

Final Review - RECOTHROM

MEMORANDUM

Food and Drug Administration
Center for Biologics Evaluation and Research

DATE: 11-NOV-07
TO: STN 125248
SPONSOR: Zymogenetics
PRODUCT: Recombinant Thrombin
ORIGINAL REVIEWER: Paul Aebersold, Ph.D
FROM: Nisha Jain, M.D., Clinical Review Branch, HFM-392
SUBJECT: Final review of the BLA (STN 125248) for, ZymoGenetics Recombinant Thrombin,
TO: Mark Shields, Regulatory Project Manager, HFM-380
THROUGH: Toby Silverman, M.D., Chief, Clinical Review Branch, HFM-392

Executive summary:

The Phase 3 trial met its primary hemostasis endpoint, based on modified ITT analysis, indicating that recombinant Thrombin is non-inferior to licensed bovine Thrombin when used according to one of its indicated methods of application, a method commonly used in clinical practice. Adverse events were on the whole similar between treatment groups and consisted primarily of ones that are not uncommon in patients undergoing surgery.

Regarding immunogenicity, antibodies to bovine thrombin developed in 43 control subjects, whereas antibodies to rThrombin developed in 3 subjects (One also had anti CHO antibodies). Subjects who developed antibodies in the rThrombin group did not neutralize native human thrombin. Additionally, no apparent correlation was observed between study drug volume administered and anti-product antibody formation. Development of antibodies in either group did not lead to any adverse events such as excessive bleeding. Limited data is available on repeat exposure to the rThrombin.

In conclusion, the data submitted support the licensure of the rthrombin for use as a general adjunct to hemostasis during surgery when control of bleeding from oozing surfaces, capillaries and small venules, by standard surgical techniques is ineffective or impractical. It may be used in conjunction with gelfoam.

Post marketing:

The sponsor has committed to conduct a post marketing study to evaluate immunogenicity and safety of re-exposure to rthrombin.

Timelines:

Protocol concept document:	2 Nov 07
Final Protocol submitted	90 days following approval
FPFV	Approximately 6 months following approval of final protocol
LPLV	Up to 25 months after FPFV
CSR	clinical study report

CSR=clinical study report, FPFV= First patient first visit, LPLV= last patient last visit
The sponsor has submitted a concept protocol on Nov 2, 2007. The phase 4 protocol is designed as an open label, multicenter study to assess immunogenicity and safety of RECOTHROM in subjects who were administered recombinant thrombin in the pivotal study and are undergoing re-treatment with the product during surgical procedure. The population sample pool will also be expanded to include subjects who were exposed to rthrombin in phase 2 and subjects undergoing any type of surgery should some of these require repeat surgery. The outlined concept of assessing immunogenicity with repeat exposure of the product in the submitted concept sheet is acceptable. The sample size with assumptions and justification will be submitted in the final protocol and will be negotiable.

PROPRIETARY NAME:

FDA has found the Proprietary name " RECOTHROM" for topical recombinant thrombin (human), acceptable.

PREA:

The sponsor has asked for deferral for pediatric studies for all age groups. The projected for submitting the pediatric plan is June 2008, with initiation of studies in September 2008 and completion by June 2009 and submission of the completed clinical study report by December 2010. A written acknowledgement for agreeing to pediatric study deferral plan has not been sent.

SUMMARY

The Phase 3 trial met its primary hemostasis endpoint, based on modified ITT analysis, indicating that recombinant Thrombin is non-inferior to licensed bovine Thrombin when used according to one of its indicated methods of application, a method commonly used in clinical practice.

Adverse events were on the whole similar between treatment groups and consisted primarily of ones that are not uncommon in patients undergoing surgery.

As expected, antibodies to bovine thrombin developed in 43 control subjects, whereas antibodies to rThrombin developed in 3 subjects.

INTRODUCTION

Recombinant Thrombin (rThrombin) is produced in Chinese hamster ovary cells from the human DNA sequence.

Bovine Thrombin-JMI is a licensed biologic. THROMBIN-JMI® is indicated as an aid to hemostasis whenever oozing blood and minor bleeding from capillaries and small venules is accessible. In various types of surgery, solutions of THROMBIN-JMI® may be used in conjunction with an Absorbable Gelatin Sponge, USP for hemostasis.

In clinical practice, thrombin is very frequently used with an Absorbable Gelatin Sponge. Such sponges are indicated as adjuncts to hemostasis and their labeling refers to use with topical thrombin, e.g.: GELFOAM. Absorbable Gelatin Sponge can be used either with or without thrombin to obtain hemostasis.

CLINICAL STUDIES

The application contains the following study reports:

A Two-Part Phase 1/2 Study of the Safety and Efficacy of Topical rhThrombin in Subjects Undergoing Surgery on the Bony Portions of the Spine

A Phase 2, Randomized, Double-Blind Study of the Safety and Efficacy of Topical rhThrombin in Subjects Undergoing Partial Hepatectomy

A Phase 2, Randomized, Double-Blind Study of the Safety and Efficacy of Topical rhThrombin in Subjects Undergoing Peripheral (Infrainguinal) Arterial Bypass Surgery

A Phase 2, Randomized, Double-Blind Study of the Safety and Efficacy of Topical rhThrombin in Subjects Undergoing Arterio-Venous Graft Formation with Synthetic Conduit for Hemodialysis

A Phase 3, Randomized, Double-Blind, Controlled, Comparative Efficacy and Safety Study of Topical Recombinant Human Thrombin (rhThrombin) and Thrombin-JMI (Bovine Thrombin) in Surgical Hemostasis

PHASE 2 TRIALS

In the Phase 2 trials, subjects in the test group received rThrombin with an absorbable gelatin sponge and subjects in the control group received an absorbable gelatin sponge alone. The sponsor considered that the licensed Thrombin-JMI in clinical practice is used mostly with an absorbable gelatin sponge, not by itself, and therefore desired to evaluate rThrombin as it would be expected to be most commonly used in surgery.

In the Phase 2 trials, over all the surgeries and evaluation sites combined, hemostasis was achieved more rapidly with rThrombin plus absorbable gelatin sponge than with absorbable gelatin sponge alone. However, there was essentially no difference in hemostasis in the two groups in spinal surgery and only a modest difference in favor of the investigational agent in liver surgery. Hemostasis was achieved more rapidly in the rThrombin group in vascular surgeries, at both the arterial and venous sites in arterio-venous graft formation and at both the proximal and distal sites in arterial bypass surgery, demonstrating a possible contribution of thrombin in reducing time to hemostasis.

FDA advised the sponsor that while a Phase 3 trial in multiple surgeries may support a general hemostasis indication, the Agency would review the results of individual surgeries on their own. If the Phase 3 findings were to reproduce the Phase 2 results, there would be no finding of benefit for rThrombin in spinal surgery and thus no contribution of spinal surgery results toward a general hemostasis indication. FDA further advised the sponsor that since the licensed Thrombin-JMI is indicated for use with an absorbable gelatin sponge, an alternative Phase 3 trial consideration would be a non-inferiority comparison of rThrombin with Thrombin-JMI, both administered with an absorbable gelatin sponge as indicated for Thrombin-JMI. The sponsor chose the non-inferiority trial design for Phase 3, presumably to reduce the possibility of an indication restricted to specific types of surgery as opposed to a general indication.

PHASE 3 TRIAL

The primary endpoint for this study was the incidence of hemostasis at 10 minutes, and the primary analysis was for non-inferiority of the investigational rThrombin compared to the

licensed bovine thrombin. Blinded study thrombin was administered with a gelatin sponge, pre-cut to the needed size. The types of surgery and associated primary hemostasis evaluation sites were:

1. Spinal; epidural venous plexus
2. Hepatic; hepatic resection site
3. Peripheral artery bypass; proximal anatomizes
4. Arteriovenous graft formation for hemodialysis; arterial anastomoses

Four hundred and sixty-three subjects were randomized, of whom 411 received blinded study thrombin. Randomization was revealed to the pharmacist in advance of surgery simply for the practical reason of preparing only one of the study thrombins, and thus not all randomized subjects ended up needing an adjunct to hemostasis (52 did not need an adjunct to hemostasis). The investigator and operating room staff were blinded at all times to study treatment. 122 subjects were treated for spine surgery, 125 for liver surgery, 88 for peripheral artery bypass surgery, and 76 in arteriovenous graft surgery.

STUDY RESULTS:

PATIENT CHARACTERISTICS

Baseline comorbidities were comparable between treatment groups overall, but differed in some aspects according to type of surgery, as would be expected. Demographic characteristics were comparable between treatment groups within each surgery type. Baseline coagulation parameters (prothrombin time, activated partial thromboplastin time, international normalized ratio, and platelet count) were comparable between treatment groups overall and within each type of surgery, as were concomitant medications that might affect coagulation (i.e., heparin, most frequently used in peripheral artery bypass surgery).

EFFICACY EVALUATION

Of 411 subjects who needed an adjunct to hemostasis 10 subjects were excluded from the primary analysis plan as they were not treated for 1 of the 4 primary bleeding sites described in the protocol. The analysis of primary endpoint presented by the sponsor is thereby a modified ITT analysis. The primary endpoint of hemostasis at 10 minutes, was achieved in 95.42% of subjects in the test group and in 95.11% of subjects in the control group. This represents a 0.3% (95% CI, -3.7% to 4.4%) difference in subjects receiving rThrombin compared to those receiving bovine thrombin, that is well within the pre-specified non-inferiority margins. If the analysis is performed on ITT population, the pre-specified non-inferiority margin will still be met (based on my calculations).

Hemostasis Within 10 Minutes ¹		
	TRADENAME (N=198) (%)	Comparator (bovine thrombin) (N=203) (%)
Overall	95.4%	95.1%
Spinal surgery	98.4%	98.4%
Hepatic resection	98.4%	96.8%
Peripheral arterial bypass	85.0%	85.7%
Arteriovenous graft formation	97.1%	97.3%

Hemostasis Within 10 Minutes ¹

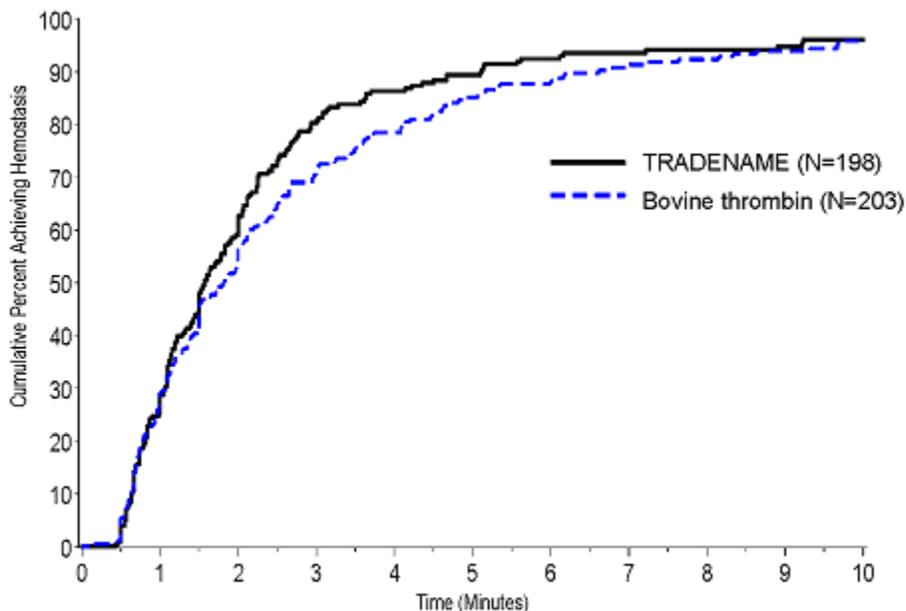
¹The primary efficacy analysis evaluated incidence of hemostasis at ≤ 10 minutes for subjects treated at 1 of 4 primary TTH bleeding site types: epidural venous plexus, hepatic resection site, peripheral arterial bypass proximal anastomosis, and arteriovenous graft arterial anastomosis (401 efficacy evaluable subjects).

Cumulative Incidence of Hemostasis Over Time ¹

Time (Minutes)	TRADENAME (N=198) n (%)	Comparator (bovine thrombin) (N=203) n (%)
1.5	95 (48%)	93 (46%)
3	160 (81%)	146 (72%)
6	183 (92%)	178 (88%)
10	189 (95%)	193 (95%)

²Includes 401 efficacy evaluable subjects.

A Kaplan-Meier curve of time to hemostasis supports that rThrombin is non-inferior to the licensed bovine thrombin. At 3 minutes hemostasis was achieved in a slightly higher proportion of subjects in the test group than in the control group.



There were no substantial differences between study centers or between surgeons in terms of treatment effect. There were also no substantial differences in treatment effect according to gender, age, anti-coagulation medication, low baseline platelets, high baseline PT, high baseline INR, or high baseline aPTT.

SECONDARY ENDPOINTS:

Health Outcomes

Hospital Stay

The average hospital stays were 5.5 days for subjects in the test group and 5.0 days for subjects in the bovine thrombin group. The average duration of additional hospital stays (not directly due to initial surgery) was also longer for subjects in the test group, 8.5 days versus 6.0 days. With regard to initial hospital stays for liver surgery, 6/62 subjects in the test group contributed significantly to the average with ≥ 20 days hospital stay each. The longest hospital stay among 62 subjects in the control group was 16 days.

Use of Blood Products

The sponsor notes that red blood cell transfusions from surgery to day 29 were approximately the same in both treatment groups, yet considers the results for subjects who received > 1000 ml to be noteworthy. On the day of surgery, 6 subjects in the bovine thrombin group received > 1000 ml of packed RBC or whole blood, compared to only 1 subject in the rThrombin group. Inclusive to day 29, the respective numbers were 13 versus 8 subjects. These anecdotal findings do not seem any more noteworthy than the longer average hospital stay for subjects in the rThrombin group.

Re-operation

The numbers of subjects who needed to be taken back to surgery were comparable between treatment group, both overall and for each type of surgery independently.

SAFETY EVALUATION

Adverse Events (AEs)

AEs are summarized by percentage of subjects who experienced them, with essentially all subjects experiencing one or more AEs. If one group experienced more adverse events per subject of a given type, this form of data presentation would mask such a difference. The sponsor was asked to submit tables of AEs by absolute number as a midcycle review information request. Tables should be submitted for all AEs in the study and for AEs in each of the four types of surgery. AEs should be grouped by system organ class and the totals for each system organ class should be included in the tables.

AEs occurring in ≥ 10 % of subjects in test group

	bThrombin	rhThrombin	Total
Preferred Term ¹	(N=206) n (%)	(N=205) n (%)	(N=411) n (%)
ANY	205 (100)	204 (100)	409 (100)
Incision site complication	130 (63)	129 (63)	259 (63)
Procedural pain	71 (34)	59 (29)	130 (32)
Nausea	73 (35)	58 (28)	131 (32)
Constipation	52 (25)	46 (22)	98 (24)
Pyrexia	40 (19)	41 (20)	81 (20)
Oedema peripheral	21 (10)	32 (16)	53 (13)
Anaemia	22 (11)	29 (14)	51 (12)
Vomiting	30 (15)	27 (13)	57 (14)
Insomnia	19 (9)	25 (12)	44 (11)
Tachycardia	18 (9)	21 (10)	39 (9)
Hypotension	18 (9)	20 (10)	38 (9)

There were 7 AEs in the test group assessed as treatment-related, versus 2 AEs in the control group. In the test group, the treatment-related AEs were arteriovenous graft thrombosis, arthralgia, ecchymosis, incision site hemorrhage, peripheral oedema, pulmonary embolism, thrombocytopenia, and vascular graft complication. In the control group, they were incision site hematoma and post procedural hematoma. However, similar AEs were in many cases reported without relation to treatment. For example, when combining arteriovenous graft thrombosis and vascular graft complication, there were 5 AEs in each group. For peripheral oedema, there were 21 AEs in the control group and 32 AEs in the rThrombin group, somewhat of an imbalance.

Thirty-six subjects in the test group had serious AEs compared to 46 subjects in the control group. Two treatment-related SAEs occurred in the rThrombin group: pulmonary embolism and arterio-venous graft thrombosis.

Events of Heightened Surveillance (EHS)

Certain adverse events were prospectively defined in Phase 3 as "adverse events of heightened surveillance" (EHS) due to their potential association with the mechanism of action of rhThrombin or bovine thrombin, known clinical sequelae of cross-reacting antibodies, the use of a gelatin sponge for delivery of rhThrombin or bovine thrombin, or based on Phase 2 results.

- Bleeding events were evaluated based on coagulopathy and bleeding sequelae noted in the literature following development of antibodies to bovine thrombin.
- Thromboembolic events were evaluated based on the mechanism of action of thrombin in clot formation
- Cardiac events were evaluated based on the principles driving the selection of thromboembolic events
- Hypersensitivity events were evaluated because the study drugs tested are protein-based therapeutics,
- Postoperative wound infections,
- Other infections not associated with postoperative wound or graft infections or abscesses were evaluated to rule out dissemination of infectious agents from a subclinical wound infection or abscess.
- Nausea and vomiting were evaluated because of the higher incidence of these events in Phase 2 studies for subjects receiving rhThrombin compared with placebo.

Table showing above EHS in phase 3 study

AE Category	bThrombin (N=206) n (%)	rhThrombin (N=205) n (%)	Total (N=411) n (%)
Subjects with any EHS	136 (66)	124 (60)	260 (63)
Bleeding	24 (12)	27 (13)	51 (12)
Cardiac	38 (18)	41 (20)	79 (19)
Hypersensitivity	37 (18)	30 (15)	67 (16)
Nausea + vomiting	83 (40)	68 (33)	151 (37)
Other infection	31 (15)	26 (13)	57 (14)
Post-op wound infection	22 (11)	19 (9)	41 (10)
Thromboembolic	10 (5)	12 (6)	22 (5)

Overall there was no difference in the occurrence of these EHS in the two treatment groups.

Thromboembolic events:

There is a slightly higher incidence of cardiac events in the test group compared to the control group but not statistically significant. Cardiac events like MI were also evaluated as TE events in the Phase 3 study. Myocardial infarctions were reported for 4 subjects in the rhThrombin group. All of the MIs were reported on Day 3, but it is possible that the MIs were evolving prior to Day 3. The verbatim term "non-ST segment elevation myocardial infarction" for 3 subjects and "myocardial infarction" for 1 subject is reported in the database. Upon review of the complete history, it appears that each of these subjects [638-P-6327 (lumbar surgery), 639-P-6290 (liver surgery with underlying neoplasm), 633-L-6043 and 606-S-6007 (PAB surgery for peripheral vascular disease)] had multiple risk factors for MI. Hence I agree with the sponsor that all 4 events were probably unrelated to study treatment.

In the Phase 2 studies, rhThrombin-treated subjects did not have an increased incidence of thromboembolic or cardiac AEs or SAEs relative to placebo. No MIs were reported during the Phase 2 studies.

Pulmonary embolism occurred in one subject (612-L-6331) in the test group. This was assessed as treatment related by the investigator (see narrative below)

Narrative

For subject 612-L-6331, the investigator assessed a possible relationship between the (blinded) study material and Grade 4 bilateral pulmonary emboli the day after surgery. The 71-year-old female had a history of diabetes, hypertension, myocardial infarction, angina, coronary artery disease, right colectomy, and hypercholesteremia. She entered the study with colon cancer metastatic to liver. Given that the subject had risk factors for an embolic event, the possible relationship to (blinded) thrombin would appear to be a cautious assessment by the investigator based on the temporal proximity to application of the study material.

Hypersensitivity reaction

Hypersensitivity reactions were similar between treatment groups (rhThrombin, n=30, 15%; bThrombin, n=37, 18%). Hypersensitivity events in the immediate postoperative period were Day 1: overall n=16, 4%; (rhThrombin n=7, 3%; bThrombin n=9, 4%). The incidence of hypersensitivity events was similar between groups from Day 1 through Day 3 and from Day 1 through Day 29. The incidence was also similar between treatment groups of each surgery type.

Nausea and Vomiting

A slightly lower incidence of nausea and vomiting was observed in the rhThrombin group compared with the bovine thrombin group (rhThrombin, n=68, 33%; bThrombin, n=83, 40%).

Postoperative Wound and Other Infections:

The incidence of postoperative wound infection was similar between treatment groups from Day 1 through Day 3 and from Day 1 through Day 29. A higher incidence of postoperative wound infections was observed for subjects who underwent hepatic resection surgery in both treatment groups compared with other surgery types. Serious adverse events of postoperative wound infection were reported with similar frequency in both treatment groups (rhThrombin n=9, 4%; bThrombin n=10, 5%) Only 1 of the serious adverse events of postoperative wound infection was reported as occurring within 72 hours of surgery, in a subject (631-S-6386), who received bovine thrombin.

Bleeding events:

No difference was observed in the overall incidence of bleeding events between treatment

groups on Day 1, from Day 1 through Day 3, and from Day 1 through Day 29. A higher incidence of bleeding events was observed in subjects who underwent PAB or AV graft surgery compared with other surgery types. Serious bleeding was reported for 5 subjects (rhThrombin n=2, 1%; bThrombin n=3, 1%). Investigators assessed each of these events as not related to study treatment.

Deaths and its causes

Study ID-Site-Subject	Study Phase	Sex/Age (yrs)	Surgery Type	Treatment Group	Cause of Death	Study Day	Associated Morbidity Conditions
499E01-616-6277	3	M/75	Hepatic resection	rhThrombin	Severe sepsis	24	Septic shock; ARDS
499E01-607-6062	3	F/78	Spinal surgery	bThrombin	Cerebrovascular accident	14	Cerebrovascular accident
499E01-618-6283	3	M/66	Hepatic resection	bThrombin	Unknown	20	Cirrhosis
499C06-611-3021	2	F/74	Hepatic resection	rhThrombin	Hepatic insufficiency (failure); sudden cardiac arrest with pulseless electrical activity	7	Hepatitis C cirrhosis with hepatocellular carcinoma status post right lobe resection
499C06-612-3011	2	M/68	Hepatic resection	Placebo	Asystole	91	Respiratory failure

ARDS = acute respiratory distress syndrome
 Source: BLA/CSS/Tab1e12_IDeath

Brief narratives of some of the SAEs

Subject 612-L-6461 had a serious adverse event of wound evisceration, but there is no narrative for this SAE. ***Narrative on this subject is not included so the sponsor should be requested to submit a narrative.***

Subject 613-L-6363 had hemorrhagic ascites and bile leak on study day 9, detected by ultrasound. The subject underwent exploratory laparotomy and had surgical repair of the bile leak. The hemorrhagic ascites was attributed to the liver disease (metastatic neuroendocrine tumor) and the bile leak was assessed as not related to study treatment.

Subject 616-L-6277 had a number of serious adverse events: atrial fibrillation (day 3), aspiration pneumonia (day 5) subsequent to post-operative bibasilar atelectasis and intubation (day 4), cardiac arrest (days 9 and 10), ARDS (day 13), sepsis (day 18), septic shock (day 18), and death (day 24). These adverse events were assessed as unlikely or not related to study treatment.

Subject 633-L-6116 experienced infectious bile leak on study day 9 and an abdominal abscess on day 15. She was diagnosed with another abdominal abscess on day 24.

Subject 633-L-6504 experienced an abdominal abscess on study day 16, following intermittent fevers. This serious adverse event was described as a well-known complication of liver resections and unrelated to study treatment.

Immunogenicity (from Pauls's memo)

Antibodies to bovine thrombin developed in 34 control subjects, whereas antibodies to rThrombin developed in 3 subjects (anti CHO antibodies). Blood samples were collected at baseline and at day 29. For subjects randomized to rThrombin, the samples were analyzed by ELISA for antibodies to rThrombin, CHO host-cell protein, and pro-thrombin activator. For subjects randomized to bovine thrombin, the samples were analyzed by ELISA for antibodies to bovine thrombin.

Sample analysis was done in three tiers. In the screening Tier 1, a single dilution of each sample was incubated in a well of the ELISA plate. The cut-off absorbance value for a positive screening result was chosen such that 90-95% of samples from untreated normal volunteers would be negative, i.e., 5-10% of samples would be presumed to be false

positives. This assumption is flawed; given that a number of subjects entered into the trial had true positive baseline values, it could be assumed that some of the normal volunteers would also have true positive values. The validation report does not present data on the selection of the cut-off screening absorbance value. True positives, if any, should have been eliminated for purposes of selecting the screening cut-off absorbance, although they would have had little influence if they represented substantially less than 5% of the samples. **As an early deficiency, the sponsor was asked if the highest of the samples used to establish the cut-off absorbance for screening were further assessed to see if they were true positives.** Their response indicates that there were no robust signals from any of the 100 normal samples used to establish the cut-off value, acknowledging that any true positives could unduly influence the choice of a cut-off value. Despite lack of robust signals, they did assess titer and specificity of samples above the 92nd percentile and found 6 samples with low-titer but specific antibodies to rThrombin. They calculated what the cut-off value would be if data from these samples were excluded and found that the cut-off value was essentially unchanged, i.e., they were not sufficiently high to significantly change the mean optical density of the group. This issue is therefore satisfactorily resolved.

In the ELISA procedures, the "recommended" test sample dilution for Tier 1 was 1:50. In the Tier 2 analysis of positive Tier 1 samples, sample dilutions were 1:50, 1:150, 1:450, and 1:1350; titer was determined as the interpolated sample dilution whose absorbance was at the cut-point. It would thus appear that samples with antibody titers less than 50 would not be reported as positive. **As an early deficiency identified, the sponsor was requested to submit data from development of this assay to explain why 1:50 was the lowest dilution tested.** The responded that at dilutions lower than 1:50, the signal to noise ratio is not optimal and that at a dilution of 1:20 or lower the non-specific background signal is unacceptably high. This answer is self serving, since they do not want to repeat the assays; but if one wants to find low titer antibodies, one of course has to test for them at low dilutions, not high dilutions. The sponsor does acknowledge that there could be different implications for repeat exposure depending on whether a subject developed no antibodies or low-titer antibodies. The more persuasive part of their response on this matter is reference to the immunogenic study in six cynomolgus monkeys that were given four weekly subcutaneous injections of rThrombin. None of the monkeys developed antibodies or showed any signs of coagulopathy. This reviewer can accept the reluctance to conduct new assays, but recommends that the labeling should state that there is no experience with repeat exposure. Perhaps the sponsor could contact subjects who were treated with rThrombin to ask if they would be willing to participate in an evaluation of repeat exposure, should they need any further surgery.

The validation report does not assess relative sensitivities of the ELISAs for antibodies to bovine or recombinant thrombin. It appears from the procedures that plates for both ELISAs were coated with -----, but without comment as to relative specific activities of the two thrombins. Non-specific background quality control samples are listed in ABBREVIATIONS/TERMINOLOGY as ----- for the recombinant thrombin ELISA, whereas ----- was used for the bovine thrombin ELISA. Positive antibody controls were raised in ----- antibodies to bovine thrombin provided higher absorbance signals at lower concentrations than ----- antibodies to recombinant thrombin. It could well be that the titers are higher for ----- anti-bovine than for ----- anti-recombinant thrombin; but if the absorbances at plateau are

different, it could also be that antigen coatings for the two ELISAs are different, leading to differing sensitivities. **The sponsor was requested to provide data for each ELISA so that the plateau absorbance is demonstrated.** The response indicates that the plateaus are the same, so this question is resolved.

Bleeding AEs occurred in 24 control subjects and 27 test subjects, with no particular differences at day 1, through day 3, or through day 29. The sponsor summarized bleeding AEs in subjects with positive anti-product antibody results, showing that 6 out of 43 control subjects with antibodies to bovine thrombin also had bleeding AEs. However, most of these intersecting events would be expected by chance (43 antibody positive / 200 subjects x 24 bleeding AEs ~ 5). In any event, bleeding AEs occurred in these subjects starting on days 1, 1, 2, 3, 5, and 8, so it would be difficult to relate development or increase in titer of antibodies to the AEs. The sponsor does not conclude that the higher frequency of anti-product antibodies in the bovine thrombin group had any clinical manifestations, but neither do they mention that the bleeding AEs in the bovine thrombin group seem to have appeared in antibody positive subjects by chance alone. ***It is recommended that FDA advise the sponsor that bleeding AEs in antibody-positive subjects do not appear to have occurred at a higher frequency than in antibody-negative subjects and that they may not advertise or promote any association of bleeding with antibody status.***

Adverse Event Conclusions

The sponsor concludes that "although differences between treatment groups were observed in some analyses, the incidences were small and the differences appear more likely attributable to background adverse events in the surgical populations rather than meaningful differences between treatments." This reviewer agrees that there is no basis to conclude superiority for the test group. Summaries by absolute number of AEs are needed to make any judgment in the other direction.

MIDCYCLE RESPONSES:

The sponsor submitted the responses to mid-cycle information request related to clinical issues on July 24, 2007 and are outlined below. The partial response to the information on CMC and facility issues were submitted in August 2, 2007, within 3 months of the action due date of the BLA. This amendment contained 2000 pages of new information on analytical testing, manufacturing and facility, which were not previously reviewed by the Agency. This amendment was classified as major amendment leading to a three month extension of the action due date.

Recommendation:

- 1. Please submit a complete narrative on the subject 612-L-6461.**

Sponsor response:

Subject 612-L-6461, a 49-year-old male randomized to the rThrombin treatment group, experienced a serious adverse event of evisceration of the wound on study day 11 post surgery. Past medical history included:

17 March 2005: a right hemicolectomy for a colon cancer with liver and pulmonary metastases

October 2005: ileostomy for small bowel rupture and fecal peritonitis

March 2006: kidney stones

17 March 2006: adrenal insufficiency, bilateral deep vein thrombosis, pulmonary embolus on leading to inferior vena cava filter placement

On study day 1 (25 May 2006) the subject underwent anatomic resection of the right anteroinferior, right posteroinferior, right posterosuperior, and right anterosuperior hepatic segments (segments V, VI, VII, and VIII) due to metastatic colon cancer to the liver. Study treatment was applied to the bleeding surface of the left superomedial and left inferomedial segments (segments IVa and IVb), hemostasis was achieved within 10 minutes, and the gelatin sponge was not removed. The subject also underwent ileostomy reversal on 25 May 2006. Post-operatively, the subject experienced non-serious events bibasilar atelectasis, bilateral pleural effusions, decreased breath sounds, edema in the pelvic region, constipation, erythema at the wound sites, incision site pain, chronic renal insufficiency, hyperglycemia, hyperkalemia, hypomagnesemia, thrombocytopenia, oliguria, a seroma, serosanguineous wound drainage, and urinary tract infection.

On study day 11, fascial dehiscence was noted and evisceration was detected during the dressing change. The subject was immediately taken to the operating room for fascia repair and intra-abdominal lavage. An abdominal exploration was performed and no intra-abdominal abscesses or interloop abscesses were identified. The wound was closed and packed with gauze. The event resolved without sequelae. Anti-product antibody results were negative at baseline and the end of the study. Concomitant medications included hydrocortisone, Insulin, spironolactone, fluconazole, albuterol

Investigator assessment: The investigator assessed the event to be grade 4 and not related to study treatment.

Sponsor Assessment: "A causal role for rThrombin is considered highly unlikely in this serious adverse event as the subject had pretreatment risk factor including underlying malignancy, multiple prior abdominal surgical procedures, history of chronic fecal peritonitis, and longstanding exposure to corticosteroids."

Reviewer's assessment: Upon review of the case history, it is unlikely that the SAE is related to the product.

2. **For subject 612-L-6331, the investigator assessed a possible relationship between the (blinded) study material and Grade 4 bilateral pulmonary emboli the day after surgery. Please submit your assessment as to the the possible relationship with the product.**

Sponsor response:

Sponsor Assessment: The temporal relationship between study-drug administration and onset of the event suggests that a causal relationship is possible, but is considered unlikely due to confounding by pre-existing risk factors for thromboembolic events. These factors include older age, metastatic colon carcinoma (usually adenocarcinoma which may secrete prothrombotic tissue factor), immobilization postsurgery, and concomitantly administered conjugated estrogen (Premarin). In addition, a causal role is considered biologically implausible as rThrombin is rapidly bound to inhibitors and cleared on exposure to circulating blood. The subject incidence of pulmonary emboli observed in the rThrombin treatment group (1/205, or approximately 0.5%) is within the range of subject incidences for pulmonary emboli described in studies of patients undergoing hepatic surgery for liver disease (0.4% (Alfieri, et al., 2001) to 0.6% (Belli, et

al., 2002)) and in a prospective epidemiologic study of patients undergoing major abdominal surgery (0.6% (Sakon, et al., 2006)).

Reviewer assessment: I agree with the sponsor that the subject had numerous predisposing risk factors for a thrombotic event. However, inadvertent intravascular injection of thrombin has the potential for causing thrombotic events such as PE. The Physicians are warned against the potential for occurrence of such events by capturing it in the PI under Warnings and Contraindication section.

- 3. Please submit a summary table of adverse events by number of adverse events, i.e., not percentage of subjects with adverse events. The table should include all specific adverse events in the study, grouped by system organ class. The total number of adverse events for each system organ class and for the entire study should also be included in the table.**

Sponsor Response:

The sponsor has submitted the above information as Table 1.

Reviewer Response:

Upon review of this table, no statistical difference with regards to safety was seen between the two treatment groups.

- 4. For subjects who developed peripheral edema in the test group, please submit in a tabular format under the following headings: Type of surgery, day when edema was recorded, cardiac and renal laboratory values, hospital stay and use of blood products.**

Sponsor Response:

The sponsor systematically reviewed all adverse event reports of TEPE to assess potential associations with TEPE and surgery type, clinical findings, time to event onset, preoperative laboratory indices of renal function, hospital length of stay for study surgery, and use of blood products. The MedDRA preferred term *Oedema peripheral* includes investigator verbatim terms which describe unilateral, bilateral, or generalized edema or swelling. Therefore, a wide range of clinical conditions are captured by the same MedDRA preferred term.

Reviewer response:

32/ 205 developed edema. The incidence of edema based on surgery type is presented in the table below:

	Subjects with TEPE ¹ n (%)
All subjects exposed to rThrombin (n=205)	32 (16)
Peripheral vascular surgery (n=82)	16 (20%)
PAB (n=44)	10 (23%)
AV graft (n=38)	6 (16%)
Nonperipheral surgery (n=123)	16 (13%)
Spine (n=61)	6 (10%)
Liver (n=62)	10 (16%)

The type of edema by surgery type is presented in the table below:

	Clinical manifestation of TEPE ¹	
	Localized n (%)	Bilateral or Generalized n (%)
All subjects exposed to rThrombin who developed TEPE (n=32)	14 (44)	18 (56)
Peripheral vascular surgery (n=16)	13 (81)	3 (19)
AV graft (n=6)	5	1
PAB (n=10)	8	2
Nonperipheral surgery (n=16)	1 (6)	15 (94)
Spine (n=6)	1	5
Liver (n=10)	0	10

The edema in subjects who underwent peripheral surgical procedures were localized to the surgical site. In non-peripheral surgeries, the edema (mostly generalized) was due to the disease process itself such as end-stage renal disease or metastatic cancer. None of the patients had a thromboembolic event. In conclusion, the edema was related to either the surgery type or due to the underlying disease and not related to the product. The imbalance seen in the incidence of edema (being higher in the test group) may be due to chance.

- You have summarized bleeding AEs in subjects with positive anti-product antibody results, showing that 6 out of 43 control subjects with antibodies to bovine thrombin also had bleeding AEs. However, most of these intersecting events would be expected by chance (43 antibody positive / 200 subjects x 24 bleeding AEs ~ 5). In any event, bleeding AEs occurred in these subjects starting on days 1, 1, 2, 3, 5, and 8, so it would be difficult to relate development or increase in titer of antibodies to the AEs. The higher frequency of anti-product antibodies in the bovine thrombin group had no clinical manifestations and bleeding AEs in the bovine thrombin group could have appeared in antibody**

positive subjects by chance alone. *Please be advised that bleeding AEs in antibody-positive subjects do not appear to have occurred at a higher frequency than in antibody-negative subjects and that they may not advertise or promote any association of bleeding with antibody status.*

Sponsor agreed.

Reviewer response:

The sponsor is advised against making any superiority claims with regards to immunogenicity because the study was not designed to assess any comparative analysis for immunogenicity data. Furthermore, the observed incidence of a positive signal in an assay may be influenced by several factors including timing of sampling, sample handling, concomitant medications, or underlying disease. Therefore, direct comparison of incidence of antibody development to recombinant or bovine thrombin or Factor V/Va following administration of rthrombin with incidence of antibody development following administration of other products may be misleading. Very limited data is available to assess immunogenicity on repeat exposure.

- 6. You have acknowledged that there could be different implications for repeat exposure depending on whether a subject developed no antibodies or low-titer antibodies, yet you do not propose to repeat the antibody assays to test for antibodies at titers lower than 1:50. Please explain how you propose to assess the potential risk of repeat exposure to recombinant Thrombin.**

The sponsor has submitted some data to support safety with regards to immunogenicity on repeat exposure.

Preclinical data:

A 6-week safety and immunogenicity study of rThrombin administered by subcutaneous (SC) injection to cynomolgus monkeys was conducted.

The study evaluated the safety (including histopathology) and potential incidence and degree of immunogenic response to rThrombin when administered via repeated SC injection to cynomolgus monkeys. Six monkeys were dosed by the SC route once weekly for 4 consecutive weeks. There were no signs of test-article-related toxicity; no animals developed antibodies to rThrombin. There were no signs of local or systemic antibody-mediated coagulopathy.

Clinical data:

7 subjects (3 subjects, Phase 3; 4 subjects, Phase 2) with pre-existing low-titer antibodies to rThrombin who participated in the rThrombin clinical studies. None of these subjects developed an increase in titer after exposure to rThrombin during surgery. No adverse events or serious adverse events related to antibody-induced coagulopathy were seen in these subjects. In addition, none of these subjects had evidence of neutralizing antibodies using a time-to-clot assay with human plasma

thrombin as the substrate.

Subject ID/Study	Screening	Baseline	Day 29
2021 (499C05) ¹	2.4	2.3	2.4
2036 (499C05)	2.0	1.9	2.0
5029 (499C08)	2.9	2.9	2.8
4026 (499C07)	1.7	NR ²	NR
639-P-6290 (499E01)	- ³	1.9	2.3
620-P-6491 (499E01)	-	2.1	1.9
624-P-6287 (499E01)	-	1.9	1.8 (NR) ⁴

¹ No subjects developed neutralizing antibodies. Data from Phase 2 Clinical Data Summary (Amendment 1; 9Aug06) Listing 4- Antibody Titers and Summary of Clinical Pharmacology Studies 2.7.2)

² NR indicates Not Reactive

³ "-" indicates sample not obtained (screening samples were not obtained in the Phase 3 trial)

⁴ (NR) indicates specificity could not be determined in Tier 3 of analysis

Plan to Assess the potential risk of re-exposure:

The sponsor plans to assess the risk of re-exposure by setting up a pharmacovigilance program. The sponsor has also proposed to conduct an open label study in the same cohort of patients who have participated in the phase 3 study and are candidates for repeat surgery. However, details of the proposal are not submitted.

7. **Please submit the case report forms of the subjects who developed antibodies in the test group.**

8. **Labeling:**

a. **Highlight section:**

i. **Indication and Usage section:**

Please delete the words, "is a coagulation factor."

Please rewrite this section as follows: "As a general adjunct to hemostasis during surgery when control of bleeding from oozing surfaces, capillaries and small venules, by standard surgical techniques is ineffective or impractical."

Please also insert the following sentence as a new paragraph:

"May be used in conjunction with an Absorbable Gelatin Sponge, USP."

ii. **Dosage and Administration section: Please bold, "For Topical Use Only". Please add the following sentence after the above sentence: "Apply on the surface of bleeding tissue only."**

iii. **Contraindication section: Rewrite this section as follows:**

- **Do not inject directly into the circulatory system.**
- **Do not use for the treatment of massive and brisk arterial bleeding.**

iv. **Warning and Precaution section: Include the following statement:
"Potential risk of thrombosis if absorbed systemically."**

v. **Please add the section, " Use in specific population**

b. Full Prescribing Information:

- i.
- ii. **Indication and Usage section: Please change as the highlight section**
- iii. **Contraindication: Please change as recommended for the highlight section**
- iv. **Warning and Precaution section: Please change as recommended for the highlight section**
- v. **Adverse Reactions section: Please follow the format as per Guidance for Industry: Adverse Reactions Section of Labeling for Human Prescription Drug and Biological Products - Content and Format. Please include the table highlighting the pre-specified events of heightened surveillance.**

The sponsor agreed to all changes except adverse reaction section. The sponsor is required to include relevant AEs that were seen in the clinical trial.

APPENDIX 1

Special Protocol Assessment

BB-IND: -----

Title: Thrombin (Human, Recombinant)

Submitted: August 15, 2005

Sponsor: ZymoGenetics, Inc.

PI: TBA

Reviewer: Paul Aebersold

Trial:

A Phase 3, Randomized, Double-Blind, Controlled, Comparative Efficacy and Safety Study of Topical Recombinant Human Thrombin (rhThrombin) and Thrombin-JMI (Bovine Thrombin) in Surgical Hemostasis

Objective:

1. To evaluate relative efficacy of the two thrombins, both administered with an absorbable gelatin sponge, as adjuncts to hemostasis in surgery.
2. To evaluate safety of rhThrombin and bovine thrombin.
3. To evaluate immunogenicity of rhThrombin and bovine thrombin.

Include:

1. Subject scheduled for one of the following surgical procedures:

Spinal surgery

Cervical, thoracic, or lumbar discectomy; corpectomy, laminectomy, lateral or interbody fusion including both anterior and posterior approaches in the cervical region.

Hepatic resection

Hepatic wedge resection or anatomic resection of 1 to 5 contiguous hepatic segments (open or hand-assisted laparoscopic); must not be combined with other abdominal procedures, except those involving only the gall bladder or bile duct. Subjects undergoing liver-related liver donation are also eligible.

Peripheral arterial bypass

Use of PTFE graft; may include revision procedures with graft-graft anastomoses.

Arteriovenous graft formation

PTFE for hemodialysis access; may include revision procedures with graft-graft anastomoses.

2. Subject 18 years old.
3. If female of child-bearing potential, negative pregnancy test within 14 days.
4. If sexually active male or sexually active female of child-bearing potential, agrees to use medically accepted form of contraception from time of consent to completion of follow-up study visits.

Exclude:

1. Known antibodies or hypersensitivity to thrombin or other coagulation factors.
2. Known sensitivity to Thrombin-JMI components or material of bovine origin.
3. Blood products within 24 hours of surgery.
4. Therapeutic surgical procedure within 30 days.
5. History of heparin-induced thrombocytopenia.
6. Known allergy to porcine collagen.
7. Factors that the investigator considers could impact safety or compliance.
8. Breast-feeding.
9. Experimental agent within 30 days.

Study:

Multicenter (up to 40 sites) study with 400 to 600 subjects, using 1000 IU/ml of rhThrombin or bovine Thrombin in combination with an absorbable gelatin sponge. At least 10% of the subjects will be assigned to each surgical setting. Not more than 40% of subjects will be in vascular surgery and not more than 50% of subjects will be in either hepatic or spinal surgery.

The primary endpoint is hemostasis at 10 minutes. The surgical areas for evaluation of hemostasis are epidural plexus bleeding in spinal surgery, large areas of diffuse bleeding from liver; and bleeding through suture holes in PTFE. If hemostasis is not achieved at 10 minutes, additional blinded study material or surgical method will be attempted first, followed by a non-thrombin containing topical hemostat, then by an alternative hemostat if needed. Factor VIIa may not be used. Other bleeding sites may be treated with blinded study material after the time to hemostasis evaluation.

A non-blinded pharmacist at each study site will reconstitute the required amount of rhThrombin or bovine Thrombin and provide the material to the investigator and surgical staff in blinded 5 mL syringes. Once the evaluation site has been identified, the syringes are to be emptied into a sterile basin and the gelatin sponge(s) (cut to the desired size if needed) immersed in the solution. Hemostasis is defined as occurring when no more blood from the bleeding site is observed seeping through or around the gelatin sponge. A gauze pad, pledget, or cottonoid is to be placed on the top of the sponge(s) and held in place with gentle pressure; this pad is to be changed every 30 seconds (or more frequently) until

hemostasis is achieved or for 10 minutes at which failure is declared. Bleeding that occurs after hemostasis is achieved and following sponge removal will not be considered a failure to achieve hemostasis.

If an eligible bleeding site is not identified, the subject will be withdrawn and replaced, with the same treatment assignment for the next subject randomized in the stratum from which the subject was withdrawn. Dynamic allocation will be used to attain an approximately equal number of subjects randomized to the two study Thrombins with each surgeon (site) and surgery type.

Evaluation:

At baseline (0 - 14 days pre-surgery): medical history, physical exam, hematology, serum chemistry, coagulation panel, immunogenicity, baseline conditions (AEs). On day 1 (surgery): surgical procedure information, AEs. On day 2 (16-48 hours post surgery closure): physical exam, hematology, serum chemistry, coagulation panel, AEs. At follow-up (day 26 - 36): physical exam, hematology, serum chemistry, coagulation, immunogenicity, AEs.

Analysis:

Blinded sample size recalculations will be performed prior to each interim analysis, which will occur at about 150 and 300 subjects. If the sample size needs to be increased to >500, a third interim analysis will be conducted. The IDMC may recommend stopping for efficacy at the 300 subject interim analysis (expecting that accrual will have reached 400 subjects) or at the third interim analysis, if the trial size is increased. The actual plan for interim analysis is to be provided in a separate SAP before the first analysis.

There is no mathematical statement of the null hypothesis. The text describes that the study is powered to exclude an absolute difference of >15% in the incidence of hemostasis at 10 minutes at an overall 0.025 level of significance, as indicated by the lower limit of the confidence interval. The four bleeding site types will be weighted equally in the estimation of the overall treatment effect. A secondary efficacy analysis will look at six bleeding sites, i.e., will consider the distal graft site in peripheral arterial bypass and the venous graft in the arteriovenous graft formation. The populations to be compared are subjects who received treatment and had a time to hemostasis recorded, whether or not censored.

Secondary endpoints are incidence and severity of adverse events, incidence and grade of clinical laboratory abnormalities, and incidence of antibodies. (One of the additional endpoints is incidence of re-operation for bleeding or thrombotic complications.) These outcomes will be summarized by treatment group. The antibody assays are to be conducted by sponsor staff who are to be given the treatment assignment for the samples.

Additional health outcome measures are duration of the surgical procedure from incision to closure; total length of hospital stay through Visit 4 (day 29); use of alternative hemostatic agents at evaluation site; use of blood products, including recombinant human clotting factors; and need for re-operation at the evaluation site for bleeding or thrombotic complications (note discrepancy between study objective above and this health outcome measure - at the evaluation site).

Qs & As:

1. Is incidence of hemostasis at 10 minutes acceptable for evaluating the comparative efficacy of rhThrombin and the licensed Thrombin-JMI?

Yes.

2. Is the plan to use a single concentration of Thrombin acceptable?

Yes. The sponsor asserts that concentrations of 100 and 250 IU/ml were less effective and that concentrations >1000 IU/ml provided no further benefit. Thrombin-JMI is recommended for use at 1000 to 2000 IU/ml where bleeding is profuse, as from abraded surfaces of liver or spleen, and is supplied with diluent for reconstitution at 1000 IU/ml. While it is theoretically possible that an exhaustive titration of concentrations in an animal model might reveal some difference between the two Thrombins, the plan to use the common 1000 IU/ml seems acceptable.

3. Is the 15% margin in absolute incidence of hemostasis for the primary efficacy analysis acceptable to conclude comparable efficacy?

Yes. There do not appear to be data available to show the effect of Thrombin-JMI when used with an absorbable gelatin sponge, for which it is indicated. FDA has advised another sponsor that a 15% margin is acceptable for comparing a new Thrombin to the licensed thrombin, both in conjunction with gelatin sponges, and that sponsor's Phase 3 trial has been underway for some time. Thus the same margin must be acceptable for this second sponsor of a new Thrombin. Earlier it did not appear to be plausible to require the other sponsor to generate data on the contribution of Thrombin to the gelatin sponge, given that the licensed Thrombin is indicated for that use. If the treatment effect were to be small to non-existent, then a new sponsor could not obtain with a reasonably-sized trial or any trial the same indication as enjoyed by Thrombin-JMI. While FDA could have required comparison of the new Thrombin alone to no treatment or to an active treatment, the earlier sponsor reported that Thrombin-JMI is used almost exclusively with absorbable gelatin sponges. Thus FDA agreed to comparing a new Thrombin with gelatin sponge to Thrombin-JMI with gelatin sponge without data to support a contribution of Thrombin to the gelatin alone. If FDA had required a three-arm study to evaluate the contribution of Thrombin to the gelatin, the outcome could be so small (or even non-existent) that a new manufacturer could never obtain the same label as Thrombin-JMI, not a regulatory outcome to be desired, because the licensed bovine Thrombin-JMI may pose more risks to subjects than the new Thrombins to be evaluated.

4. Is the one-sided 0.025 level of significance acceptable?

Yes.

5. Is a single final analysis of all subjects stratified by surgery type acceptable, as opposed to individual analyses of each surgery type?

Yes. This design is commonly accepted by CDRH for adjuncts to hemostasis. However, CBER has long recognized that an investigational adjunct to hemostasis could conceivably be ineffective in one surgery type yet receive a general labeling in surgery because of statistically strong results in other types of surgery. CBER would not recommend a general label in such a case where the data actually show that the

adjunct is *not* effective in general. Thus this sponsor should be advised to present confidence intervals for the four surgery types separately and that CBER does not approve biologics simply on the basis for meeting a specific endpoint. That said, it is difficult for CBER to say prospectively what worse outcome for rhThrombin in one type of surgery would raise a concern about a general label.

6. Are the inclusion/exclusion criteria appropriate?

Yes. CBER has previously advised the sponsor that the two types of graft surgery are not viewed as particularly different.

7. Are the enrollment limits acceptable?

No. It would not be acceptable to have, for example, 10% of subjects (only 20 in the test group) in liver surgery. The point of limiting the two graft surgeries to a combined maximum of enrollment was to assure better balance than 10% in one of the other types of surgery. Basically, it would be preferred to have 33% in liver surgery, 33% in spinal surgery, and 34% in the two graft surgeries. The sponsor should propose an algorithm to obtain something close to those percentages.

8. Is dynamic allocation stratified by surgeon and surgery type acceptable?

Presumably; this reviewer will defer, however, to the statistical reviewer.

9. Is the plan to replace subjects who are not treated acceptable?

It is possibly acceptable to this reviewer. It is not an intent-to-treat analysis. Although not stated, it would appear that the sponsor is concerned about the wastage (cost) of preparing the two materials as A and B and having the subject randomized only when an acceptable evaluation site is identified. Since the question is asked, however, one has to ask why any subjects might not present an evaluable bleeding site during surgery? Bleeding through suture holes in PTFE is practicably legendary, and oozing bleeding from cut liver has been evaluate in fibrin sealant studies without any such withdrawal plan.

10. Is the use of unblinded pharmacists acceptable?

Yes.

11. Is the method for assessing time to hemostasis within 10 minutes acceptable?

Yes, as the primary analysis. However, hemostasis is defined when no blood is observed seeping through or around the gelatin sponge, yet the sponge is to be removed and any bleeding that re-occurs is not to be considered a failure. The sponsor should be advised to record recurrences of bleeding following sponge removal and to present comparative data on this observation. In the worst case possible, that bleeding

always recurred in the test group but never in the control group, the test agent's effectiveness would certainly be called into question.

12. Are the scope and frequency of planned safety assessments appropriate?

Yes.

13. Is the plan to assess antibodies based on assigned treatment acceptable?

More explanation is needed from the sponsor. It is not stated how the blood samples will be provided without subject identifiers, because the identifiers presumably must be on the tubes at the time of collection. What is the process by which hospital labels are replaced and what is the coding process? It is probably acceptable for the laboratory to run assays appropriate to the type of Thrombin, e.g., there is no point to assessing anti-CHO antibodies for a control subject. However, the possibility of bias always creeps in and it would be preferable to run all assays on all samples. This would show cross-reactivity of any antibodies to the Thrombin products. The other issue is that certain sponsor personnel will know of any immunogenicity issues and will know them before the IDMC knows of them. However, such antibodies were not an issue in the Phase 2 trials.

14. Is the proposed statistical approach acceptable?

This question is too broad; it is akin to asking if the protocol is acceptable.

15. Are the proposed populations for safety and efficacy analysis acceptable?

No. At a minimum, subjects who received study material must be included in the efficacy analysis, whether or not time to hemostasis is recorded. At a maximum, the primary analysis must be intent to treat and the sponsor will have to absorb the cost of preparing both study drugs prior to last minute randomization. The safety population, all subjects treated with blinded study drug, is acceptable.

16. Is the approach to handling missing data acceptable?

No. Investigators must understand that there is a penalty for unacceptable study conduct, i.e., for not recording data. A sensitivity analysis must be conducted for missing data, to include a worst-case scenario.

17. Is the proposed method of sample size recalculation acceptable?

This reviewer will defer to the statistical reviewer. However, it is very disturbing that an "unblinded" sample size analysis can be conducted on data that are going assessed for efficacy in the second interim analysis. Is the sponsor asserting that there is no penalty for the efficacy/futility looks?

18. Do the composition of the IDMC and its charter provide appropriate safety monitoring while maintaining blinded treatment assignments?

The question raises questions. How in the world could the IDMC review antibody data - anti-bovine Thrombin, anti-rhThrombin, anti-CHO, and anti-TPA - without becoming unblinded? Further, it is not clear where in the protocol there is a statement that the interim analysis are to remain blinded.

The IDMC charter is also unacceptable on a number of matters (independence).

19. Is the proposed approach for setting stopping boundaries for comparable efficacy and futility acceptable?

Why is the question about the approach to setting boundaries rather than about the boundaries themselves? The major problem with this question is that the SAP for the interim analyses has not been submitted.

20. Is it appropriate to define inappropriate bleeding as not sufficient to require use of a topical hemostat or hemorrhage/brisk bleeding that requires more significant intervention?

No. Hemorrhage/brisk bleeding should **not** be raised as a question, because adjuncts to hemostasis are not intended for such bleeding, but rather for the mild to moderate residual bleeding after surgical modalities of suture, ligature, and cautery have been used. It would be preferable to define appropriate bleeding, as mild to moderate bleeding remaining after brisk bleeding has been controlled by standard surgical modalities. The current definition implies that a subject with brisk bleeding is ineligible for the study.

21. Is the algorithm for using alternative hemostatic measures acceptable?

No. Detail is lacking. If hemostasis is not achieved at 10 minutes and further blinded study material is to be used, is the original sponge removed or is a further sponge soaked in Thrombin placed over the first sponge? Also, it is odd to suggest that bleeding not stopped by a gelatin sponge soaked in Thrombin will be treated with a gelatin sponge alone; such a rescue, if successful, would certainly make one wonder how much Thrombin is contributing to hemostasis. However, the sponsor should simply record and ultimately report all rescue measures.

The second tier of rescue methods includes adjuncts to hemostasis that contain plasma-derived human or bovine thrombin. This situation is problematic for interpretation of safety and in particular of antibody data. For example, if bovine thrombin is used as a rescue, any antibodies that might be generated might show cross-reactivity to rhThrombin; the sponsor would no doubt want to brush them off as having been raised by the bovine thrombin, which raises questions about the relative sensitivity of the assays for Thrombin-JMI and rhThrombin. It is not clear that any of the surgeries in this study cannot be accomplished successfully without thrombin-containing products, and it is vastly preferable that such not be used as rescue

methods. However, if surgeons think they must use them, then FDA will have no real choice other than to attribute antibodies to rhThrombin to the investigational product, absent any prospective plan for evaluation of alternative explanations.

22. Are the four surgeries sufficient to support a general adjunct to hemostasis label claim in conjunction with an absorbable sponge?

Yes, even if the two graft surgeries are considered as one surgery type.

23. If superiority efficacy of rhThrombin is observed, would that support a claim?

This reviewer will defer to the statistical reviewer. However, testing for superiority and then, if superiority is not found, testing for non-inferiority makes sense, whereas the converse seems suspicious. Since there is no hypothesis to support testing for superiority, this proposal appears to want to make hay out of a possible accidental finding.

24. Is it acceptable for the labeling to reflect stability of rhThrombin (longer after reconstitution than Thrombin-JMI) rather than the conditions of use in the study (constrained by blinding to the shorter time for Thrombin-JMI).

This is not a Special Protocol Assessment question per se, but a product question driven by the trial design. One question back to the sponsor would be: have they **ever** in Phase 2 trials used material stored at room temperature for 8 hours?

25. Is the minimum number of subject exposures to rhThrombin in Phase 3, combined with those in Phase 1 and Phase 2 (approximately 300) sufficient for licensure?

It is sufficient to support a marketing application.

26. Is the Phase 3 protocol, together with the one Phase 1 study and four Phase 2 studies, sufficient to support licensure?

It is sufficient to support a marketing application.