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EHTIC study: Evaluation of a new hemostatic agent based on tissue factor in skin grafting procedures

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ABSTRACT

Background: Excessive bleeding is a major concern in scar debridement and grafting procedures. TT-173 is a new topical hemostatic agent based on recombinant human tissue factor that has shown promising results in patients who underwent tooth extraction. EHTIC study sought to evaluate the efficacy and safety of TT-173 to reduce the bleeding in donor sites of skin grafting procedures.

Methods: EHTIC study was a phase II, randomized, parallel, double blind, placebo controlled trial. Patients received TT-173 (n=38) or placebo (n=33) sprayed over donor site after graft harvest. Time to hemostasis and incidence of adverse events were recorded. Systemic absorption of the product and its immunogenicity were also measured during the follow up of the subjects.

Results: Treatment with TT-173 significantly reduced the bleeding time from 7 to 3 min (Log-Rank $p < 0.0001$). Moreover, bleeding stopped within the 10 min of evaluation period in all the patients that received TT-173. In contrast, 24.24% of patients from placebo group required additional measures to arrest hemorrhage (Fisher $p = 0.0013$). Product related adverse events, systemic absorption into blood stream, interferences with the healing of the donor site or immunogenic reaction against TT-173 were not observed.

Conclusion: The new hemostatic agent TT-173 has proven efficacious and safe to reduce the bleeding from donor site. This study paves the way for further investigation of the product as topical hemostatic treatment in plastic surgery and other surgical indications.

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1. Introduction

Excessive bleeding is one of the major concerns associated with skin grafting procedures, as it frequently requires the transfusion of allogenic blood and, in the latter instance, influences the surgical strategy and limits the total surface treatable in a single session [1]. The tangential debridement of burned tissue or scars is the major cause of hemorrhage associated with these procedures. However a significant amount of blood can also be lost from the donor areas if large graft surface is to be collected [2]. Excessive bleeding may also occur both in donor and receptor areas as a consequence of the reduced hemostatic function of severely burned patients [3].

Several strategies to improve bleeding control in skin grafting procedures have been suggested including systemic, subcutaneous and topical treatments. In this way, the use of topical or subcutaneous vasoconstrictors such as epinephrine has been proved useful to limit the blood loss [4–8]. Alternatively, the bioactive agents such as liquid thrombin and fibrin sealants have been evaluated in several clinical studies that showed the efficacy of these products in donor or receptor sites [9–13]. Although helpful, these treatments are far from perfect and the development of new therapeutic strategies to improve the bleeding control in skin grafting procedures is highly desirable. In fact, epinephrine is inevitably absorbed into systemic circulation and, in some instances, can lead to systemic side effects [14,15]. Compared with epinephrine, thrombin formulations are expensive and their use can trigger immunogenic reactions against factor V or thrombin itself. This adverse event has been reported mainly in formulations containing thrombin derived from bovine blood and, in last instance, can lead to secondary coagulopathy [16,17]. This risk is thought to be lower for formulations containing human or recombinant thrombin [18,19].

Fibrin sealants are effective to limit the blood loss and may be an alternative to staples and sutures to fix the skin graft in receptor area [20–22]. However, its use in the context of skin grafting procedures is expensive, especially if large areas of the body should be treated. Secondly, they contain thrombin and eventually other coagulation factors that could trigger immunogenic reactions and immune mediated coagulopathy. Finally, the fibrinogen used in fibrin sealants is obtained from human blood and, in consequence, there is a potential risk of transmission of infectious agents [23–25]. Several quality controls and procedures are necessary to minimize the chance of this transmission and, in consequence, fibrin sealants are among the most expensive hemostatic agents available. In general, recombinant products are less expensive and simpler to produce than those obtained from human blood, but different recombinants can significantly differ in their manufacturing requirements and associated costs.

TT-173 is a new hemostatic agent based on tissue factor (TF) that activates the extrinsic pathway of coagulation. The product consists in a modified version of recombinant tissue factor (rhTF) incorporated into lipid microvesicles. It is formulated as a lyophilized powder that should be reconstituted and used as a suspension in a similar way as bovine, human or recombinant thrombin [26]. The use of TF as a topical hemostatic treatment has been proposed in the past [27]. However, TF is a transmembrane protein that must be integrated into a lipid bilayer and properly folded in order to be active [28]. This has limited the development of bioactive, pharmaceutical grade TF for clinical use. TT-173 was produced

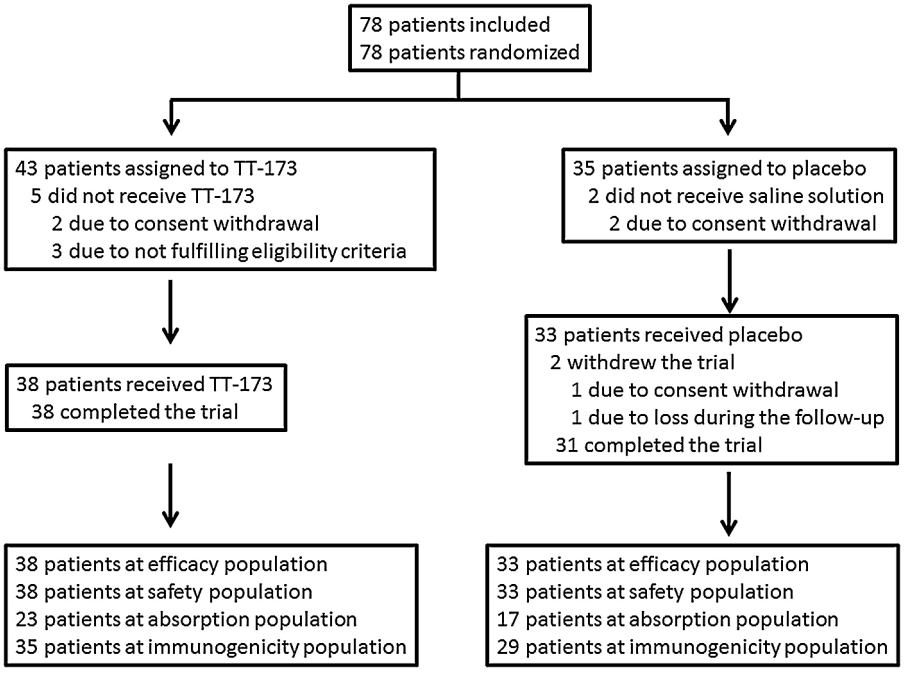

Fig. 1 – Distribution of the patients recruited in the study.

in *Saccharomyces cerevisiae* that express a modified form of human tissue factor. The posterior purification and lyophilization of cell membranes enable the obtention of bioactive, stable at room temperature and pharmaceutical grade recombinant TF to be used as topical hemostatic agent [29].

TT-173 has been previously evaluated in a phase I, randomized, open label and placebo controlled clinical trial that recruited subjects undergoing tooth extraction. In this study, the administration by dropping the product over the alveolus reduced the bleeding time after dental extraction without associated adverse events or other safety concerns [30].

Due to the promising results obtained in the phase I study, a second trial was carried out in order to evaluate the efficacy and safety of the product in major surgery. In this way, the EHTIC study was designed to evaluate the efficacy, safety, systemic absorption and immunogenicity of TT-173 in patients subjected to skin grafting. This was a Phase II trial that compared the product against placebo in order to obtain a proof of concept of the potential usefulness of TT-173 in grafting procedures. Results of this phase II could pave the way for further phase III trials to evaluate the efficacy and safety of TT-173 in comparison with standard of care in larger number of patients.

2. Methods

2.1. Ethical considerations

This trial complied with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and its amendments and was performed according to applicable regulations (European directive 2001/20/EC and Spanish Royal Decree 223/2004, of February 6, 2004). The trial was registered in the www.clinicaltrials.gov database with the number NCT02012569 and was approved by the Spanish Medicine Agency (AEMPS) and the respective Ethical Committees of participating centers. Informed consent was obtained from all the patients recruited before the realization of any study procedure.

2.2. Study design

This Phase II multicenter clinical trial was designed as a double blind, placebo controlled, prospective and randomized study to evaluate the safety, efficacy, immunogenicity and systemic absorption of a dose of 148 μg of TT-173 applied over a donor site of 100 cm^2 (Fig. 1).

The study comprised 7 visits: an initial visit to evaluate the inclusion and exclusion criteria, to obtain the informed consent and to collect the basal samples to quantify the systemic absorption and the immunogenic response against the product; a second visit where the surgery was carried out, treatment was applied and time to hemostasis was recorded; four follow up visits after 1, 2, 7 and 14 days where the safety and systemic absorption were evaluated; and a final visit after one month where safety was evaluated again and samples for immunogenicity characterization were collected (Table 1).

2.3. Patients

Patients over 18 years of age undergoing to a split thickness skin grafting procedure were recruited. The most common

Table 1 – Study design and timeline of procedures.

	Visit 1 (V1) Screening −4 days to 0 day	Visit 2 (V2) Intervention 0 day	Visit 3 (V3) 1 day ±6h	Visit 4 (V4) 2 days ±12h	Visit 5 (V5) 7 days ±2 days	Visit 6 (V6) 14 days ±2 days	Visit 7 (V7) 35 days ±7 days
Informed consent	X						
Eligibility criteria	X						
Medical history	X						
Concomitant treatments	X	X	X	X	X	X	X
Demographic data: age, sex, weight, height	X						
Vital signs	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X
Serology: HBV, HCV, HIV	X						
Hemogram	X	X	X	X	X	X	X
Biochemistry	X	X	X	X	X	X	X
Coagulation tests	X	X	X	X	X	X	X
Pregnancy test	X						X
ECG	X						
Study treatment administration		X					
Pharmacokinetics	X	X	X	X	X		
Evaluation of hemostasia of donor site		X					
Evaluation of epithelization of donor site					X	X	X
Immunogenicity	X						X
Adverse events review		X	X	X	X	X	X

causes for the surgery were burn or traumatic injuries but patients treated for tumor or infection sequelae were also candidates for enrolment. Patients (n=78) were recruited from 8 Spanish University Hospitals: La Fe University and Polytechnic Hospital (n=20), La Paz University Hospital (n=19), University Hospital Complex of A Coruña (n=15), University Hospital of Getafe (n=11), University Hospital Virgen del Rocío (n=5), University Hospital of Bellvitge (n=4), Miguel Servet University Hospital (n=3), Cruces University Hospital (n=1).

Volunteers were randomized to treatment or control groups (43 and 35 patients, respectively). Subjects were excluded from the selection if they presented with a lesioned body surface over 30%, personal or family history of coagulation disorders, affectation by hematological, respiratory, cardiologic, active oncological disease, renal insufficiency or active infection. Other exclusion criteria were the presence of uncontrolled diabetes type II, clinically relevant systemic alterations, treatment with antiplatelet agents, anticoagulants, active drug abuse and hypersensitivity to yeasts.

2.4. Collection of the graft

Collection of donor graft was performed with electrical dermatome under general epidural or regional blocking anesthesia. A donor site of 100 cm^2 was used to evaluate the efficacy. Subcutaneous infiltrations of any kind were not permitted in the area used for bleeding time determination.

2.5. TT-173 administration procedure

After obtaining the graft, 1 mL of TT-173 solution (37 μg/mL) was applied to donor site using a 1 mL Luer-lock syringe coupled to nozzle cap diffuser from a distance of approximately 20 cm. The product was applied for one minute without applying compression or covering the donor site to facilitate their contact with blood (Fig. 2). After that time, the area was slightly compressed with the aid of cotton gauzes and the duration of the bleeding was recorded. With this maneuver, the excess of blood was removed to enable the inspection of the surface of the injury. This process was repeated three more times until applying a total dose of 148 μg/4 mL with four syringes. The patients randomized to the control group received the same volume of saline solution applied in the same way.

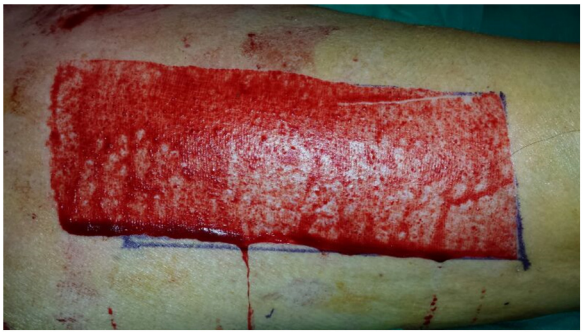

Fig. 2 – A 100 cm^2 donor site treated with TT-173 after bleeding cessation showing the coagulated blood all over the surface of the lesion.

2.6. Randomization

The 1:1 allocation of the subjects to TT-173 and saline solution was performed at the statistical department of the Contract Research Organization (PIVOTAL S.L., Madrid, Spain), irrespective of subjects' characteristics and blinded for the investigators involved in the study. Randomization was performed in blocks and the subjects were stratified according to center, skin lesion cause (burn versus trauma/other) and lesion size.

The subjects were randomized during the screening period, and received the TT-173 product or the saline solution according with the randomization list. This list was generated with Oracle® and the patient randomization codes were provided to the site's pharmacy by an onsite RDC (Remote Data Capture) system. The four syringes loaded with TT-173 or saline solution were filled in the pharmacy, transferred to surgical room using a cool bag and used during the following four hours after product reconstitution.

2.7. Concomitant medications

Antibiotics, NSAIDs, paracetamol, opioids and metamizole were permitted during all the study period. At the end of bleeding time determination, the donor site was managed according to the standard procedures of participating centers. Oral anticoagulants, antiplatelet drugs and unfractioned heparin were not permitted from one week before the inclusion to 24 h after intervention. Fractioned heparins at prophylactic doses indicated to prevent postsurgical pulmonary thromboembolism (20–40 mg of enoxaparin) were permitted during all the study.

2.8. Efficacy evaluation

The efficacy was evaluated by determination of the time to hemostasis every minute for a maximum of 10 min. If the bleeding persisted after this time, rescue measures such as compression or bandaging were applied according to the surgeon criteria. Every minute the donor site area was carefully dried with the aid of conventional cotton gauze avoiding lateral friction and taking special care to not remove the formed clots. This maneuver required only 3–5 s and was performed to eliminate the excess of uncoagulated blood and exudate in order to enable the visualization of the donor surface to evaluate the bleeding. Hemorrhage persistence was determined at surgeon criteria by visual identification of bleeding points. In each participating center all the patients were recruited and evaluated by the same investigators. In centers with more than one investigator, at least one of them was present in all the evaluations performed in his center.

2.9. Safety evaluation

Safety evaluation was conducted by the measurement of physiological parameters (Tª, arterial pressure, heart rate, and hemoglobin O_2 saturation) and adverse events recording. Additionally, blood determinations including coagulation parameters (aPTT, PT, thrombin time, fibrinogen and D-dimer), full blood count and serum biochemistry (urea,

creatinine, glucose, transaminases, gamma-GT, alkaline phosphatase, sodium, potassium, chloride, calcium, total cholesterol, albumin, total and direct bilirubin, lactate dehydrogenase, erythrocyte sedimentation rate and C-reactive protein) were performed before surgery and at 2, 24, 48 h, 15 and 30 days after treatment.

2.10. Evaluation of systemic absorption

In order to evaluate the systemic absorption of the product, citrated total blood samples were collected before treatment, at 2 h and 1, 2 and 15 days after TT-173 application. Two commercially available ELISA kits (IMUBIND® Tissue Factor Kit American Diagnostica, Lexington, MA, USA; *S. cerevisiae* Host Cell Proteins Assay® Cygnus Technologies, Southport, NC, USA) were used for the quantification of TF and *S. cerevisiae* proteins in total blood samples.

The Limit of Detection (LOD) and the Lower Limit of Quantification (LLOQ) determined for the experimental conditions of the study were 3.375 pg/mL and 22.404 pg/mL for IMUBIND® Tissue Factor Kit and 9.463 ng/mL and 26.520 ng/mL for *S. cerevisiae* Host Cell Proteins Assay®.

2.11. Immunogenicity evaluation

The occurrence of immunogenic response against the product was evaluated before and one month after treatment. Competitive ELISAs were designed, validated and used to evaluate the production of antibodies against rhTF, the whole TT-173 and the His-tag peptide. An increment in serum immunoreactivity higher than 20% (coefficient of variation of the method) compared with its respective basal value or with naïve serum control was considered as indicative of positive immune response.

2.12. Statistical analysis

Shapiro–Wilk test was used to contrast if the continuous measures followed a Normal distribution. To analyze the relationship between continuous variables and the treatment group, Student's t-test was used if the variable followed the Normal distribution. Otherwise, the non-parametric Wilcoxon test was applied. The variation of continuous variables with respect to the baseline determination was contrasted using the paired t-tests or the non-parametric Wilcoxon Signed-Rank test. The association between categorical variables and the treatment group was evaluated using the Chi-square test or Fisher's test, as applicable. To analyze repeated measures of a continuous variable, the ANOVA model was applied if the variable followed the normal distribution and a Mixed Model otherwise. For categorical variables (previously dichotomized), a logistic regression model of repeated measures was used. A survival analysis using a Kaplan–Meier model, stratified by the treatment group, was carried-out to analyze bleeding time determinations. In this case, the comparison between treatment arms was performed by means of Log-Rank test. A bilateral risk of $\alpha = 5\%$ was fixed for all analyses performed, and a signification level of 95% (alpha = 0.05) was considered as statistically significant. All analyses were performed by using the SAS version 9.3 statistical package.

3. Results

3.1. Population characteristics

Study arms were balanced without any statistically significant differences between groups in demographic variables and population characteristics (Table 2). Most of the subjects were caucasoid (TT-173: 97.37%, placebo: 96.97%); male (TT-173:76.32%, placebo 66.67%) and mean ± SD ages were 47.92 ± 13.02 and 41.42 ± 14.75 years for TT-173 and placebo groups, respectively. The main reason for skin graft was burns (TT-173: 84.21%, placebo: 84.86%) followed by trauma (TT-173:10.53%, placebo: 9.09%). Other causes of lesion were reported in 5.26% and 6.06% of the subjects from each treatment arm.

The mean body surface affected by skin lesions was also similar between groups, 6.51% and 5.83% in subjects treated with TT-173 and placebo, respectively. Skin grafts were obtained from limbs and trunk, but in the vast majority of the cases were harvested from lower limb (TT-173: 97.37%, placebo: 93.94%).

3.2. Efficacy

The mean ± SD and median bleeding times were 3.53 ± 2.1 min and 3.00 min (Q1, Q3: 2.00, 3.00), for those subjects treated with TT-173. Those times were significantly shorter ($p < 0.0001$) than those recorded in placebo group, in which the mean ± SD and median times were, respectively, 6.73 (2.80) min and 7.00 min (Q1, Q3: 4.00, 10.00).

Kaplan–Meier analysis showed a statistically significant increased hemostasis probability for the subjects treated with TT-173 with a hazard ratio (HR) in front of controls of 0.308 (95% CI: 0.179 to 0.530; log-rank <0.0001) (Fig. 3).

In fact, satisfactory hemostasis was achieved within 1.00–4.00 min in most of the subjects (73.68%) treated with TT-173. By contrast, in the subjects treated with placebo, hemostasis

Table 2 – Characteristics of the patients included in the study. No statistically significant differences in population characteristics were observed between groups.

Subject characteristics	TT-173 n=38	Placebo n=33
Age (years)		
Mean (SD)	47.92 (13.02)	41.42 (14.75)
Gender, n (%)		
Female	9 (23.68)	11 (33.33)
Male	29 (76.32)	22 (66.67)
Cause of skin lesion, n (%)		
Burn	32 (84.21)	28 (84.85)
Trauma	4 (10.53)	3 (9.09)
Other	2 (5.26)	2 (6.06)
Percentage of body surface affected		
Median (Q1, Q3)	4 (1.5, 8)	4 (2, 7)
Source of skin graft, n (%)		
Upper limb	1 (2.63)	1 (3.03)
Lower limb	37 (97.37)	31 (93.94)
Trunk	0 (0.00)	1 (3.03)

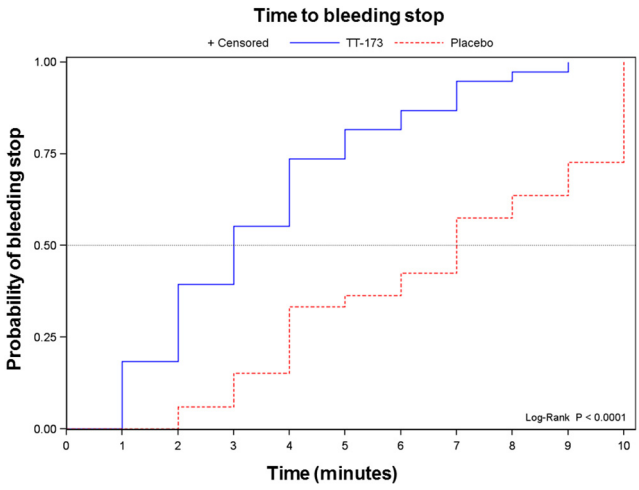

Fig. 3 – Kaplan-Meier analysis of bleeding time.

was reached from 5.00 to 10.00 min (66.66%). In 7 out of 38 subjects from treatment group (18.42%) the bleeding stopped during the first minute of evaluation, while it persisted for longer times in all the subjects treated with placebo. Overall, bleeding lasted less than 10 min in all subjects treated with TT-173, whereas in 27.27% (9 out of 33) of the subjects of placebo group, hemostasis was reached after 10 minutes (Fig. 4). In consequence, no subjects from TT-173 arm required rescue treatment at the end of observation, measures that were necessary in 24.24% of the subjects that received placebo (p=0.0013).

3.3. *Safety*

The number of subjects that experienced at least one adverse event was significantly higher in the placebo group. 24 out of 38 subjects (63.16%) treated with TT-173 had at least one adverse event during the trial compared to 28 out of 33 subjects (84.85%) treated with placebo (Chi-Square, p=0.0395). In total, 159 AEs were reported, 89 from subjects treated with TT-173 and 70 from subjects treated with placebo. All AEs were grade 1 (mild) or grade 2 (moderate), except for 2 subjects treated with placebo who suffered grade 3 (severe) AEs, consisting in dizziness and headache, respectively.

Only 1 subject treated with TT-173 and 1 treated with placebo experienced related AEs consisting in grade 1 pyrexia in both cases. Both events resolved, but in the subject treated with TT-173 lasted 2 days while in the subject that received placebo lasted 22 days. There were no grade 4 (life-threatening) AEs and no subject withdrew the trial due to AEs.

The most frequent AEs were pyrexia, pain, pruritus, nausea, vomiting, wound complication and procedure related pain (Table 3).

5 serious adverse events (SAEs) were reported, 3 from one subject treated with TT-173, 1 from another subject also from treatment group and 1 from a subject that received placebo. All SAEs were unrelated to the study drug and resolved. There were no deaths during the trial.

Results obtained from total blood count, serum biochemistry and coagulation parameters were similar between patients treated with TT-173 or placebo and did not reach statistical significance between treatment arms.

Finally, no differences regarding the epithelization of the donor site were observed. At day 35, almost all wounds were completely epithelized, 97.37% and 90.91% of the subjects from the TT-173 and placebo arms, respectively.

3.4. *Pharmacokinetics*

rhTF and yeast protein concentrations in all samples from treated patients were below the lower limit of detection and below the limit of quantification. In isolated cases, low concentrations of TF were detected. However, the results were not suggestive to be the consequence of treatment

Table 3 – Adverse events observed during the study. No statistically significant differences were observed between groups.

	TT-173 (n=38)			Placebo (n=33)		
	Grade 1–2 n (%)	Grade 3 n (%)	Total n (%)	Grade 1–2 n (%)	Grade 3 n (%)	Total n (%)
Pyrexia	14 (36.8)	0 (0.00)	14 (36.8)	5 (15.2)	0 (0.00)	5 (15.2)
Pain	10 (26.3)	0 (0.00)	10 (26.3)	9 (27.3)	0 (0.00)	9 (27.3)
Wound complication	3 (7.9)	0 (0.0)	3 (7.9)	4 (12.1)	0 (0.0)	4 (12.1)
Procedural pain	3 (7.9)	0 (0.0)	3 (7.9)	3 (9.1)	0 (0.0)	3 (9.1)
Hematoma	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.1)	0 (0.0)	2 (6.1)
Wound secretion	2 (5.3)	0 (0.0)	2 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)
Anemia	7 (18.4)	0 (0.0)	7 (18.4)	8 (24.2)	0 (0.0)	8 (24.2)
Nausea	5 (13.2)	0 (0.0)	5 (13.2)	1 (3)	0 (0.0)	1 (3)
Vomiting	4 (10.5)	0 (0.0)	4 (10.5)	1 (3)	0 (0.0)	1 (3)
Constipation	2 (5.3)	0 (0.0)	2 (5.3)	2 (6.1)	0 (0.0)	2 (6.1)
Pruritus	8 (21.1)	0 (0.0)	8 (21.1)	4 (12.1)	0 (0.0)	4 (12.1)
Dizziness	4 (10.5)	0 (0.0)	4 (10.5)	1 (3)	1 (3)	2 (6.1)
Phlebitis	2 (5.3)	0 (0.0)	2 (5.3)	2 (6.1)	0 (0.0)	2 (6.1)
Anxiety	1 (2.6)	0 (0.0)	1 (2.6)	3 (9.1)	0 (0.0)	3 (9.1)
Insomnia	2 (5.3)	0 (0.0)	2 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)
Back pain	2 (5.3)	0 (0.00)	2 (5.3)	0 (0.00)	0 (0.00)	0 (0.00)

absorption due to the lack of temporality of the findings in relation to product administration. Therefore, under the assayed experimental conditions, TT-173 was not significantly absorbed into the blood stream.

3.5. *Immunogenicity*

The ELISAs determinations carried out with serum samples to detect antibodies against TF, whole TT-173 and His-Tag from patients treated with TT-173 did not reveal any increase in serum immunoreactivity one month after product application. In consequence, under assayed conditions, antibody formation against TT-173 or its constituents did not occur.

4. Discussion

Our results demonstrate that treatment with TT-173 significantly reduces the bleeding time of donor site (Fig. 3). In fact, bleeding stopped within 1.00–4.00 min in most of the subjects treated with TT-173. By contrast, most of the patients that received placebo only achieved hemostasis after 5.00–10.00 min. In addition, no subjects treated with TT-173 needed rescue treatment at the end of evaluation whereas 24.24% of the patients that received placebo required it for bleeding cessation (Fig. 4). The reduction of bleeding observed in our trial was higher than that reported for thrombin in similarly designed studies suggesting that TT-173 could be more effective than thrombin to improve bleeding control during skin debridement and grafting procedures [9,11].

The present study was a phase II trial to evaluate TT-173 for the first time in skin grafting procedures. In order to obtain a proof of concept of the efficacy of the product in this indication the comparison was done against a placebo. Further phase III studies should evaluate the product against standard of care and eventually other hemostatic agents. For its mode of application and characteristics, the most logical comparator could be liquid thrombin at least in those countries in where these formulations are available. Comparisons against epinephrine could also be carried out since it is used regularly in clinical practice. However epinephrine is a vasoconstrictor agent and the hemostatic effect of TT-173 could be synergic. In consequence the most effective approximation to reduce the blood loss could be the combined use of epinephrine and TT-173.

Under the experimental conditions of the trial, TT-173 presented a very favorable safety profile without concerns of local tolerance and did not interfere with the healing of the donor site. The number and characteristics of reported adverse events were comparable in both groups (Table 3). Only one adverse event, grade 1 pyrexia, was considered related to TT-173 by the investigators. Nevertheless, pyrexia is very common in patients who suffered burns and one episode of pyrexia related with the treatment was also reported in the placebo group.

The favorable safety profile of TT-173 was further reinforced by the absence of analytical alterations related with the treatment. Moreover, no differences in analytical parameters were observed between both treatment arms. Additionally, ELISA determinations for detecting the presence of

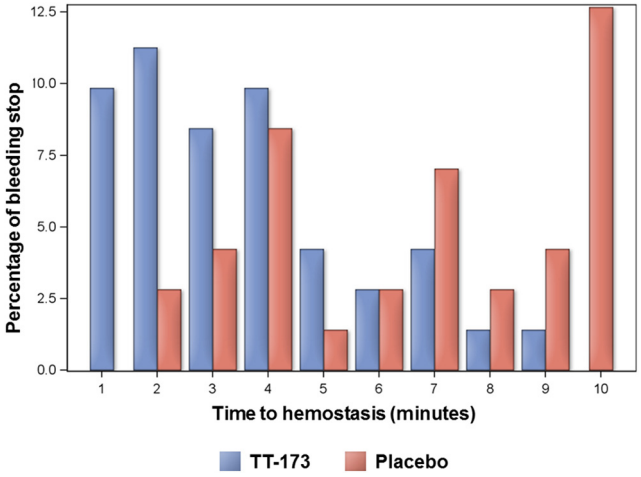

Fig. 4 – Percentage of patients that stopped bleeding at each minute of evaluation showing the elevate number of patients in control group that required additional hemostatic measures.

circulating rhTF or yeast proteins indicate that TT-173 is not significantly absorbed into the blood stream.

TT-173 is a recombinant product produced in yeast and host cell proteins participate in the maintenance of the micro vesicle structure and cannot be eliminated without altering the biologic activity of the product. Moreover rhTF contained in TT-173 presents structural modifications to increase its specific activity. Therefore, it could be hypothesized that TT-173 application could induce an immune response against TF or associated yeast proteins and the evaluation of this risk should be taken into consideration during the clinical development. However, in this study, immunogenic reaction against the product was not observed in the patients that were allocated in the treatment group.

Taken together, the results of the EHTIC study indicate that TT-173 shows an excellent safety profile and that it significantly reduces the bleeding time from the donor site. Furthermore, the lack of immunogenic response would constitute a major advantage as compared to thrombin formulations as their major safety concern is the rise of immunogenic reaction and eventually the development of autoimmune coagulopathy. In a similar way, the absence of systemic absorption would allow the extensive use of the product without risks of systemic side effects, which is a clear advantage in comparison to epinephrine. Moreover, TT-173 activates blood coagulation forming a clot firmly adhered to the bleeding surface (Fig. 2). This mechanism of hemostasis is less prone to rebound effects than the vasoconstriction induced by epinephrine and related compounds, thus reducing the risk of postsurgical bleeding.

Compared with fibrin sealants, TT-173 could be a much cheaper alternative to improve the bleeding control and is more user friendly. TT-173 does not contain any animal or human component, thus ruling out any potential risks of pathogen transmission and the need of strict controls and procedures to avoid it. Moreover, as it is produced in yeast, it is easier to grow in large quantities as compared to recombinant thrombin which is produced in mammalian cells.

Results of the EHTIC study are very promising, but further research is necessary to determine the clinical benefit of TT-173 in skin debridement and other procedures of reconstructive surgery. In fact, the surface of tissue exposed to the product in the present study was only 100cm^2 and the systemic absorption of TT-173 could be higher if larger areas were treated. Moreover, higher doses of TT-173 could be necessary to reduce the bleeding during the debridement of recipient areas and, eventually, this could increase the odds of systemic absorption, immunogenic reactions or systemic adverse events. In this study, a limited number of patients were recruited and cannot be discarded that additional adverse events could emerge if a larger population was exposed to the product. This would be particularly likely in the event of administering higher doses or application in wider severed areas. This could be mostly relevant for detecting events occurring with very low frequency such as clinically relevant immunogenic reactions.

5. Conclusions

TT-173 reduces the bleeding in donor site and could become an alternative to other topical treatments such as thrombin, epinephrine and fibrin sealants. Further investigations are necessary to replicate these findings in lesion debridement and to evaluate the safety of the product in a larger sample of patients.

Conflict of interest

Santiago Rojas, Jesus Murat and Ramón López are employed by Thrombotargets Europe. Other authors have no conflict of interest.

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Appendix A.

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