin	5405	346	0.346	1000

^a Units/m² of whole body surface area

^b Units/kg based on 0.32 m² for a 5 kg animal

^c Nominal bovine thrombin concentration based on label claim

M/F = male/female

Animals were dose once weekly for four weeks

Results:

SC administration of rhThrombin or bovine thrombin to monkeys for 4 weeks at a dose level of 5405 U/m² (346 U/kg) did not result in antibody (neutralizing or non-neutralizing) response to rhThrombin or bovine thrombin. No abnormal findings were observed in any groups for: Serum chemistry, hematology, coagulation, Gross necropsy or histopathology at days 1,15, 18, 22, 23, 26, 29 or 42. The dose administered in this study represents approximately 1643.5 U of rhThrombin absorbed.

Reports on assay validation are listed in the Toxicology table on page 4 of this review.

Please see CMC review for evaluation/validation of relevant chemistry assays.

Hematologic values:

Table 4: Individual Animal and Group Mean Hematology Values
Study Number: 1509-175

Study number: 1909-175		White Blood Cell Count			
Animal Number	Sex	10 ³ /μL			
		Day -7	Day 1 (predose)	Day 1 (6 hr postdose)	Day 1 (24 hr postdose)
Group 1: Control (0 Units/m ²)					
F22143M	M	11.2	12.2	15.2	9.5
F22144M	M	10.4	15.5	23.6	13.9
F22138M	M	9.0	11.9	14.2	8.9
F22699F	F	9.0	10.3	9.9	8.4
F22670F	F	11.7	9.2	11.5	10.9
F22192F	F	18.2	12.1	11.8	12.4
Mean		11.6	11.9	14.4	10.6
S.D.		3.4	2.1	4.9	2.1
Group 2: Bovine Thrombin (5,405 Units/m ²)					
F22145M	M	9.7	12.2	13.0	9.1
F22740M	M	6.7	11.6	13.0	7.8
F20802M	M	11.2	9.7	14.3	9.9
F217108F	F	10.8	12.9	16.4	13.3
F21798F	F	14.4	12.8	13.8	14.7
F22172F	F	9.6	8.7	13.3	10.7
Mean		10.4	11.3	14.0	10.9
S.D.		2.5	1.7	1.3	2.6
Group 3: rhThrombin (5,405 Units/m ²)					
F21302M	M	8.7	10.1	9.7	8.1
F21717M	M	8.1	9.0	9.4	6.3
F21716M	M	5.9	5.7	7.3	4.6
F217100F	F	19.8	13.7	14.1	10.0
F21772F	F	10.4	11.9	13.6	11.5
F221104F	F	8.9	11.0	13.0	13.7
Mean		10.3	10.2	11.2	9.0
S.D.		4.9	2.7	2.8	3.4

Coagulation values:

Table 5: Individual Animal and Group Mean Coagulation Values
Study Number: 1509-175

Study Number: 1503-175					
		Fibrinogen Concentration			
Animal	Sex	mg/dL			
Number		Day -7	Day 1 (predose)	Day 1 (6 hr postdose)	Day 1 (24 hr postdose)
Group 1: Control (0 Units/m ²)					
F22143M	M	216	203	173	200
F22144M	M	254	277	216	291
F22138M	M	220	262	192	232
F22699F	F	199	198	126	178
F22670F	F	244	254	214	239
F22192F	F	310	222	151	205
Mean		236	236	179	224
S.D.		47	33	36	40
Group 2: Bovine Thrombin (5,405 Units/m ²)					
F22145M	M	237	264	230	272
F22740M	M	297	280	237	280
F20802M	M	207	246	156	262
F217108F	F	218	260	172	254
F21798F	F	332	257	239	358
F22172F	F	244	216	218	235
Mean		256	257	209	277
S.D.		49	24	36	43
Group 3: rhThrombin (5,405 Units/m ²)					
F21302M	M	230	275	129	297
F21717M	M	224	232	163	222
F21716M	M	214	251	156	218
F217100F	F	275	341	230	275
F21772F	F	205	277	216	317
F221104F	F	185	185	146	194
Mean		222	260	173	254
		30	52	40	49

Gross Necropsy findings:

Table 6: Individual Animal Gross Necropsy Observations

Study Number: 1509-175

Animal Number	Sex	Organ	Observations
Group 1: Control (0 Units/m²)			
F22143M	M	adrenal	focus, left, red, 2 mm, single
		stomach	focus, red, 4 mm diameter, mucosa, single
F22144M	M	mandibular lymph node	size increased, bilateral
		thyroid	size increased, bilateral, mild
		epididymis	nodule, 1 cm x 0.6 cm, left epididymal fat
		spleen	size increased, mild
		spleen	cleft, 2 cm in length
		spleen	focus, white, 3 mm, capsule
		spleen	accentuated follicular pattern
F22138M	M	ileocecocolic valve	discoloration, red, 3 mm diameter
		injection site	scaly, tan, minimal
		pancreas	nodule, dark red, 3 mm diameter
		stomach	nodule, dark red, 2 mm, serosal surface
F22699F	F	colon	focus, red, 2 mm, multiple, greater than 20
		lung	focus, black, 1 mm, all lobes, less than 10
		kidney	discoloration, white, right, capsule, 2 mm, single
F22670F	F	colon	nodule, grey, 3 mm, submucosa, single, (probable Oesophagostomum)
F22192F	F	colon	no visible lesions
		colon	focus, red, multiple, less than 20, mucosa
Group 2: Bovine Thrombin (5,405 Units/m²)			
F22145M	M	epididymis	nodule, tan, 3 mm, pedunculated, bilateral
		sublumbar lymph node	size increased, mild
		colon	discoloration, red, pinpoint to 2 mm, mucosa, multifocal, mild, throughout colon
		brain	discoloration, black, multifocal, mild, frontal cortex, bilateral
F22740M	M	ileocecocolic valve	discoloration, red, mucosa, focal, mild
F20802M	M	epididymis	nodule, red, 6 mm, pedunculated, left
		tonsil	size increased, bilateral, mild
		lung	focus, red, 2 mm, left diaphragmatic lobe, single
		colon	discoloration, red, mucosa, multifocal, pinpoint, minimal, small area
F217108F	F	spleen	nodule, tan, 4 mm, blade edge
		lung	cyst, 2 mm, right diaphragmatic lobe
		mesenteric lymph node	size increased, mild
F21798F	F	colon	discoloration, red, mucosa, multifocal, minimal, throughout colon
F22172F	F	omentum	nodule, red, 3 mm
		ovary	cyst, 6 mm, right
		ovary	cyst, 6 mm, paraovarian, right
		colon	discoloration, red, mucosa, multifocal, mild, throughout colon
Group 3: rhThrombin (5,405 Units/m²)			
F21302M	M	skin	discoloration, left cheek, red, 2 cm diameter
		skin	discoloration, red, left anterior shoulder, 2 x 3 cm
		pancreas	nodule, dark red, 3 mm diameter
		thyroid	size increased, bilateral, mild
		tongue	discoloration, black, focal, 3 mm diameter, serosal surface
		ileocecocolic valve	discoloration, red, 2 cm
F21717M	M	spleen	size increased, mild
		ileocecocolic valve	discoloration, red, 1 cm
F21716M	M	epididymis	nodule, tan, 3 mm, pedunculated, left
F217100F	F		no visible lesions
F21772F	F	skin	swelling, right arm, elbow, anterior, edema, discoloration of muscle
		spleen	accentuated follicular pattern
		colon	focus, red, 1 mm, mucosa, diffuse, greater than 20
F221104F	F	pancreas	nodule, red, 2 mm

Histopathology findings:

STUDY ID : rhTHROMBIN IMMUNOGEN
 FATE: ALL
 DAYS ON TEST: ALL

STUDY NUMBER: 1509175

SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1M	2M	3M
		(1)	(2)	(3)
NUMBER OF ANIMALS:		3	3	3
BRAIN	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		1	0	3
MINERALIZATION		1	0	0
HEMORRHAGE		0	0	1
SPINAL CORD	# EX	3	3	3
SCIATIC NERVE	# EX	3	3	3
DEGENERATION		0	1	0
THYROID	# EX	3	3	3
ECTOPIC THYMUS		1	0	1
MONONUCLEAR CELL INFILTRATE		2	3	0
FOLLICULAR DEGENERATION		0	2	0
PARATHYROID	# EX	2	3	3
CYST		1	0	0
INFLAMMATION, ACUTE		0	0	1
ADRENAL	# EX	3	3	3
PITUITARY	# EX	3	3	3
KIDNEY	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		2	3	3
MINERALIZATION, PAPILLA		0	1	0
GLOMERULOSCLEROSIS		0	1	1
CAST, PROTEIN		0	1	0
URINARY BLADDER	# EX	3	3	3
TESTIS	# EX	3	3	3
INCOMPLETE SPERMATOGENESIS, IMMATURE		3	2	3
FIBROUS NODULE		0	0	1
EPIDIDYMISS	# EX	3	3	3
FIBROUS TAG		0	1	0
MONONUCLEAR CELL INFILTRATE		1	0	0

(1) - VEHICLE CONTROL
 (2) - BOVINE THROMBIN, 5,405 U/m2

(3) - rhTHROMBIN, 5,405 U/m2

STUDY ID : rhTHROMBIN IMMUNOGEN
 FATE: ALL
 DAYS ON TEST: ALL

STUDY NUMBER: 1509175

SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1M	2M	3M
		(1)	(2)	(3)
NUMBER OF ANIMALS:		3	3	3
PROSTATE	# EX	#	#	#
MONONUCLEAR CELL INFILTRATE		3	3	3
GLANDULAR DILATATION		0	0	1
SEMINAL VESICLE	# EX	3	3	3
TRACHEA	# EX	3	3	3
LUNG	# EX	3	3	3
INFLAMMATION, CHRONIC		2	1	1
FIBROSIS, PLEURA		0	1	0
MONONUCLEAR CELL INFILTRATE, PERIVASCULAR		0	0	1
HEART	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		1	2	1
AORTA	# EX	3	3	3
FIBROSIS, INTIMAL		1	1	1
SKIN	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE, PERIVASCULAR		1	0	0
INFLAMMATION, ACUTE		0	0	1
HEMORRHAGE		0	0	1
MAMMARY GLAND	# EX	3	1	2
DILATATION, DUCT		1	1	0
TONGUE	# EX	3	3	3
GRANULOMA		0	0	1
SKELETAL MUSCLE	# EX	3	3	3
ESOPHAGUS	# EX	3	3	3
INFLAMMATION, CHRONIC		1	0	0
STOMACH	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE, PERIVASCULAR		0	1	0

(1) - VEHICLE CONTROL

(3) - rhTHROMBIN, 5,405 U/m2

(2) - BOVINE THROMBIN, 5,405 U/m2

STUDY ID : rhTHROMBIN IMMUNOGEN

STUDY NUMBER: 1509175

FATE: ALL

DAYS ON TEST: ALL

SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1M	2M	3M
		(1)	(2)	(3)
NUMBER OF ANIMALS:		3	3	3
DUODENUM	# EX	3	3	3
JEJUNUM	# EX	3	3	3
HEMORRHAGE, MESENTERY		0	1	0
ILEUM	# EX	3	3	3
COLON	# EX	3	3	3
HEMORRHAGE		1	2	0
CECUM	# EX	3	3	3
CILIATE PARASITE, INTRALUMINAL (BALANTIDIUM)		3	1	2
EOSINOPHILIC CELL INFILTRATE		1	0	0
RECTUM	# EX	3	3	3
MEDIASTINAL LYMPH NODE	# EX	3	3	3
LYMPHOID HYPERPLASIA		0	1	1
ERYTHROCYTOSIS		3	2	3
MESENTERIC LYMPH NODE	# EX	3	3	3
VASCULITIS, EOSINOPHILIC, MESENTERIC VESSEL		1	0	0
THYMUS	# EX	3	3	3
INVOLUTION		2	3	1
CYST		0	1	1
HEMORRHAGE		1	1	0
SPLEEN	# EX	3	3	3
LYMPHOID HYPERPLASIA		0	1	0
MANDIBULAR SALIVARY GLAND	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		3	3	3
PANCREAS	# EX	3	3	3
ECTOPIC SPLEEN		1	0	1

(1) - VEHICLE CONTROL

(3) - rhTHROMBIN, 5,405 U/m2

(2) - BOVINE THROMBIN, 5,405 U/m2

STUDY ID : rhTHROMBIN IMMUNOGEN
 FATE: ALL
 DAYS ON TEST: ALL

STUDY NUMBER: 1509175

SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1M (1)	2M (2)	3M (3)
NUMBER OF ANIMALS:		3	3	3
LIVER	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		3	0	3
GALLBLADDER	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		1	0	0
EYE	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE, CILIARY BODY		1	0	1
STERNUM (BONE & MARROW)	# EX	3	3	3
LYMPHOID NODULE		1	0	0
RIB (COSTOCHONDRAL JUNCT)	# EX	3	3	3
FEMUR (EPIPHYSEAL PLATE)	# EX	3	3	3
INJECTION SITE	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE, PERIVASCULAR		3	3	3
ILEOCECAL VALVE	# EX	1	1	2
HEMORRHAGE		1	1	2
MANDIBULAR LYMPH NODE	# EX	1	0	0
LYMPHOID HYPERPLASIA		1	0	0
ERYTHROCYTOSIS		1	0	0
TONSIL	# EX	0	1	0
LYMPHOID HYPERPLASIA		0	1	0
SUBLUMBAR LYMPH NODE	# EX	0	1	0
AMYLOID DEPOSITION		0	1	0
ERYTHROCYTOSIS		0	1	0

(1) - VEHICLE CONTROL

(2) - BOVINE THROMBIN, 5,405 U/m2

(3) - rhTHROMBIN, 5,405 U/m2

STUDY ID : rhTHROMBIN IMMUNOGEN
 FATE: ALL
 DAYS ON TEST: ALL

STUDY NUMBER: 1509175

SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1F (1)	2F (2)	3F (3)
NUMBER OF ANIMALS:		3	3	3
BRAIN	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		1	2	3
MINERALIZATION		0	1	0
CYST, MENINGES		0	1	0
SPINAL CORD	# EX	3	3	3
SCIATIC NERVE	# EX	3	3	3
THYROID	# EX	3	3	3
ECTOPIC THYMUS		0	0	1
MONONUCLEAR CELL INFILTRATE		1	0	1
FOLLICULAR DEGENERATION		1	1	1
CYST		0	1	0
PARATHYROID	# EX	2	2	2
ADRENAL	# EX	3	3	3
HYPERPLASIA, CORTEX		0	0	1
MINERALIZATION		1	0	0
PITUITARY	# EX	3	3	3
CYST		0	2	0
KIDNEY	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		3	3	3
GLOMERULOSCLEROSIS		0	0	1
SCAR, CORTEX		0	0	1
CAST, PROTEIN		0	1	0
URINARY BLADDER	# EX	3	3	3
CERVIX	# EX	3	3	3
UTERUS	# EX	3	3	3
OVARY	# EX	3	3	3
MINERALIZATION		2	3	1

(1) - VEHICLE CONTROL

(2) - BOVINE THROMBIN, 5,405 U/m2

(3) - rhTHROMBIN, 346 U/KG

STUDY ID : rhTHROMBIN IMMUNOGEN STUDY NUMBER: 1509175

FATE: ALL

DAYS ON TEST: ALL

SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:

NUMBER OF ANIMALS:

1F (1) 2F (2) 3F (3)
3 3 3

OVARY	#	#	#	#
CYST	# EX	3	3	3
		0	1	0
VAGINA	# EX	3	3	3
TRACHEA	# EX	3	3	3
LUNG	# EX	3	3	3
BRONCHIOLECTASIS		1	0	0
MONONUCLEAR CELL INFILTRATE, PERIVASCULAR		0	1	0
HEART	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		1	1	1
AORTA	# EX	3	3	3
SKIN	# EX	3	3	3
INFLAMMATION, ACUTE		0	0	1
HEMORRHAGE		0	0	1
MAMMARY GLAND	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		0	1	0
TONGUE	# EX	3	3	3
GRANULOMA		0	0	1
REGENERATION, MUSCLE		1	0	0
SKELETAL MUSCLE	# EX	3	3	3
ESOPHAGUS	# EX	3	3	3
CANDIDIASIS		0	0	1
STOMACH	# EX	3	3	3
DUODENUM	# EX	3	3	3
JEJUNUM	# EX	3	3	3

(1) - VEHICLE CONTROL

(2) - BOVINE THROMBIN, 5,405 U/m2

(3) - rhTHROMBIN, 346 U/KG

STUDY ID : rhTHROMBIN IMMUNOGEN

STUDY NUMBER: 1509175

FATE: ALL

DAYS ON TEST: ALL

SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1F (1)	2F (2)	3F (3)
NUMBER OF ANIMALS:		3	3	3
ILEUM	# EX	3	3	3
COLON	# EX	3	3	3
HEMORRHAGE		1	1	1
GRANULOMA, PARASITIC (OESOPHAGOSTOMUM)		1	0	0
CILIATE PARASITE, INTRALUMINAL (BALANTIDIUM)		0	0	1
CECUM	# EX	3	3	3
CILIATE PARASITE, INTRALUMINAL (BALANTIDIUM)		1	2	3
RECTUM	# EX	3	3	3
MEDIASTINAL LYMPH NODE	# EX	3	3	3
LYMPHOID HYPERPLASIA		0	3	0
ERYTHROCYTOSIS		1	3	2
MESENTERIC LYMPH NODE	# EX	3	3	3
LYMPHOID HYPERPLASIA		1	2	0
THYMUS	# EX	3	3	3
INVOLUTION		3	0	1
CYST		0	2	2
HEMORRHAGE		1	1	0
SPLEEN	# EX	3	3	3
MANDIBULAR SALIVARY GLAND	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		2	3	3
MINERALIZATION		0	1	0
PANCREAS	# EX	3	3	3
LIVER	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		3	2	3
LIPIDOSIS		1	0	0
NECROSIS		0	1	0

(1) - VEHICLE CONTROL

(3) - rhTHROMBIN, 346 U/KG

(2) - BOVINE THROMBIN, 5,405 U/m2

STUDY ID : rhTHROMBIN IMMUNOGEN		STUDY NUMBER: 1509175		
FATE: ALL				
DAYS ON TEST: ALL		SEX: FEMALE		
INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS				
GROUP:		1F	2F	3F
		(1)	(2)	(3)
NUMBER OF ANIMALS:		3	3	3
		#	#	#
GALLBLADDER	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE		0	2	1
EYE	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE, CILIARY BODY		2	0	0
STERNUM (BONE & MARROW)	# EX	3	3	3
LYMPHOID NODULE		0	0	1
RIB (COSTOCHONDRAL JUNCT)	# EX	3	3	3
FEMUR (EPIPHYSEAL PLATE)	# EX	3	3	3
INJECTION SITE	# EX	3	3	3
MONONUCLEAR CELL INFILTRATE, PERIVASCULAR		3	3	3
OMENTUM	# EX	0	1	0
ECTOPIC SPLEEN		0	1	0

Conclusion:

The surgical exposures resulting from absorption of rhThrombin from the site of injury are estimated from clinical studies as follows: (spine) 8.6 mL x 1000 U/mL x 0.0037 (0.37%) absorption from injury site = 31.82 max 48 mL x 1000 U/mL x 0.0037 absorption from injury site = **177.6 U**; (liver) 17.2 mL x 1000 U/mL x 0.0037 absorption from injury site = 63.64 max 40 mL x 1000 U/mL x 0.0037 absorption from injury site = **148.0 U**; (Peripheral arterial) 10.3 mL x 1000 U/mL x 0.0037 absorption from injury site = 38.11 max 28 mL x 1000 U/mL x 0.0037 absorption from injury site = **103.6 U**; (Ateriovenous graft) 8.8 mL x 1000 U/mL x 0.0037 absorption from injury site = 32.56 max 20 mL x 1000 U/mL x 0.0037 absorption from injury site = **74.0 U**. Exposures in surgical bleeding are supported by the pivotal non-clinical toxicology study demonstrating safety at 346 U/kg dosed SC. This accounts for 1643.5 U absorbed in the pivotal non-clinical study or 9.25 fold the estimated maximum clinical trial exposure.

Local tolerance:

Dermal – Study 1499-003 FHSA Primary skin irritation/corrosion of rhThrombin

Objective: This study was designed to demonstrate the degree of skin irritation caused by rhThrombin at three different concentrations compared to commercially available bovine thrombin.

Methods:

Study design:

<i>n</i>	Treatment	Concentration ^a (U/mL)	Dose /site (mL)	Route of Administration	Duration of Exposure	Score (Hours Post-Dosing)
6	rhThrombin	100	0.5	Topical	24 hours	24 and 72
6	rhThrombin	1000	0.5	Topical	24 hours	24 and 72
6	rhThrombin	2000	0.5	Topical	24 hours	24 and 72
6	Thrombin Placebo (Vehicle)	N/A	0.5	Topical	24 hours	24 and 72

^a nominal concentration

Scoring and evaluation of skin reactions:

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation (preventing grading of erythema)	4
Edema Formation	Value
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised about 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Adapted from 16 CFR Part 1500.41.

Results:

The primary and average scores are listed shown in the following tables. Slight erythema was observed at abraded sites for two animals in the bovine thrombin group. No other significant local (topical) application site abnormalities were noted.

Cumulative primary dermal irritation scores for rhThrombin 100U/mL, 1000, 2000 U/mL:

Rabbit Number	Sum of 24 & 72 hr scores		Primary Irritation Score ¹
	Erythema (A)	Edema (B)	
34467	0	0	0
34468	0	0	0
34469	0	0	0
34470	0	0	0
34471	0	0	0
34472	0	0	0
Sum:			0
Mean Primary Irritation Score: ²			0

1. Sum of erythema and edema divided by 4
2. Sum of Primary Irritation Scores divided by 6

Rabbit Number	Sum of 24 & 72 hr scores		Primary Irritation Score ¹
	Erythema (A)	Edema (B)	
34453	0	0	0
34464	0	0	0
34485	0	0	0
34465	0	0	0
34454	0	0	0
34466	0	0	0
Sum:			0
Mean Primary Irritation Score: ²			0

1. Sum of erythema and edema divided by 4
2. Sum of Primary Irritation Scores divided by 6

Rabbit Number	Sum of 24 & 72 hr scores		Primary Irritation Score ¹
	Erythema (A)	Edema (B)	
34458	0	0	0
34459	0	0	0
34447	0	0	0
34461	0	0	0.25
34462	0	0	0.25
34463	0	0	0
Sum:			0.5
Mean Primary Irritation Score: ²			0.1

1. Sum of erythema and edema divided by 4
2. Sum of Primary Irritation Scores divided by 6

Conclusion: Topical administrations of 100, 1000 and 2000 U/mL were non-irritating to normal and abraded skin of six test rabbits.

Ocular – Study 1499-004 – Ocular irritation test (ISO) of rhThrombin

Objective: This study was designed to assess the potential for rhThrombin to cause ocular irritation topical application.

Methods:

Study design:

<i>n</i>	Treatment Right Eye	Concentration ^a (U/mL)	Dose (mL)	Treatment Left Eye	Dose (mL)	Score (Hours Post-Dosing)
3	rhThrombin	100	0.1	Control	None	1, 24, 48, 72
3	rhThrombin	1000	0.1	Control	None	1, 24, 48, 72
3	rhThrombin	2000	0.1	Control	None	1, 24, 48, 72
3	Thrombin Placebo (Vehicle)	N/A	0.1	Control	None	1, 24, 48, 72

^a nominal concentration

Scoring of ocular lesions:

Observation	Value ^{1,2}
Cornea	
Degree of opacity (most dense area used)	
No opacity	0
Scattered or diffuse areas, details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2*
Opalescent areas, no details of iris visible, size of pupil barely discernible	3*
Opaque, iris invisible	4*
Area of cornea involved	
One-quarter (or less), not zero	0
Greater than one-quarter, but less than half	1
Greater than half, but less than three-quarters	2
Greater than three quarters, up to whole area	3
Iris	
Normal	0
Folds slightly above normal, congestion, swelling, circumcorneal injection, (any or all or combination of these); iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*
Conjunctivae	
<u>Redness</u> – refers to palpebral and bulbar conjunctivae, excluding cornea and iris	
Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easy discernable	2*
Diffuse beefy red	3*
<u>Chemosis</u>	
No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids about half closed to completely closed.	4*
<u>Discharge</u>	
No discharge	0
Any amount different from normal (does not include small amount observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

1. ISO 10993-10:2002(E)

2. Positive reactions are started.

Results:

Preliminary eye irritation scores for 100 U/mL, 1000 U/mL and 2000 U/mL (Dose 0.1 mL):

Cytotoxicity:

Conclusion: Neither rhThrombin at concentration of 100, 1000 and 2000 U/mL (0.1 mL) nor thrombin Placebo (vehicle) caused a positive ocular irritation response in the eyes of test animals.

R = Right Eye, L = Left Eye																	
Animal Number	Sex	Wt. (kg)	Time After Dosing(hrs)	Cornea Opacity	Cornea Percent Area	Iris Redness	Conjunctivae Chemosis	Discharge	Animal Number	Sex	Wt. (kg)	Time After Dosing(hrs)	Cornea Opacity	Cornea Percent Area	Iris Redness	Conjunctivae Chemosis	Discharge
34483	F	2.3	1	0 0	0 0	0 0	0 0	0 0	34484	F	2.2	1	0 0	0 0	0 0	0 0	0 0
			24	0 0	0 0	0 0	0 0	0 0				24	0 0	0 0	0 0	0 0	0 0
			48	0 0	0 0	0 0	0 0	0 0				48	0 0	0 0	0 0	0 0	0 0
			72	0 0	0 0	0 0	0 0	0 0				72	0 0	0 0	0 0	0 0	0 0
34490	F	2.3	1	0 0	0 0	0 0	0 0	0 0	34486	F	2.2	1	0 0	0 0	0 0	0 0	0 0
			24	0 0	0 0	0 0	0 0	0 0				24	0 0	0 0	0 0	0 0	0 0
			48	0 0	0 0	0 0	0 0	0 0				48	0 0	0 0	0 0	0 0	0 0
			72	0 0	0 0	0 0	0 0	0 0				72	0 0	0 0	0 0	0 0	0 0
34455	M	2.0	1	0 0	0 0	0 0	0 0	0 0	34452	M	2.1	1	0 0	0 0	0 0	0 0	0 0
			24	0 0	0 0	0 0	0 0	0 0				24	0 0	0 0	0 0	0 0	0 0
			48	0 0	0 0	0 0	0 0	0 0				48	0 0	0 0	0 0	0 0	0 0
			72	0 0	0 0	0 0	0 0	0 0				72	0 0	0 0	0 0	0 0	0 0
34488	F	2.3	1	0 0	0 0	0 0	0 0	0 0									
			24	0 0	0 0	0 0	0 0	0 0									
			48	0 0	0 0	0 0	0 0	0 0									
			72	0 0	0 0	0 0	0 0	0 0									

ZGI 1499-003 (GLP) and RES 1499-002 (Non-GLP) – rhThrombin cytotoxicity – ISO elution test

Objective: This study was performed to determine the in vitro biologic reactivity of mammalian cell cultures to rhThrombin at three different concentrations using mouse L929 fibroblast cells cultured for 48 hours.

Methods:

Experimental design:

Treatment	Initial Concentration ^a (U/mL)	Percent of MEM/Test article	Final concentration ^b (U/mL)	Incubation Period (hours)	Scoring (post incubation period) (hours)
rhThrombin	100	75/25	25	48 ± 2	24 ± 1 and 48 ± 2
rhThrombin	1000	75/25	250	48 ± 2	24 ± 1 and 48 ± 2
rhThrombin	2000	75/25	500	48 ± 2	24 ± 1 and 48 ± 2
Thrombin Placebo (Vehicle)	N/A	75/25	N/A	48 ± 2	24 ± 1 and 48 ± 2

^a nominal concentration before dilution in MEM

^b nominal concentration after dilution in MEM

Results:

In mouse L292 cells, rhThrombin was found to be cytotoxic at concentrations of 250 and 500 U/L. The definition of cytotoxicity was defined by abnormal morphological changes in the cells after 24 and 48 hours of incubation. This finding was comparable to commercially available bovine Thrombin.

Reactivity grades for elution test:

Grade	Reactivity	Condition of Cell Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis or empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

Test results:

Treatment	Reactivity 24 hrs	Grade 24 hrs	Reactivity 48 hrs	Grade 48 hrs
rhThrombin 25 U/mL ^a , Dish #1	None	0	None	0
rhThrombin 25 U/mL, Dish #2	None	0	None	0
rhThrombin 25 U/mL, Dish #3	None	0	None	0
rhThrombin 250 U/mL, Dish #1	Mild	2	Severe	4
rhThrombin 250 U/mL, Dish #2	Mild	2	Severe	4
rhThrombin 250 U/mL, Dish #3	Mild	2	Severe	4
rhThrombin 500 U/mL, Dish #1	Severe	4	Severe	4
rhThrombin 500 U/mL, Dish #2	Severe	4	Severe	4
rhThrombin 500 U/mL, Dish #3	Severe	4	Severe	4
Thrombin Placebo (vehicle), Dish #1	None	0	None	0
Thrombin Placebo (vehicle), Dish #2	None	0	None	0
Thrombin Placebo (vehicle), Dish #3	None	0	None	0
Controls				
Positive #1	Moderate	3	Severe	4
Positive #2	Moderate	3	Severe	4
Positive #3	Moderate	3	Severe	4
Negative #1	None	0	None	0
Negative #2	None	0	None	0
Negative #3	None	0	None	0
Reagent #1	None	0	None	0
Reagent #2	None	0	None	0
Reagent #3	None	0	None	0

^a Final concentration of rhThrombin in culture media

Conclusion:

Based on cellular morphology changes, a final concentration of 25 U/mL rhThrombin and Thrombin placebo met ISO 10993-5 requirements and are not classified as cytotoxic. In contrast rhThrombin at final concentrations of 250 and 500 U/L was found to cause marked

cytotoxicity to L929 cells following continuous exposure over 48 hours. These findings are consistent with the known biology of thrombin to alter cell shape and cellular attachment.

Impurity Toxicity:

1499-006 A toxicity study of single intravenous, SC or repeated SC doses of prothrombin activator in male and female ----- mice

Objective: To evaluate the toxicity of PTA by single IV, SC and repeated SC dosing to determine toxicity and immunogenicity. This study is supported by **ZGI 1499-008**.

Groups and Dosing:

Group/ Color Code	Dosing Day 1 (M/F)	Dosage/ Route (mg/kg)	Dose Concentration (mg/mL)	Dose Volume ^a (mL/kg)	Sacrifice		Eartag No.	
					Terminal (M/F, Day)	Recovery (M/F, Day)	Females	Males
Main Study								
1 / white	18/18	0 (iv)	0	5	9/9/group, Day 2	9/9/group, Day 14	B731 - B748	B621 - B638
2 / yellow	18/18	0.0005 (iv)	0.0001	5			B749 - B766	B639 - B656
3 / green	18/18	0.0025 (iv)	0.0005	5			B767 - B784	B657 - B674
4 / blue	18/18	0.0075 (iv)	0.001	5			B785 - B802	B675 - B692
5 / red	18/18	1.0 (iv ^a)	0.2	5			B803 - B820	B693 - B710
5a/red	6/6	1.0 (sc)	0.2	5	3/3/group, Day 2	3/3/group, Day 14	B835 - B840	B725 - B730
Satellite Study								
Group/ Color Code	Dosing Days 1, 8, 15, 22 (M/F)	Dosage/ Route (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Sacrifice (M/F, Day)	Eartag No.		
						Females	Males	
6 / gray	7/7	0 (sc)	0	5	7/7 group, Day 35	B821 - B827	B711 - B717	
7 / black	7/7	1.0 (sc)	0.2	5		B828 - B834	B718 - B724	

^a Total dose volume (mL) was calculated based on the most recent body weight. Dose volumes were rounded (up) to the next readable syringe increment (100µl) according to SOP GES044.

^b Dosing of females in Group 4 was stopped after one animal was dosed.

^c Intravenous injection (received the test-article intravenously in error)

^d Subcutaneous injection (given to available spare animals)

* Animals received dose intravenously instead of subcutaneously

Results:

There were no signs of toxicity observed at dosing levels evaluated (maximum dose 1.0 mg/kg)

Conclusion:

Absorption of up to 66.5 mg in a 70 kg adult (70kg PTA x 0.95 = 66.5) appears to be safe. All animals treated at any dose level developed anti-PTA antibodies. Residual PTA limits should be set such that levels of PTA in the end product result in a 10 fold less exposure of PTA following topical use.

Labeling:
