Prospective, Randomized, Phase II, Non-Inferiority Study to Evaluate the Safety and Efficacy of Topical Thrombin (Human) Grifols as Adjunct to Hemostasis During Vascular, Hepatic, Soft Tissue, and Spinal Open Surgery

Harold Minkowitz, MD, Jordi Navarro-Puerto, MSc, Shankar Lakshman, MD, Sonia Singla, MD, Charles Cousar, MD, Robin Kim, MD, FACS, Alan Villavicencio, MD, Levester Kirksey, MD, Jaume Ayguasanosa, MD, on behalf of the Clinical Investigation Study Group on Topical Thrombin (Human) Grifols in Surgery

BACKGROUND: Thrombin-based formulations have been used for topical hemostasis in surgery for decades. However, the number of randomized clinical trials comparing bovine vs human thrombin is limited.

STUDY DESIGN: A randomized, double-blind, non-inferiority phase II study evaluated the hemostatic efficacy and safety of plasma-derived topical thrombin (human) Grifols (TTH-Grifols; Instituto Grifols SA) vs bovine THROMBIN JMI (BT-JMI; GenTrac Inc) (2:1 ratio) in vascular, hepatic, soft tissue, and spinal operations. The primary efficacy end point was the percentage of patients achieving hemostasis at target bleeding sites with mild to moderate bleeding (response) within 5 minutes (T_5) of treatment application. Non-inferiority was met if the lower limit of the 95% CI of the response ratio of TTH-Grifols relative to BT-JMI by T_5 exceeded 0.8. Secondary efficacy variables were the cumulative response by 3 and 4 minutes (T_3 and T_4), and the number of treatment failures. Safety parameters were assessed.

RESULTS: Randomized patients in TTH-Grifols and BT-JMI groups were n = 137 and n = 68, respectively. In modified intention-to-treat population, rates of hemostasis by T_5 were 78.3% (94 of 120) in TTH-Grifols and 80.3% (49 of 61) in BT-JMI (response ratio: 0.973; 95% CI 0.833 to 1.135). Rates of hemostasis in vascular, hepatic, soft tissue, and spinal operations ranged from 75.0% to 82.5% for TTH-Grifols and from 54.5% to 91.7% for BT-JMI. No significant differences in adverse events were observed between treatment groups. Antibodies to bovine factor V antigen were detected in 2 patients exposed to BT-JMI and in none exposed to TTH-Grifols.

CONCLUSIONS: The TTH-Grifols was safe and well tolerated as a local hemostatic agent and was non-inferior to BT-JMI. No antibodies to thrombin developed in TTH-Grifols-treated patients. (J Am Coll Surg 2019;229:497–507. © 2019 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

Disclosure Information: This study was funded by Grifols, the manufacturer of topical thrombin (human) Grifols, Medical writing support was provided by Gines Escolar, MD, PhD, under the direction of the authors, with funding from Grifols. Drs Ayguasanosa and Navarro-Puerto are employed by Grifols. Dr Villavicencio received research funding from Grifols, paid to his institution. Dr Minkowitz's institution receives clinical trial grant money from Research Concepts, GP LLC.

Disclosures outside the scope of this work: Dr Villavicencio received honoraria from Leading Edge Spinal Implants and received research funding from Pfizer, Empirical Spine, and Globus Medical, paid to his institution. Dr Minkowitz is a paid consultant to AcelRX, Heron, Avenue, Concentric, Takeda, Sorrento, and Acacia, and receives payment for lecture from AcelRX, Heron, Avenue, Acacia, and Merck.

ClinicalTrials.gov identifier NCT02014402.

ICMJE data-sharing statement available online as eDocument 1.

Members of the Clinical Investigation Study Group on Topical Thrombin (Human) Grifols in Surgery who co-authored the manuscript are listed in the Appendix.

Received April 4, 2019; Revised July 12, 2019; Accepted July 12, 2019.

From the Anesthesia Department, Memorial Hermann-Memorial City Medical Center, Houston, TX (Minkowitz), Grifols Bioscience Research Group, Grifols, Barcelona, Spain (Navarro-Puerto, Ayguasanosa), Lotus Clinical Research, Pasadena, CA (Lakshman, Singla), River City Clinical Research, Jacksonville, FL (Cousar), University of Utah Hospital, Salt Lake City, UT (Kim), Boulder Neurosurgical and Spine Associates, Boulder, CO (Villavicencio), and Cleveland Clinic Heart and Vascular Institute, Cleveland, OH (Kirksey).

Correspondence address: Harold Minkowitz, MD, Department of Anesthesiology, Memorial Hermann-Memorial City Medical Center, 921 Gessner Rd, Houston, TX 77024. email: harold@minkowitzMD.com

https://doi.org/10.1016/j.jamcollsurg.2019.07.008
ISSN 1072-7515/19

Abbreviations and Acronyms

AE	= adverse event
AR	= adverse reaction
BT-JMI	= bovine THROMBIN-JMI
FV	= Factor V
ITT	= intent to treat
mITT	= modified intent to treat
T_3	= 3 minutes after start of treatment
T_4	= 4 minutes after start of treatment
T_5	= 5 minutes after start of treatment
TBS	= target bleeding site
$T_{Closure}$	= time of completion of the surgical closure
TEAE	= treatment-emergent adverse event
T_{Start}	= start of treatment
TTH-Grifols	= topical thrombin (human) Grifols

Bleeding is a major complication of surgical procedures and is associated with increased morbidity and mortality.[1] Excessive bleeding impairs visualization of the surgical field, prolongs time spent in the operating room, can enhance the rate of re-interventions, and overall has a negative impact on clinical and financial outcomes.[2] A retrospective analysis of databases in 600 hospitals throughout the US indicates that bleeding events and use of transfusion products are common in hospitalized surgical patients and vary according to surgical procedure (general 27.5%, solid organ 28.5%, vascular 31.5%, and spinal surgery 15.0%).[3]

Numerous strategies are available for local control of surgical bleeding that persists after the standard approaches with cautery, ligature, or other conventional hemostatic methods have been applied. Active topical hemostatic agents, foam patches, fibrin glues, or combinations have been used to facilitate hemostasis when classic strategies prove insufficient.[4,5] The aim of these local hemostatic agents is to promote the rapid formation of a more resilient hemostatic plug of platelets and fibrin that will act as a barrier and limit excessive bleeding. Topical hemostats reduce blood loss, avoid the adverse effects of systemic hemostatic drugs, and shorten the time to hemostasis and overall time spent in the operating room.[6] Use of local hemostatic agents can reduce time spent in the ICU and overall hospitalization time and, consequently, is medically and economically valuable.[7]

Thrombin is classified as an active local hemostatic agent that reproduces the final steps of the coagulation mechanisms and the effects of which can be enhanced when used in combination with mechanical products, such as gelatin, collagen, or cellulose sponges.[6] Thrombin of bovine origin has been available in the US market as a commercial adjunct to hemostasis for more than 70 years.[8,9] These bovine thrombins have been used extensively, alone or in combination with other hemostatic agents, in a variety of surgical settings, including general, cardiovascular, neurological, transplantation, and orthopaedic surgical procedures. Extensive use of earlier thrombins of bovine origins resulted in a series of adverse events due to the formation of antibodies that cross-reacted with human coagulation factors.[10,11] Presence of bovine coagulation Factor V (FV) was elevated in the older commercial preparations, but improvements in methods of manufacturing significantly reduced contaminating proteins, resulting in more purified, less immunogenic bovine thrombins.[12] Current evidence reviewed does not support a definitive association between perioperative or postoperative generation of antibodies and an increased risk of adverse events in surgical patients treated with newly developed topical bovine thrombin preparations.[13] In fact, bovine THROMBIN JMI (BT-JMI; GenTrac Inc) has become the standard comparator in randomized clinical trials powered to demonstrate non-inferiority in hemostatic efficacy parameters.[14-16] Regulatory authorities require that clinical investigation of agents used for topical hemostasis meet specific guidelines and recommend that the efficacy of these therapies be assessed using objective clinical end points and adequate comparators.[17,18]

Topical thrombin (human) Grifols (TTH-Grifols; Instituto Grifols SA) is a new formulation of highly purified plasma-derived human thrombin supplied as a lyophilized powder in vials for use as a topical hemostat. Once reconstituted, TTH-Grifols can be applied on surgical injuries directly or in combination with gelatin sponges. We hypothesized that the new formulation with purified human thrombin in TTH-Grifols could be as effective and safe as a standard bovine thrombin preparation when used for local hemostasis. To test this hypothesis, we evaluated TTH-Grifols in different types of operations.

METHODS

Objectives and study design

The purpose of this study was to demonstrate that TTH-Grifols is both effective (ie achievement of hemostasis without occurrence of re-bleeding until time of completion of the surgical closure [$T_{Closure}$]) and safe (ie clinical safety, viral safety, and immunogenicity) when used as an adjunct to hemostasis during vascular, hepatic, soft tissue, and spinal surgical procedures. To accomplish these objectives, we compared TTH-Grifols with BT-JMI in a non-inferiority, randomized, double-blind clinical trial carried out in 30 centers in the US (ClinicalTrials.gov identifier NCT02014402). Participants in the study

were randomized in a 2:1 ratio to TTH-Grifols and BT-JMI treatment groups.

This non-inferiority study was designed to provide at least 80% power to demonstrate non-inferiority of TTH-Grifols to BT-JMI. Non-inferiority was defined in terms of the lower limit of a 2-sided 95% CI for the ratio of percentage of patients achieving hemostasis success by 5 minutes in the TTH-Grifols group divided by the corresponding percentage in the BT-JMI group (response ratio). If the lower limit for the 95% CI analysis was >0.8, then non-inferiority was deemed to have been demonstrated. After the non-inferiority of TTH Grifols to BT-JMI was established, its superiority could be additionally claimed if the 95% CI for the response ratio was entirely >1. To derive the required sample size for this clinical trial design, it was additionally assumed that across all surgical specialties studied (vascular, hepatic, soft tissue, and spinal), the true response rate at 5 minutes for the treatment groups would be 85%. With these assumptions and a 2:1 randomization ratio, it was estimated that a cumulative sample size of 180 patients (120 in TTH-Grifols group and 60 in BT-JMI group) would provide 80% power to establish non-inferiority. In each of the 4 operation subtypes (vascular, hepatic, soft tissue, and spinal), the expected sample size was approximately 45 patients (30 treated with TTH-Grifols and 15 treated with BT-JMI). There was not a maximum number of permitted randomized patients at each operation subtype protocol. However, the minimum number of patients who were randomized and treated with the study drug was at least 30 (20 for TTH-Grifols and 10 for BT-JMI).

The study was approved by the IRBs of the participating sites, and was conducted in accordance with local regulations and with the ethical principles of the current Declaration of Helsinki. Written informed consent was obtained from adult patients and from the parents or legal guardian on behalf of pediatric patients. Patients were monitored for up to 30 days (range 26 to 34 days) after the surgical procedure.

Patient population

The clinical study included both adult and pediatric patients who required an elective (non-emergency), open (non-laparoscopic; non-endovascular) surgical procedure from among the following categories: vascular open operation, which is a surgical procedure involving a native artery graft end to side proximal anastomosis using coated or uncoated polytetrafluoroethylene graft; hepatic open operation, which is a hepatic resection of at least 1 anatomic hepatic segment or equivalent tissue volume; soft tissue open operation, which is a surgical procedure involving nonparenchymous soft tissue; and spinal surgery, which is a spinal surgical procedure in which the epidural venous plexus was exposed. Additional inclusion criteria were preoperative hemoglobin ≥9.0 g/dL and fibrinogen level ≥150 mg/dL within 24 hours before the surgical procedure. Exclusion criteria were traumatic injury or infective process in the anatomic surgical area, history of severe reactions to any blood-derived product, and pregnancy.

Patients scheduled for the different surgical procedures were screened in 2 visits, 1 within 21 days and another 24 hours before the procedure. Randomized assignment to the study groups was performed after the second visit.

Randomization

Qualified patients were randomized in a 2:1 ratio to the TTH-Grifols and BT-JMI treatment groups, stratified by type of operation (vascular, hepatic, soft tissue, and spinal) and subtype of operation in certain cases (vascular, peripheral arterial bypass, and extremity vascular access for hemodialysis) and soft tissue operation (mastopexies and abdominoplasties and non-mastopexies and non-abdominoplasties). Randomizations were performed at the study center's pharmacy using an Interactive Response Technology system, which provided the randomization number and assigned the corresponding thrombin treatment. The assigned study drug was then reconstituted by pharmacy staff at each study center and supplied to the investigators in syringes labeled with coded information to mask the treatment identification. Investigator, study nurses, or testing laboratories were blinded to the treatment group and could not identify the agent being assigned.

Identification and grading of target bleeding sites

A specific bleeding area/site was defined as the target bleeding site (TBS) for each surgical procedure in patients already randomized when it was determined by the surgeon that control of bleeding by conventional surgical techniques (including suture, ligature, and cautery) was ineffective or impractical and required an adjunct treatment to achieve hemostasis. Once the TBS was identified, the surgeon then rated the intensity of bleeding at the TBS according to a 3-point scale as mild, moderate, or severe. Only mild or moderate bleeding cases were included in the study. If the nature of the bleeding was initially severe, the surgeon can use standard surgical modalities (eg compression, sutures, or ligation of vessels) to control the bleeding. If the nature of the bleeding became mild or moderate after these measures have been taken, the patient would be considered eligible for randomization

and treatment. If the nature of the bleeding remained severe, the patient should not be treated and would be considered an intraoperative screen failure. For hepatic and soft tissue procedures, the size of the approximate bleeding surface was also determined according to a 3-point scale (small, medium, or large). Details on the evaluations of bleeding intensities for similar surgical procedures have been provided in previous literature.[14,15,19-22]

Intensity of bleeding was always evaluated by the surgeon for each surgical setting. For vascular open operations, anticoagulation with heparin before arterial clamping was allowed according to the standard practice. A TBS was identified in applicable peripheral vascular procedures involving an end to side arterial anastomosis using coated or uncoated polytetrafluoroethylene grafts in aortic, iliac, and femoral combined localizations. For hepatic open operations, a TBS in the parenchymous, raw cut liver surface should have been planned during recruitment to be eligible for randomization and treatment with the investigational product. For soft tissue open operations, the TBS in the appropriate soft tissue should have been present and should not have included leakage of lymph fluid, urine, or gastrointestinal contents. For spinal operations, a TBS in the epidural venous plexus should have been planned during recruitment. For hepatic, soft tissue, and spinal operations, intensities of the bleeding at the eligible TBS were rated by the surgeon using the following 3-point scale: mild, oozing and capillary; moderate, gradual and steady; or severe, brisk and forceful. For vascular open operations, the intensity of bleeding at the TBS was rated as: mild, bleeding that affected $<25\%$ of the suture line or consisted of <5 suture-line bleeds (non-pulsatile, non-spurting bleeding); moderate, nonspurting bleeding that affected at least 25% of the suture line, consisted of at least 5 suture-line bleeds, or consisted of 1 pulsatile suture-line bleed; or severe, bleeding that consisted of >1 pulsatile suture-line bleed or at least 1 continuous suture-line bleed. The use of intra- or postoperative blood products was recorded for both treatment groups.

Investigational treatments

The TTH-Grifols and BT-JMI preparations were supplied as glass vials containing freeze-dried powder for reconstitution according to manufacturer's instructions. The BT-JMI was considered the standard of care topical hemostat and was used as a control. Each topical hemostat was applied to the TBS using gelatin sponges (Gelfoam; Pharmacia and Upjohn) cut to the desired size and shape to cover the entire bleeding surface and soaked in the respective hemostat solution before administration. Treated patients could receive up to 6 vials of 5 mL (5,000 IU) of the corresponding topical hemostat. The number and size of Gelfoam sponges, as well as the approximate amount of product applied to the TBS, were recorded.

Hemostatic evaluations

Evolution of bleeding at the TBS was monitored during a 5-minute period after the application of each treatment. Hemostasis was defined as an absence/cessation of bleeding at the TBS within the 5-minute observational period, according to the surgeon's judgment, so that surgical closure of the exposed field could be started. Hemostasis was assessed from the start of treatment application (T_{Start}) at the TBS and at 3 minutes after T_{Start} (T_3); 4 minutes after T_{Start} (T_4); and 5 minutes after T_{Start} (T_5). Any bleeding from the TBS that had previously achieved hemostasis that required additional hemostatic intervention was classified as "re-bleeding." If the TBS re-bled within the 5-minute observational period, but cessation of bleeding was again achieved within the 5-minutes time frame, the effective hemostatic time point was taken to be the time when cessation of re-bleeding occurred.

Efficacy end points

The primary efficacy variable was the percentage of patients for each treatment group achieving hemostasis (yes/no) at the TBS by T_5 without occurrence of re-bleeding until $T_{Closure}$. Secondary efficacy variables were the cumulative percentage of patients achieving hemostasis at the TBS by the time points T_3 and T_4. Treatment failure was defined as: persistence of bleeding beyond T_5; breakthrough (brisk and forceful) bleeding at the TBS that jeopardized the safety of the patients according to the investigator's judgment at any moment during the 5-minute observational period and $T_{Closure}$; occurrence of re-bleeding at the TBS in the period between T_5 and $T_{Closure}$; requirements of alternative hemostatic treatments or maneuvers at the TBS during the 5-minute observational period and until the completion of the surgical closure; or need for re-application of study treatment.

Safety variables

The safety variables evaluated were vital signs, physical assessments, evaluation of adverse events (AEs), AEs potentially related to the study product (adverse reactions [ARs]), and serious AEs. The AEs were classified as treatment-emergent AEs (TEAEs) or non-treatment-emergent AEs (non-TEAEs), depending on the comparison of AE onset date and time with the start of study

treatment. A TEAE was defined as an AE that occurred on or after the start of study treatment, up to and including the date of the day-30 visit. Modifications in routine clinical laboratory tests were monitored.

Presence of viral markers (parvovirus B19) was analyzed using nucleic acid testing or viral serology methods. A new positive result for this viral marker was defined as a positive post-baseline value after a negative or missing baseline result. Any relevant quantitative increase in the marker was classified as qualitatively positive.

Immunogenicity studies specifically aimed at the detection of antibodies against human or bovine thrombin, or to human or bovine coagulation FV were carried out. If a sample tested positive for any of the previous antibodies, all previous samples from that patient were checked. The sample was considered positive only if all previous samples had been reported negative. The titer and neutralizing capacity of the antibodies in the positive samples were determined.

Study populations and statistical analysis

The following 4 analysis populations were initially defined in the protocol: the intent to treat (ITT) population, defined as all patients enrolled and randomized into the study; the modified ITT (mITT) population included all patients who were randomized into the study and treated with any amount of TTH-Grifols or BT-JMI; the per-protocol population, defined as the mITT population excluding any patients with 1 or more major protocol deviations that could impact the evaluation of efficacy data, as determined at a data review meeting before unblinding; and the safety population, included all patients who received any amount of TTH-Grifols or BT-JMI. The efficacy analysis was prespecified in the protocol to be performed using the patients included in the mITT population. The primary efficacy end point was analyzed using Cochran-Mantel-Haenszel test stratified by type of operation (vascular, hepatic, soft tissue, and spinal). Non-inferiority was considered to have been demonstrated if the lower limit of the 95% CI of the response ratio by T_5 (weighted across the 4 types of operation) exceeded 0.8. Analyses relating to secondary efficacy variables, the cumulative response (percentage of patients achieving hemostasis) by other individual assessment times and the percentage of treatment failures were summarized and analyzed using the Cochran-Mantel-Haenszel test. SAS software (SAS Institute), version 9.2, was used for statistical analyses.

Safety analyses were based on the safety population defined here. The incidence and severity of TEAEs, suspected ARs, and SAEs were summarized by treatment group. Non-TEAEs and TEAEs were summarized separately. At each level of summarization, a patient was counted once per system organ class or preferred term (per MedDRA; www.meddra.org) using the most severe AE. The incidences of intraoperative, surgical, and nonsurgical TEAEs/suspected ARs were also summarized separately. Vital signs and physical assessment findings were also collected and reported using descriptive statistics.

RESULTS

Study patients

Flow of patients through the study is shown in Figure 1. Overall, 231 patients were screened and 205 were randomized into the study (ITT population) from January 2014 (first patient enrolled) to November 2015 (last patient competed). A total of 137 patients were randomized to the TTH-Grifols treatment group, including 17 who were not dosed, resulting in an mITT population of 120 patients. The per-protocol population included 104 (86.7%) patients from the mITT population after 16 (13.3%) were excluded for at least 1 major protocol deviation that might have had an impact on the primary efficacy assessment. In the BT-JMI treatment group, 68 patients were randomized, including 7 who were not dosed, resulting in the mITT population of 61 patients. Eleven of 61 (18.0%) patients had at least 1 major protocol deviation (mostly procedural, visit window, investigational product, and informed consent) that might have had an impact on the primary efficacy assessment and were excluded, resulting in the per-protocol population of 50 (82.0%). Five of 61 (8.2%) patients in the BT-JMI treatment group were dosed, but prematurely discontinued due to withdrawal of consent (2 patients), lost to follow-up (2 patients), or an AE (1 patient). Overall, a total of 181 patients of the mITT population in both treatment groups ($n = 120$ and $n = 61$) were included in the safety analysis.

Baseline characteristics

As summarized in Table 1, demographic and baseline characteristics of patients included in the mITT population appeared comparable between the 2 treatment groups attributable to the stratified randomization in the study. Overall, recruited patients were mostly adults (mean $\pm$ SD age 55.8 $\pm$ 16.1 years). Most of the patients in the mITT population were white (81.2%). Bleeding intensities and distributions of TBS sizes in participating patients were similarly distributed for both treatment groups. Mean exposure to the hemostats investigated was 8.8 mL for TTH-Grifols and 8.4 mL for BT-JMI, with a similar number of collagen Gelfoam sponges

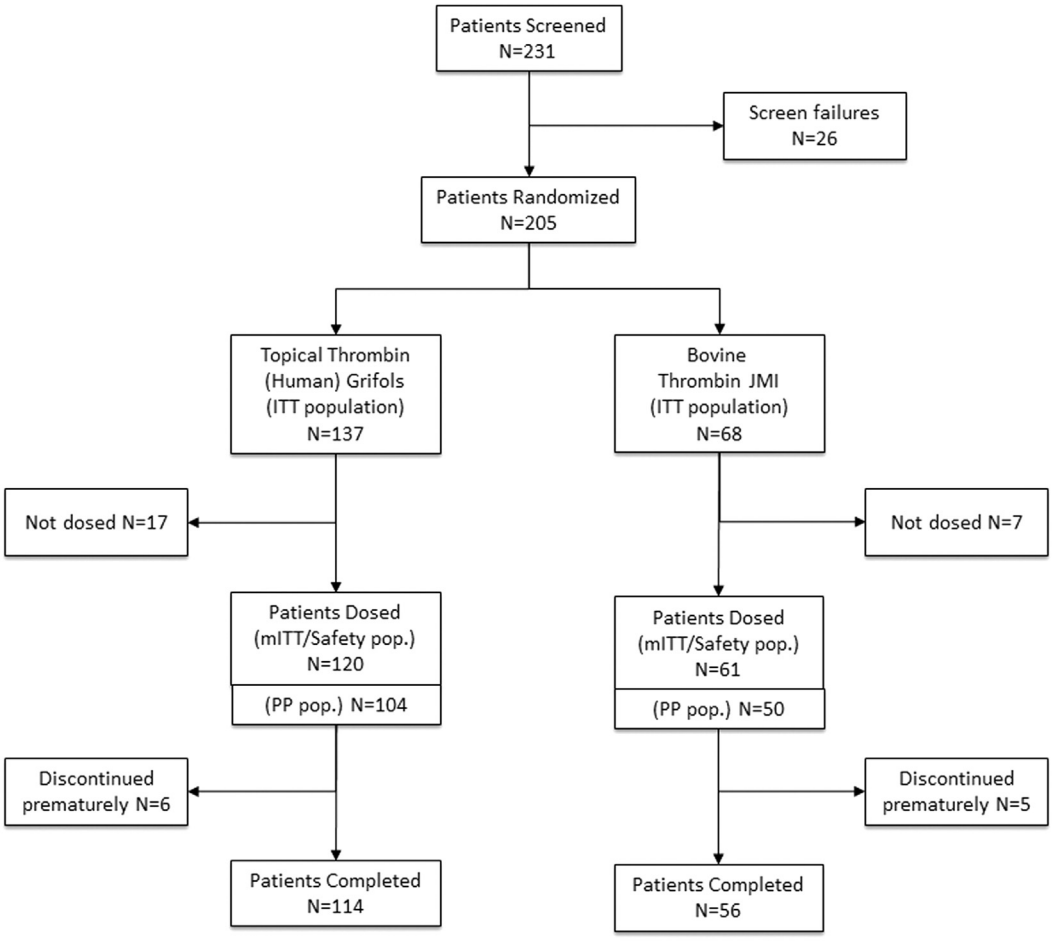

Figure 1. Flow diagram showing the distribution of patients into the 2 study treatments and the different analysis populations. Patients were randomized 2:1 to treatment with TTH-Grifols or BT-JMI. Efficacy and safety analyses were based on the mITT population. ITT, intent-to-treat population; mITT, modified intent to treat population; PP, per-protocol population.

used in patients who received TTH-Grifols or BT-JMI (83.3% and 80.3% of the patients, respectively).

Efficacy assessment

Primary efficacy

As summarized in Table 2, percentages of patients achieving hemostasis at the TBS by T_5 were 78.3% (94 of 120) in the TTH-Grifols treatment group and 80.3% (49 of 61) in the BT-JMI treatment group. The response ratio by T_5 was 0.973 (95% CI 0.833 to 1.135), showing that TTH-Grifols is non-inferior to BT-JMI and that the primary efficacy objective was met in the mITT population. Percentages of patients achieving hemostasis at the TBS by T_5 in vascular, hepatic, soft tissue, and spinal operations ranged from 73.9% to 82.5% in the TTH-Grifols treatment group and from 54.5% to 91.7% in the BT-JMI treatment group (see Table 2 for details).

Secondary efficacy

The percentages of patients achieving hemostasis by T_3 in the mITT population in the TTH-Grifols and BT-JMI treatment groups were 61.7% (74 of 120) and 63.9% (39 of 61), respectively. The response ratio by T_3 was 0.959 (95% CI 0.765 to 1.202). Percentages of patients achieving hemostasis by T_4 in the mITT population in the TTH-Grifols and BT-JMI treatment groups were 70.8% (85 of 120) and 73.8% (45 of 61), respectively. The response ratio by T_4 was 0.955 (95% CI 0.796 to 1.146). Percentages of patients with treatment failure in the mITT population in the TTH-Grifols and BT-JMI treatment groups were 21.7% (26 of 120) and 19.7% (12 of 61), respectively. In the TTH-Grifols group, the most common cause of treatment failure was persistent bleeding (12.5% [15 of 120]), and in the BT-JMI group it was the use of additional/alternative hemostatic treatment (14.8% [9 of 61]).

Table 1. Demographic and Baseline Characteristics of the Modified Intent-to-Treat Populations Included in Efficacy and Safety Evaluation

Characteristic	TTH-Grifols (n = 120)	BT-JMI (n = 61)	Total (n = 181)
Male, n (%)	50 (41.7)	19 (31.1)	69 (38.1)
Age, y, mean (SD)	55.5 (16.0)	56.4 (16.5)	55.8 (16.1)
Hispanic, n (%)	12 (10.0)	6 (9.8)	18 (9.9)
Race, n (%)			
White	97 (80.8)	50 (82.0)	147 (81.2)
Black or African American	19 (15.8)	7 (11.5)	26 (14.4)
Asian	2 (1.7)	2 (3.3)	4 (2.2)
American Indian or Alaskan Native	1 (0.8)	1 (1.6)	2 (1.1)
Native Hawaiian/Pacific Islander	1 (0.8)	0 (0)	1 (0.6)
Other	0 (0)	1 (1.6)	1 (0.6)
Weight, kg, mean (SD)	82.6 (19.3)	76.6 (21.8)	80.6 (20.3)
TBS bleeding intensity, n (%)			
Mild	66 (55.0)	29 (47.5)	95 (52.5)
Moderate	54 (45.0)	32 (52.5)	86 (47.5)
TBS size, n (%)			
Small ($\leq$10 cm^2)	29 (67.4)	13 (59.1)	42 (64.6)
Medium (>10 cm^2 and $\leq$100 cm^2)	13 (30.2)	7 (31.8)	20 (30.8)
Large (>100 cm^2)	1 (2.3)	2 (9.1)	3 (4.6)
Type of operation, n (%)			
Vascular	20 (16.7)	11 (18.0)	31 (17.1)
Hepatic	20 (16.7)	10 (16.4)	30 (16.6)
Soft tissue	23 (19.2)	12 (19.7)	35 (19.3)
Spinal	57 (47.5)	28 (45.9)	85 (47.0)

BT-JMI, bovine THROMBIN JMI; TBS, targeted bleeding site (size applies to hepatic and soft tissue operations); TTH-Grifols, topical thrombin (human) Grifols.

Safety assessments

A summary of safety assessments is provided in Tables 3 and 4. The percentages of patients for whom TEAEs were reported were similar between treatment groups at approximately 85% to 88%. In the TTH-Grifols treatment group, 15.8% of patients experienced a suspected AR compared with 26.2% of patients in the BT-JMI treatment group. No patients experienced a confirmed AR. Severe AEs were reported in 8.3% of patients in the TTH-Grifols treatment group and in 9.8% of patients in the BT-JMI treatment group. One patient in the BT-JMI treatment group was discontinued due to an AE. Overall, the TEAEs showed no consistent treatment-related pattern for any particular type of event. The frequencies of TEAEs overall were similar between treatments.

The most common TEAEs (experienced by $\geq$5% patients within a treatment group) were procedural pain, nausea, constipation, pruritus, muscle spasms, insomnia, pyrexia, and vomiting. No substantial differences in TEAE incidences were noted between treatment groups. The majority of TEAEs (99% in the TTH-Grifols treatment group and 98% in the BT-JMI treatment group) were either mild or moderate in severity. Four (3.3%) patients in the TTH-Grifols treatment group and 3 (4.9%) patients in the BT-JMI treatment group experienced a severe TEAE. No deaths were reported in the TTH-Grifols treatment group. Two deaths were reported in the BT-JMI treatment group (1 spinal surgery patient who experienced severe plasma cell myeloma starting on day 24 and died on day 279 and 1 vascular surgery patient who experienced severe pneumonia starting on day 12 and died on day 80); neither event was considered related to the study treatment.

No relevant differences were noted in vital sign data or clinical assessments. Changes in laboratory parameters over time and shifts to abnormally low or high values are typical of these operations and were similar between treatment groups at all time points. Mean changes from baseline for glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, total bilirubin, BUN, creatinine, sodium, potassium, chloride, calcium, aPTT ratio, and prothrombin international normalized ratio were small in both treatment groups at all time points (data not shown). Patients were monitored for potential transmission of parvovirus B19. No

Table 2. Analysis of Hemostasis at Target Bleeding Site Stratified by Type of Operation (Modified Intent-to-Treat Population)

	TTH-Grifols (n = 120)		BT-JMI (n = 61)		
Variable	**n/N**	**%**	**n/N**	**%**	**Response ratio* (95% CI)**
Patient achieving hemostasis by 5 min	94	78.3	49	80.3	0.973 (0.833−1.135)
Type of operation					
Vascular	15/20	75	6/11	54.5	—
Hepatic	15/20	75	8/10	80	—
Soft tissue	17/23	73.9	11/12	91.7	—
Spinal	47/57	82.5	24/28	85.7	—

*Ratio of percentages of patients achieving hemostasis in the 2 treatment groups (TTH-Grifols relative to BT-JMI) weighted across the 4 types of operations.
BT-JMI, bovine THROMBIN JMI; TTH-Grifols, topical thrombin (human) Grifols.

treatment-emergent parvovirus B19 infection was detected by viral nucleic acid test or viral serology methods.

Immunogenicity

No immunogenicity response to TTH-Grifols treatment was observed. Two patients in the BT-JMI treatment group (3.2% of treated patients) were found to have a low titer antibody to bovine FV. One of these patients was negative for antibodies at baseline, but was positive at postoperative day 14 with a low titer and was negative again at postoperative day 30. The other patient was negative for antibodies to bovine FV at baseline and postoperative day 14, but was positive at day 30 with a low titer antibody.

DISCUSSION

The current study has compared the clinical safety and the hemostatic efficacy of TTH-Grifols with a reference bovine thrombin product. Primary efficacy analysis of hemostasis showed that the human thrombin formulation in TTH-Grifols is non-inferior to the bovine thrombin used as comparator (BT-JMI) in the patients studied. Percentages of patients achieving hemostasis within 5 minutes of treatment application were similar between both treatment groups. The TTH-Grifols was safe and well tolerated. Overall, data suggest that TTH-Grifols could be a safe and effective local hemostatic agent in patients undergoing vascular, hepatic, soft tissue, and spinal operations.

Table 3. Summary of Treatment-Emergent Adverse Events in the Safety Population: Patients Who Received Any Amount of the Hemostatic Agents Investigated

Variable	**TTH-Grifols (n = 120)**	**BT-JMI (n = 61)**
Patient with any TEAE, n (%)	105 (87.5)	52 (85.2)
Total no. of TEAEs	351	211
Patient with any suspected AR, n (%)	19 (15.8)	16 (26.2)
Total no. of suspected ARs	56	36
Patient with any AR, n (%)	0	0
Total no. of ARs	0	0
Patient with any SAE, n (%)	10 (8.3)	6 (9.8)
Total no. of SAEs	13	8
Patient with any serious suspected AR, n (%)	1 (0.8)	0
Total no. of serious suspected ARs	1	0
Patient with any TEAE with outcome of death, n (%)	0	2 (3.3)
Patient with any TEAE leading to discontinuation from study, n (%)	0	1 (1.6)
Total no. of TEAEs leading to discontinuation from study, n (%)	0	1
Patient with any intraoperative TEAE, n (%)	36 (30.0)	23 (37.7)
Total no. of intraoperative TEAEs*	71	40
Patient with any surgical TEAE, n (%)	89 (74.2)	47 (77.0)
Total no. of surgical TEAEs*	206	109
Patient with any nonsurgical TEAE, n (%)	61 (50.8)	34 (55.7)
Total no. of nonsurgical TEAEs	145	102

*TEAE might not be exclusive to 1 category. Missing start time can cause a TEAE to be assigned to intraoperative and/or surgical.
AR, adverse reaction; BT-JMI, bovine THROMBIN JMI; SAE, serious adverse event; TEAE, treatment-emergent adverse event; TTH-Grifols, topical thrombin (human) Grifols.

Table 4. Treatment-Emergent Adverse Events Reported in ≥5% Patients Within a Treatment Group in the Safety Population*

	TTH-Grifols (n = 120)		BT-JMI (n = 61)	
Preferred term	**n**	**%**	**n**	**%**
Procedural pain	67	55.8	38	62.3
Nausea	33	27.5	16	26.2
Constipation	12	10.0	8	13.1
Pruritus	12	10.0	4	6.6
Muscle spasm	11	9.2	6	9.8
Insomnia	11	9.2	4	6.6
Pyrexia	7	5.8	5	8.2
Vomiting	6	5.0	10	16.4

*Safety population included patients who received any amount of the hemostatic agents investigated.
BT-JMI, bovine THROMBIN JMI; TTH-Grifols, topical thrombin (human) Grifols.

Although bovine-derived thrombin products have become the standard hemostatic agent for local hemostasis for many years, there are few randomized clinical trials comparing its hemostatic efficacy with thrombins of human origin.[14,15,23] The previously referenced studies have demonstrated non-inferiority of newer human plasma-derived or recombinant human thrombin compared with licensed bovine thrombin in terms of efficacy as adjunct to hemostasis in surgical setting similar to those investigated in our current trial. The hemostatic results in our study with TTH-Grifols are comparable with results reported for bovine or human thrombin products. In a previous clinical study, the effectiveness of plasma-derived human thrombin was compared with that of bovine thrombin for achieving hemostasis in spinal, cardiovascular, and general or post-traumatic elective surgical procedures.[15] In another comparative trial, efficacy, safety, and immunogenicity of recombinant human thrombin and bovine thrombin as adjuncts to hemostasis were compared in the setting of liver resection, spine, peripheral arterial bypass, and dialysis access operations.[14] In that study, the incidence of hemostasis after product application was evaluated for 10 minutes. Percentages of patients achieving hemostasis observed at 5 minutes for TTH-Grifols and BT-JMI in our current studies are fully compatible with the cumulative incidence of hemostasis at equivalent observation times for human, recombinant human, or bovine thrombin in the trials mentioned previously.

No substantial differences in the incidence of adverse events were noted between treatment groups. An evaluation of the safety of recombinant human thrombin from pooled data in 8 clinical trials reported adverse events for ≥10% of the patients,[24] a percentage compatible with the 8.3% of AEs found in the group of patients treated with TTH-Grifols in our current study. The majority of TEAEs reported in both treatment groups in our study were either mild or moderate in severity and similar to those communicated in clinical trials using local hemostats on similar surgical procedures.[14,15,23,24] Two deaths were reported, both in the BT-JMI treatment group, but were considered not related to the study treatment.

The thrombin component of TTH-Grifols undergoes 3 dedicated viral safety steps with validated capacity for elimination and/or inactivation of potential pathogens using patented technologies.[25] The safety of the human thrombin in the TTH-Grifols—as a component of a fibrin sealant—has already been confirmed in randomized clinical trials.[19-22] No treatment-emergent infection was detected by viral nucleic acid testing or viral serology methods in any of the study groups.

The immunogenicity of topical thrombin agents is a matter of concern.[16,26] Two patients in the BT-JMI group (3.2% of treated patients) showed low-level titers of antibodies to bovine FV with no major clinical transcendence. No immunogenic response was observed in patients exposed to TTH-Grifols. The low immunogenic profile of TTH-Grifols in our current study would be similar to that reported in previous studies with human or recombinant topical thrombin. It was suggested that patients with repeated perioperative exposure to topical bovine thrombin have a 3- to 10-fold greater risk for development of antibodies to topical bovine thrombin than do patients with no history of procedure-related exposure to this agent.[13] In the clinical trials mentioned previously,[14,15] percentages of the patients exposed to bovine thrombin who showed detectable levels of antibodies to the product ranged from 12.7% to 21.5%, figures that exceed by several times those observed in our current study for the bovine thrombin preparation (3.2%). Although homologous thrombin preparations offer an aprioristic advantage of being less immunogenic than bovine thrombin,[6,9,24] it seems evident that the improvements in the purification of bovine products have greatly reduced the immunogenic potential of the earlier preparations.[12,27,28]

Fibrin and platelets are the main components of hemostasis.[29] Thrombin plays a double role in hemostasis by activating platelets in the vicinity of damaged vascular areas and by promoting the polymerization of fibrinogen into fibrin network that will consolidate hemostasis.[30] Clot formation can be reduced to several seconds when elevated concentrations of thrombin are present locally.[31] Thrombin facilitates platelet adhesion onto damaged vascular surfaces, even in the absence of plasma adhesive

proteins.[32] Vascular surgery patients present specific challenges to hemostasis due to the blood pressure at vascular sites and exposure to antithrombotic therapies during the procedures. A post-hoc subgroup analysis of a previous trial was conducted for patients undergoing peripheral arterial bypass or arteriovenous graft.[14,23] Results from that analysis indicated that recombinant human thrombin provided more rapid onset of hemostasis in patients undergoing arteriovenous graft. Interestingly, in the vascular surgery setting investigated in our study, percentages of patients achieving hemostasis at the TBS by T_5 suggested a difference for the TTH-Grifols treatment group (75.0%) compared with the BT-JMI treatment group (54.5%). Additional studies should address the clinical relevance of these differences.

There are several limitations in the current study, such as the subjectivity of the surgeons judgment who evaluated the bleeding intensity. The protocol relied on the ability and expertise of the experienced surgeon to detect a TBS and evaluate the intensity of bleeding for a specific surgical intervention. No inter-rater reliability tests were performed. These are inherent limitations in previous studies with local hemostatic agents. The inclusion of only mild to moderate bleeding cases, the statistical focus on multiple surgical settings combined, and failure to use an ITT approach are additional limitations.

Studies in experimental models indicate that topical thrombin can reliably control the pharmacologic effects of the association of anticoagulant and antiplatelet therapies.[33] Thrombin-based topical hemostats can circumvent the reduction in thrombin generation associated with current anticoagulant treatments, allowing the performance of minimally invasive procedures and avoiding the thrombotic risks associated with the disruption of anticoagulant treatments. Specifically designed studies aimed at evaluating the effectiveness of thrombin-based hemostats in this subgroup of patients deserve future consideration.

CONCLUSIONS

Results of the current study suggest that TTH-Grifols was safe and well tolerated as a local hemostatic agent in surgical procedures, such as vascular, hepatic, soft tissue, and spinal. The hemostatic efficacy of TTH-Grifols in mild or moderate bleeding was non-inferior to the BT-JMI used as a reference treatment. No antibodies to thrombin developed in TTH-Grifols-treated patients.

Author Contributions

Study conception and design: Navarro-Puerto, Ayguasanosa

Acquisition of data: Minkowitz, Lakshman, Singla, Cousar, Kim, Villavicencio, Kirksey

Analysis and interpretation of data: Navarro-Puerto, Ayguasanosa

Drafting of manuscript: Minkowitz, Navarro-Puerto, Ayguasanosa

Critical revision: Minkowitz, Navarro-Puerto, Lakshman, Singla, Cousar, Kim, Villavicencio, Kirksey, Ayguasanosa, the Clinical Investigation Study Group on Topical Thrombin (Human) Grifols in Surgery

Acknowledgment: The investigators thank the patients for their indispensable contribution. The following centers that contributed with patients are acknowledged: Summerlin Hospital Office, Las Vegas, NV; Tucson Orthopedic Institute, Tucson, AZ; Jacksonville Center for Clinical Research, Jacksonville, FL; Cedars Sinai Medical Center, Los Angeles, CA; Northwest Orthopedic Specialists, PS, Spokane, WA; University of Maryland School of Medicine, Baltimore, MD; University of Florida, Gainesville, FL; University of North Carolina, Chapel Hill, NC; Northwestern University Feinberg School of Medicine, Chicago, IL.

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APPENDIX

The Clinical Investigation Study Group on Topical Thrombin (Human) Grifols in Surgery: Christopher D Anderson, MD, FACS (University of Mississippi Medical Center, Jackson, MS); Daniel Labow, MD, FACS (Mount Sinai School of Medicine, New York, NY); Thomas Fishbein, MD, FACS (MedStar Georgetown Transplant Institute, Washington, DC); Patricia Sheiner, MD (Hartford Hospital, Hartford, CT); Harry Lockstadt, MD (Bluegrass Orthopedics & Hand Care Research, Lexington, KY); Kecia Courtney, BSc, Junliang Cheng, PhD, Gladis Barrera, MSc, and Waleska T Henriquez, BSc (Grifols Bioscience Research Group, Grifols, Barcelona, Spain).