Safety evaluation of a lyophilized platelet-derived hemostatic product

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BACKGROUND: Hemorrhage causes significant morbidity and mortality in people aged <65 years. A lyophilized platelet-derived hemostatic agent (Thrombosomes) demonstrated hemostatic efficacy in animal models. We report the results of the first safety trial of autologous Thrombosomes given to normal subjects.

STUDY DESIGN AND METHODS: Ten subjects received autologous Thrombosomes prepared from their apheresis platelets, and five control subjects received a buffer solution. There were five cohorts, with three subjects per cohort (two in the Thrombosomes group and one in the control group). Doses escalated from 1/1,000 to 1/10 of a proposed efficacious dose. Cohorts 4 and 5 received the highest dose, but in Cohort 5, one-half the dose was infused 2 hours apart. Cohorts 1 through 3 were monitored for 42 days, Cohorts 4 and 5 were monitored for 60 days using hematology, coagulation, and chemistry assays and antibody testing.

RESULTS: There were no serious adverse events (AEs) and no subject withdrawals. There were eight treatment-related AEs (TRAEs) in 5 of 15 subjects (33%) (four in the Thrombosomes group and one in the control group). Of four subjects receiving the highest doses, three had TRAEs. One had elevated D-dimer, prothrombin fragment 1 + 2, and white blood cell count (subject had concurrent upper respiratory tract infection); one had T-wave inversions in precordial leads V2 and V3 without elevated troponin or symptoms; and one had a platelet autoantibody without change in platelet count. All subjects' TRAEs resolved by Day 21.

CONCLUSION: There were no serious AEs in this small study. Thrombosomes were considered safe at the doses assessed. Future, larger trials will be needed to further assess safety and efficacy.

Traumatic injury resulting in uncontrolled hemorrhage is a worldwide public health concern in individuals aged <46 years and is considered a major cause of preventable death.[1–4] A platelet-derived hemostatic agent, Thrombosomes, has been developed by freeze drying platelets. This agent has the potential to treat uncontrolled, noncompressible bleeding (e.g., internal hemorrhage), diffuse vascular bleeding, or bleeding associated with platelet dysfunction.[5]

ABBREVIATIONS: AE = adverse event; aPTT = activated partial thromboplastin time; BSA = bovine serum albumin; CBC = complete blood cell; CRC = Clinical Research Center; DMC = Data Monitoring Committee; EDTA = ethylenediamineteraacetic acid; EKG = electrocardiogram; FDA = US Food and Drug Administration; FITC = fluorescein isothiocyanate; GP = glycoprotein; HLA = human leukocyte antigen; Ig = immunoglobin; hs = high sensitivity; INR = international normalized ratio; MFI = mean fluorescent intensity; NZWR = New Zealand white rabbit; PE = phycoerythrin; PF 1 + 2 = prothrombin fragments 1 and 2; PI = principle investigator; PT = prothrombin time; TAT = thrombin-antithrombin; TEAE = treatment-emergent AE; TRAE = treatment-related AE; URI = upper respiratory tract infection; WBC = white blood cell.

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In thrombocytopenic New Zealand white rabbits (NZWRs) subjected to an "ear cut through injury" model, human Thrombosomes have reduced blood loss by ≥80%.[6] The lowest observed effective dose approximated 1.89×10^8 particles per kg or approximately 1% of a normal rabbit's platelet count. Human Thrombosomes given to NZWRs were nonimmunogenic and demonstrated a circulating half-life of approximately 24 hours.[7] In a nonhuman primate (*Macaca mulatta*) involving partial hepatectomy, human Thrombosomes demonstrated a strong trend to reduce blood loss when administered 15 to 20 minutes after the onset of bleeding, supporting the hemostatic efficacy seen in the rabbit model.[5]

The Phase 1 dose escalation trial reported herein was the first ever study of Thrombosomes given to humans to evaluate safety, potential thrombogenicity, and immunogenicity of autologous Thrombosomes given to healthy subjects. The trial was designed as an exploratory investigational new drug application, and, in compliance with US Food and Drug Administration (FDA) Guidance, the starting dose was approximately 1/1000th up to 1/10th of the estimated hemostatically effective dose based on the NZWR studies.

MATERIALS AND METHODS

Study design

This was a single-site, single-blind, randomized, controlled safety study of ascending microdoses of Thrombosomes divided into five cohorts, with three subjects per cohort. The study was reviewed and approved by the Western Institutional Review Board (approval 20141084). Each Thrombosomes infusion contained platelet-derived particles manufactured from autologous apheresis platelets collected within approximately 12 weeks before transfusion. In Cohorts 1 through 4, two subjects received ascending microdoses of autologous Thrombosomes or one control subject received the buffering solution used to stabilize/resuspend the Thrombosomes. In Cohort 5, two subjects received the same Thrombosomes dose as in Cohort 4, but one-half the dose was given twice, 2 hours apart. To allow review of results, sequential subjects were at least 3 days apart. The principle investigator (PI) determined the severity and causality of the AEs in a blinded manner, according to a predetermined ad hoc algorithm. The Data Monitoring Committee (DMC) consisted of two physicians who were experts in the field of transfusion medicine and one statistician. Because of the small sample size, the DMC reviewed all blinded data, including AEs and laboratory data, by subject (including the PI's adjudication) before advancing to the next cohort.

Subject selection

Eligibility criteria

Healthy subjects were enrolled if able to do the following: donate a single or double unit of apheresis platelets (minimum, 3.0×10^{11} or 6.3×10^{11} platelets, respectively); provide informed consent; commit to study visits; and not take nonsteroidal anti-inflammatory or aspirin-containing drugs. Subjects had a negative serum pregnancy test result, if a premenopausal woman, and agreed to practice medically acceptable contraception.

Exclusion criteria

Subjects were excluded if: they were aged <18 years or >45 years; they were pregnant or previously pregnant; they received prior blood products; they exhibited abnormal vital signs; they had antiplatelet or anti–human leukocyte antigen (HLA) antibodies; they smoked within the prior 6 months; they had a family or a personal history of venous or arterial thrombosis before the age of 50 years; they had abnormal laboratory assays or electrocardiogram (EKG); they had any medical illness; they had splenectomy/splenomegaly; or they had a history of taking antiplatelet agents, any anticoagulant, or platelet function inhibitors.

Apheresis collection

Plateletpheresis was performed using the Trima Accel Version 6.0. The target platelet count was 3.0 to 6.3×10^{11}. Platelets were tested for infectious diseases and shipped overnight to Cellphire, Inc. for processing into Thrombosomes.

Thrombosomes manufacturing

The units were stored at 22°C in a Helmer Platelet Incubator with constant agitation for no more than 2 days before starting manufacturing. Platelets were tested for acceptability by measuring platelet swirling, pH, and particle count using a hematology analyzer (Beckman Coulter Ac·T 10 or Ac·T diff Hematology Analyzer; Beckman Coulter); size distribution; surface markers (CD41a, CD42b, and CD62P); and annexin V binding by flow cytometry (Beckman Coulter Gallios flow cytometer). After pH adjustment and removal of supernatant plasma, the platelets were resuspended in a trehalose-containing proprietary buffer. After incubation, platelets were diluted to approximately 2.0×10^6 per μL with a proprietary bulking agent, dispensed into glass vials, and lyophilized with a proprietary process using a Millrock Stellar Lyophilizer (Millrock Technology Inc.). Vials were stoppered under vacuum and transferred to a dry heat oven for annealing. After manufacturing, a sample was sent for sterility and endotoxin testing (Wuxi Apptec). Thrombosomes were also tested for aggregation to thrombin, collagen, and arachidonic acid (AggRAM, Helena Laboratories); clot strength was determined by Thromboelastograph (Hemostasis Analyzer Model 5000, Haemonetics Corporation); and thrombin generation was performed using either the Technoclone method (Technoclone GmbH) or the Calibrated Automated Thrombogram (Stago Diagnostica Inc.).

Release criteria for each assay were established for both the apheresis platelets and the finished product on the basis of data from Thrombosomes manufactured from pools of five apheresis donors.

Platelet antibody assay

Platelet antibodies were detected by flow cytometry using a fluorescent mixture of Fluorescein isothiocyanate (FITC)-labeled $F(ab')_2$ goat anti-human immunoglobin (Ig) G (Fc) and Phycoerythrin (PE)-labeled $F(ab')_2$ donkey anti-human IgM (Fc). Platelets were prepared by differential centrifugation of whole blood ($365 \times g$) in a Sorvall RC5 (Thermo Fisher Scientific) drawn in Ethylenediaminetetraacetic acid (EDTA). The platelet-rich plasma was concentrated ($1548 \times g$) to pellet the platelets, resuspended in buffer (0.1 mol/L Tris, pH 7.5, EDTA, and NaCl), and adjusted to a concentration of $300 \times 10^6/\mu L$. Then, 300 μL of platelets and 300 μL of citrated plasma were incubated in a 37°C H_2O bath for 30 minutes. A Sepharose 2B column was equilibrated in 1.6% bovine serum albumin (BSA), and 0.015 mg tromethamine (Tris) buffer was used to separate platelets from unbound plasma proteins. These column-purified platelets were incubated with the fluorescent mixture, washed one time with the 1.6% BSA buffer, and analyzed on a FACSCanto (BD Biosciences). A sample was positive if the log fluorescence was at least 3 SDs above the calculated cutoff for the run using the Levey-Jennings multirole procedure (Westgard Rules).

Unfortunately, Thrombosomes could not be separated from unbound plasma proteins using the column. Therefore, Thrombosomes were adjusted to a concentration of $2 \times 10^5/\mu L$, and 100 μL was incubated with 100 μL of plasma. Thrombosomes plasma samples were washed two times with buffer; 20 μL of FITC goat anti-human IgG (Fc) was incubated with the Thrombosomes suspended in 100 μL of buffer and was washed with buffer. Analysis was performed on the FACSCanto. The cutoff was established using Thrombosomes and individual normal negative control plasma values. If the mean fluorescent intensity (MFI) exceeded the negative plasma MFI by 3 SDs, the assay was positive. A known positive antibody sample was also tested with each day's runs. All Thrombosomes testing was performed in triplicate and was reported as a mean. Analyzing subject samples using a glycoprotein (GP)–specific assay to detect anti-GP IIb to IIIa and anti-GP Ib to IX antibodies was considered for further evaluation if a clinically significant antibody was detected by the standard method previously described.

HLA antibody

Detection of antibodies to class I HLA was performed using a Luminex-based assay (LABScreen Single Antigen HLA Class I kit; One Lambda, Inc.). Test serum was incubated for 30 minutes with microparticles coated with purified HLA antigens. Unbound antibodies were washed off the beads, PE-labeled goat anti-human IgG was added to the microparticles, and they were incubated another 30 minutes. The microparticles were washed again and analyzed with a Luminex analyzer. A sample was positive if the fluorescence of any microparticle population (each of 97 populations was coated with a different HLA) was higher than the local cutoff values, compared with negative controls.

Randomization and blinding

Randomization codes for eligible subjects were provided in sealed envelopes. Subjects and staff interacting with subjects were blinded to material administered. Staff responsible for dose preparation wrapped each syringe (test article or control) in aluminum foil and labeled with the designated recipient's identification number before transferring it to the intravenous administration staff for infusion. In addition, all doses were diluted in 10 mL of control buffer to maintain blinding.

Doses administered

A starting dose of 1.41×10^7 total Thrombosomes (equivalent to 2.01×10^5 Thrombosomes per kg in a 70-kg subject) was 1/1,000th of the equivalent projected human therapeutic dose (2.06×10^8 Thrombosomes per kg). Ten subjects received ascending doses of Thrombosomes from 1/1,000th to 1/10th the projected minimum effective dose, and five received the control buffering solution. All doses were diluted in 10 mL of the control buffer.

CRC admission

Subjects were admitted to the Clinical Research Center (CRC) at the University of Washington Medical Center (Seattle) for preinfusion and 24-hour postinfusion monitoring. Preinfusion, the following evaluations were performed: complete medical history and physical examination, baseline global neurologic assessment, vital signs, pulse oximetry, laboratory tests (prothrombin time/international normalized ratio [PT/INR], activated partial thromboplastin time [aPTT], D-dimer, platelet aggregation, fibrinogen, thrombin-antithrombin [TAT], prothrombin fragments 1 and 2 [PF 1 + 2], complete blood cell [CBC] count [which was performed {at select times} at two different laboratories to provide a comparison of results over time from CBC testing performed at two locations], urinalysis, serum pregnancy test, high sensitivity (hs) troponin I, chemistry 12 panel, γ-glutamyl transferase, lactate dehydrogenase, total cholesterol, and urine drug screen), microscope examination for anisocytosis and platelet clumping, antibody testing (platelet and Thrombosomes autoantibodies and alloantibody and HLA assays), infectious disease screening, Holter monitoring, and 12-lead EKGs. These studies and adverse event (AE) assessments were periodically repeated for up to 42 days

for Cohorts 1 through 3 and up to 60 days for cohorts 4 and 5. (Supplemental Data 1).

Grading of AEs

Severity assessment of AEs:

- Mild-transient deviation from subject's normal clinical course not requiring medical intervention.
- Moderate-deviation from subject's normal clinical course and may require medical intervention (including additional samples for laboratory testing).
- Severe-deviation from the subject's normal clinical course and may represent a major threat of mortality or morbidity.
- Life threatening-subject has immediate risk of death.

Thrombosomes reconstitution

Thrombosomes were reconstituted before administration by adding sterile water, and they were transfused within 1 hour.

Study endpoints

The primary end point was product safety and tolerability. Secondary endpoints were hematologic and immunologic responses to transfusion.

Statistical analysis

Descriptive statistical analyses were performed for select laboratory data (hematology, coagulation, and chemistry measures) over time. Comparative statistical analyses using t tests or Mann-Whitney U tests for select laboratory values, including platelet count, PT, aPTT, INR, D-dimer, and fibrinogen, over time were performed for normally or non-normally distributed data; significance was set at 0.05.

RESULTS

Subjects

We screened 90 healthy subjects; 55 did not meet inclusion criteria or declined to consent, of the 35 subjects who were enrolled, 2 subjects failed to complete apheresis, and 18 subjects were withdrawn. The following were the reasons for withdrawal: Thrombosomes did not meet acceptance criteria (n = 11), subject withdrew (n = 3), subject was not eligible (n = 2), abnormal baseline EKG (n = 1), and study was completed before infusion (n = 1). The remaining 15 subjects were infused.

More subjects were men (60%), their mean age was 27.7 years (range, 20-42 years), and their mean weight was 208 pounds, or 94 kg (Table 1). Demographic data were comparable for the test and control groups.

Results of infusion

Table 2 summarizes data on Thrombosomes infusions. Dose escalation by cohort started at 1/100,000 of the maximum safety dose given in the animal studies (one time) and increased incrementally as 1, 10, 30, and 100 times in Cohorts 4 and 5 of the starting dose of 1.5×10^5 per kg. All subjects received the target dose for their cohort on the basis of actual subject weight and estimated blood volume. The mean weight in Cohort 5 (176 pounds, or 80 kg) was lower than in Cohort 4 (229 pounds, or 104 kg), resulting in a higher dose per kg in Cohort 5 (2.03×10^6 particles/kg) versus Cohort 4 (1.55×10^6 particles/kg), although both cohorts were targeted to receive the same total dose.

Vital signs

Blood pressure, heart rate, respiration rate, temperature, and pulse oximetry were obtained at specified intervals (Supplemental Data 1), and all were within normal ranges.

Physical examination

There were no clinically significant changes in general physical examinations or global neurologic assessments for any subject.

12-Lead EKG and Holter monitoring

One subject in Cohort 5 receiving Thrombosomes developed a T-wave abnormality, which was recorded as an AE, possibly related to investigational product and moderate in severity (detailed information given later).

Hematology

There were no clinically significant decreases in hemoglobin, hematocrit, red blood cell, or platelet counts. One subject in Cohort 4 receiving Thrombosomes developed a mild elevated white blood cell (WBC) count; however, the subject also had an upper respiratory tract infection at the time of the increase (detailed information given later).

Antibody assays

One subject in Cohort 5 receiving Thrombosomes demonstrated low levels of IgG on her autologous platelets and tested positive for an antibody to her autologous Thrombosomes at baseline (detailed information given later).

Coagulation assays

One subject in Cohort 1 receiving Thrombosomes and one subject in Cohort 2 receiving control developed an elevated TAT. One subject in Cohort 4 receiving Thrombosomes had a single D-dimer value above normal. This subject also had signs of an active infection at the time of testing but no evidence of a thromboembolic event (detailed information given later).

One subject in Cohort 2 receiving control developed an elevated PF 1 + 2. One other subject in Cohort 4 receiving

TABLE 1. Baseline data

Variable	Thrombosomes (N = 10)	Control (N = 5)	All subjects (N = 15)
Sex			
Female	3 (30)	3 (60)	6 (40)
Male	7 (70)	2 (40)	9 (60)
Age (years)			
Mean (SD)	28 (7)	26 (3)	28 (6)
Median	28	27	27
Minimum, maximum	20, 42	21, 29	20, 42
Race*			
American Indian or Alaskan Native	1 (10)	0 (0)	1 (7)
Asian	1 (10)	2 (40)	3 (20)
Black or African American	3 (30)	0 (0)	3 (20)
Native Hawaiian or other Pacific Islander	0 (0)	0 (0)	0 (0)
White	8 (80)	3 (60)	11 (73)
Other	0 (0)	0 (0)	0 (0)
Ethnicity: Hispanic or Latino origin			
Yes	1 (10)	0 (0)	1 (7)
No	9 (90)	5 (100)	14 (93)
Not reported	0 (0)	0 (0)	0 (0)
Height (inches/cm)			
Mean (SD)	70 (5)/178 (13)	69 (3)/175 (8)	69 (4)/175 (10)
Median	69/175	69/175	69/175
Minimum, maximum	64, 78/163, 198	67, 74/170, 188	64, 78/163, 198
Weight (pounds/kg)			
Mean (SD)	207 (29)/94 (13)	210 (37)/95 (17)	208 (31)/94 (14)
Median	206/93	222/101	208/94
Minimum, maximum	148, 250/67, 113	170, 259/77, 118	148, 259/67, 118

* More than one category may have been selected by a subject; therefore, the percentages may total >100%.
[1] Data are given as number (percentage) of each group unless otherwise indicated.

TABLE 2. Thrombosomes infused

	Thrombosomes				
Variable	Cohort 1 (N = 2)	Cohort 2 (N = 2)	Cohort 3 (N = 2)	Cohort 4 (N = 2)	Cohort 5 (N = 2)
Total volume infused (mL)†	10.5 (2.1)	10.5 (0.7)	10.0 (0.0)	10.0 (0.0)	20.0 (0.0)
Total Thrombosomes infused (particles × 10^6)	13.5 (0.6)	182.9 (48.0)	496.7 (58.3)	1610.0 (141.4)	1600.0 (169.7)
Subject weight (kg)	208.4 (37.7)	204.9 (15.1)	214.8 (20.9)	229.05 (29.6)	176.1 (40.4)
Total particles infused per kg body weight (particles × 10^6/kg)	0.2 (0.0)	2.0 (0.7)	5.2 (1.1)	15.5 (0.6)	20.3 (2.5)
Total blood volume (L)*	5.8 (0.6)	6.2 (0.7)	6.0 (0.1)	5.5 (0.1)	4.4 (0.7)
Total particles infused per mL blood volume (particles × 10^6/mL)	1.4 (0.2)	17.3 (3.4)	49.7 (5.9)	161.0 (14.1)	160.0 (17.0)

Data are given as the mean ± 1 SD.
* Blood volume calculated as 70 mL/kg.
† All doses were prepared to a standard volume of 10 mL; for subjects in Cohort 5, the dose was split into two 5 ml aliquots and administered during two infusions 2 hours apart.

Thrombosomes had an increased PF 1 + 2 value at baseline that showed further elevations, peaking at 24 hours after infusion (detailed information given later).

No clinically significant changes in PT, INR, aPTT, fibrinogen, or platelet aggregation assays were observed.

Chemistry

There were no clinically significant changes in chemistry values, including hs troponin, or urinalysis.

Statistical analyses of select laboratory data

No statistically significant differences were detected after analyses; given the small sample size, this was not unexpected.

Adverse events

All the AEs were mild or moderate in severity, with no serious AEs and no deaths; no subject discontinued study participation. Forty AEs were considered treatment emergent (TEAEs) in 12 of 15 subjects (80%) (three in the control

TABLE 3. Adverse events

Variable	Thrombosomes (N = 10)	Control (N = 5)	All subjects (N = 15)
No. of AEs reported			
• TEAEs*	29	11	40
• TRAEs†	6	2	8
At least one TEAE‡	9 (90)	3 (60)	12 (80)
• Mild	3 (30)	2 (40)	5 (33)
• Moderate	6 (60)	1 (20)	7 (47)
At least one TRAE‡	4 (40)	1 (20)	5 (33)
• Mild	0 (0)	1 (20)	1 (7)
• Moderate	4 (40)	0 (0)	4 (27)

Percentages are based on the number of subjects in each treatment group. Subjects are only counted once in each category. There were no severe, life-threatening, or fatal AEs in either the Thrombosomes or the control group.

* TEAE is an event that emerges during treatment, having been absent pretreatment (i.e., an AE with an onset date and time on or after the first dose of study product); or an AE/medical condition that worsens relative to the pretreatment state (i.e., increases in severity or frequency after the initiation of treatment).

† TRAE is a TEAE considered related or possibly related to the study drug by the investigator.

‡ Data are given as number (percentage) of subjects.

group and nine in the Thrombosomes group) (Table 3). The most frequently reported TEAEs (occurring in two or more subjects) included dizziness and headache, increased PF 1 + 2, nausea, fall, and nasal congestion. Eight of the TEAEs were considered by the investigator as related or possibly related to the infusion in 5 of 15 subjects (33%) (one in the control group and four in the Thrombosomes group; i.e., these were considered treatment-related AEs (TRAEs), and three subjects had their treatment unblinded) (Table 4). Overall, three of four subjects (75%) who received the highest level of Thrombosomes in Cohorts 4 and 5 had TRAEs of a positive platelet autoantibody, abnormal EKG T-wave inversion, or elevated level of PF 1 + 2, D-dimer, or WBCs.

Treatment-related AE description by subject

One subject (Subject 5) in Cohort 1 receiving Thrombosomes experienced moderate dizziness that was possibly related, which started approximately 4 hours after infusion and resolved within 1 minute. The PI designated this as moderate severity because the site increased vital sign monitoring and provided oral hydration (Table 4).

One subject (Subject 63) in Cohort 4 receiving Thrombosomes was also diagnosed as having an upper respiratory tract infection (URI) at 24 hours after infusion on the basis of clinical symptoms of sinus and nasal congestion, and this URI was considered not related. However, this subject had TRAEs of elevated D-dimer level (without changes in PT, aPTT, or fibrinogen), PF 1 + 2 level (value before apheresis was also elevated), and WBC count within 24 hours of infusion that were considered of moderate severity and that resolved without sequelae (Supplemental Data 2).

One subject (Subject 83) in Cohort 5 receiving Thrombosomes was a 28-year-old woman who had a normal EKG at screening and right before infusion (baseline) (Fig. 1). The subject did not have any known risks for thromboembolic disease. At 1 and 2 hours after her second

TABLE 4. Treatment-related adverse events

Subject no.	Cohort	Randomization	AE-preferred term (verbatim term)	Causality	Reasons for moderate severity	Unblinding*
5	1	Thrombosomes	Dizziness	Possibly related	Increased vital sign monitoring and oral hydration	No
63	4	Thrombosomes	D-dimer increased	Possibly related	Additional laboratory testing	Yes to determine what additional testing should be performed, if any
63	4	Thrombosomes	Elevated PF 1 + 2	Possibly related	Additional laboratory testing	See above
63	4	Thrombosomes	WBC count increased	Possibly related	Additional laboratory testing	See above
83	5	Thrombosomes	Electrocardiogram T-wave abnormality	Possibly related	Additional cardiac testing	Yes to consider if changes to protocol and investigational brochure were required, if any
90	5	Thrombosomes	Autoantibody positive (platelet autoantibody)	Related	Additional laboratory testing	No
11	2	Control	TAT III complex increased	Related	N/A-mild severity	No
11	2	Control	Prothrombin level increased (PF 1 + 2)	Related	N/A-mild severity	No

* One other subject (not in table) with a headache and numbness in maxillary area of face on Day 42 after infusion had his treatment unblinded. Headache was similar to almost daily headaches, and AE was determined not related.

N/A = not applicable.

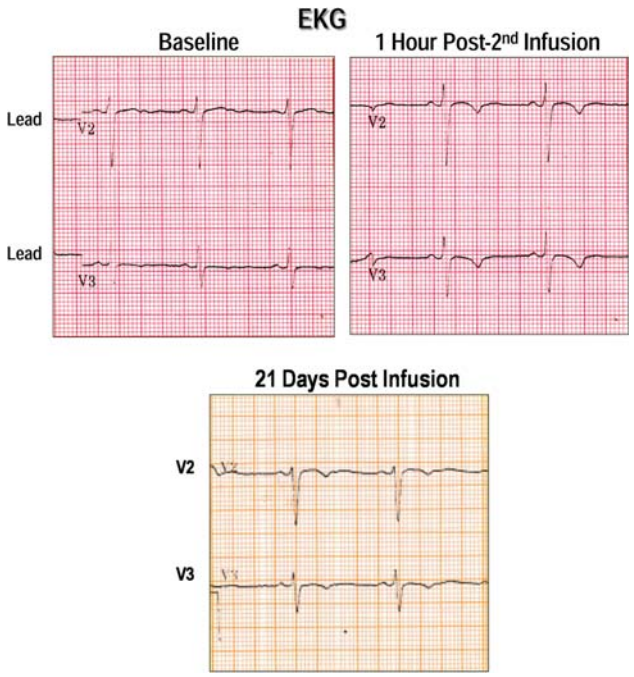

Fig. 1. EKG TEAEs in Subject 83. The subject had two normal EKGs before infusion. At 1 hour after her second Thrombosomes infusion in Cohort 5, she demonstrated T-wave inversions in leads V2 and V3, which resolved by Day 21. [Color figure can be viewed at wileyonlinelibrary.com]

Thrombosomes infusion, the subject had T-wave inversions in leads V2 and V3, which resolved by Day 21 (Fig. 1). The baseline and 1- and 2-hour post second infusion EKGs were all performed by the same nurse using the same EKG machine. By Day 21, these inversions were much less. All EKGs were reviewed by three cardiologists (two were blinded to study group treatment) who suggested these changes were possibly a normal variant. In addition, a stress echocardiogram performed at 5 months after infusion demonstrated no cardiac pathology, and a lead placement study revealed similar T-wave inversions in leads V1 through V3 when the precordial leads were placed in a rib interspace that was too low. The subject's hs troponin levels did not change and were within the normal range. The subject had no clinical symptoms and no changes in vital signs or pulse oximetry during her hospitalization. This event was considered possibly related to the investigational product and was designated as moderate in severity because additional laboratory testing was performed. This subject also experienced a moderate TEAE of nausea during a blood draw after infusion that was considered unrelated.

One subject (Subject 90) in Cohort 5 receiving Thrombosomes had a TRAE of a low-level positive platelet IgG autoantibody test (35 times lower than the positive control). Screening and baseline platelet autoantibody results were negative (MFI, 3), but the autoantibody test became positive at Day 7, remained positive on Days 14 and 21, and returned to negative on Days 42 and 60 (Supplemental Data 3, Fig. 2). There were no changes in platelet counts. Test results for autoantibody against autologous Thrombosomes were positive at baseline and Days 7, 14, and 21; they became negative on Days 42 and 60. Positive control MFI values were 140 to 300, whereas the subject's highest MFI was 7. There were no alloantibodies against donor platelets

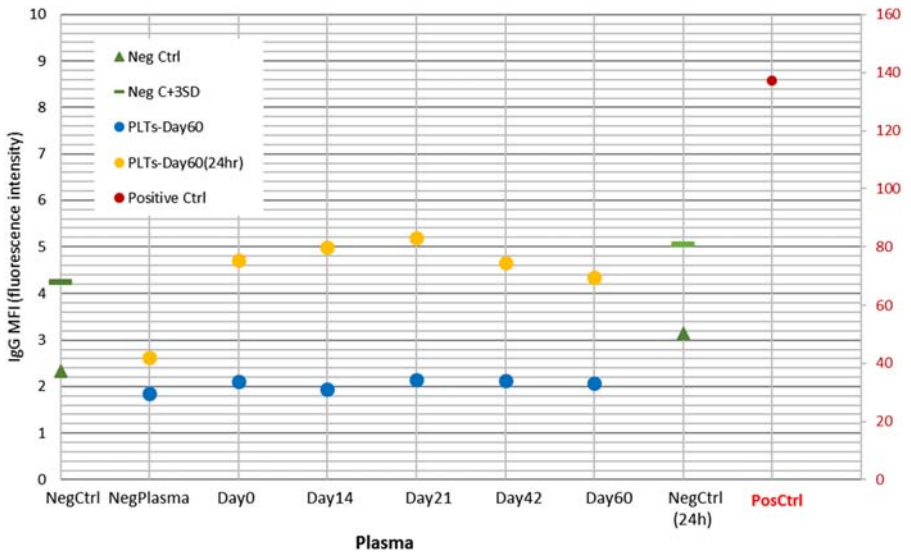

Fig. 2. Autoplatelet antibody test results through Day 60 in Subject 90. The subject's platelets (PLTs) exhibited MFI results that were positive (Pos; MFI values greater than the mean of the negative control [Neg Ctrl] plus 3 SDs on Days 7, 14, and 21) and were negative on screening, baseline, and Days 42 and 60 after infusion. Positive control MFI values were 140 to 300, whereas the subject's highest MFI was 7; mean Neg Ctrl plus 3 SDs was selected as the cutoff to indicate a positive result and ranged from 4.0 to 5.6

or pooled random donor Thrombosomes. The results of HLA antibody tests done on Days 21, 42, and 62, and of tests for platelet-specific antibodies and red blood cell alloantibodies or autoantibodies done at Day 14, were all negative. This AE was designated as moderate in severity because additional laboratory testing was performed.

Additional TEAEs in this subject (Subject 90), considered to be either unlikely or not related to treatment, included urticaria on Day 13 after the use of topical sunscreen, menorrhagia, and dysmenorrhea (there was no history of menorrhagia or dysmenorrhea); signs of hyperventilation (dyspnea, nausea, and dizziness) on Day 26; a fall on Day 51; gastritis on Day 54; and a second event of menorrhagia and dysmenorrhea on Day 56.

One subject in Cohort 2 (Subject 11) receiving control had normal TAT and PF 1 + 2 values at baseline that increased above their normal ranges at 1 hour and both returned to normal by 24 hours after infusion (Supplemental Data 2). These changes in coagulation tests were deemed related and mild in severity. WBC counts also tended to increase and decrease in the same time frames as the coagulation assays but were never outside the normal range. Approximately 5 weeks after infusion, the subject experienced left facial numbness that lasted approximately 1 hour, concurrent with a headache similar to previous headaches (before enrollment in the study) that the subject experienced. Because of the time interval between infusion and symptoms, these findings were considered unrelated.

DISCUSSION

This study reports data on the first human infusion of lyophilized platelets (Thrombosomes). This was a dose escalation study of five Thrombosomes cohorts given ascending doses of 1/1,000th to 1/10th of the minimum hemostatically effective dose on the basis of animal studies.[5] Because of concerns that the activated lyophilized platelets might be thrombogenic, the FDA required that the subjects be hospitalized with constant monitoring of vital signs, including O_2 saturation and Holter monitoring. Other monitoring for the first 24 hours included frequent EKGs, a large panel of laboratory assays (coagulation, chemistries, cardiac, and testing to detect the formation of antibodies to the subject's platelets, allogeneic platelets, HLA, red blood cells or Thrombosomes), and neurologic assessments. Follow-up evaluations were performed over the following 42 days (Cohorts 1-3) and 60 days (Cohorts 4 and 5) (assessment tests and timing are outlined in Supplemental Data Table 1). The possible immunogenicity of Thrombosomes was a concern, and, although subjects were given autologous Thrombosomes, antibody studies were completed to determine if an immunogenic neoantigen was formed during manufacturing. Transfusion of allogeneic Thrombosomes manufactured from platelet pools could induce antiplatelet antibodies from intact cells or particulate antigens. Future clinical studies will be powered to detect the incidence of antibody formation to infusion of allogeneic Thrombosomes.

To mitigate the potential for thrombogenic or immunologic effects, the study subjects were carefully selected to be aged <45 years, with no personal or family history of thrombotic events before the age of 50 years, and they had to have had no prior pregnancies or transfusions.

Demographic data did not differ between test and control subjects (Table 1). All 15 subjects who received transfusion completed the study without serious AEs or deaths. Preinfusion and postinfusion platelet counts were not changed. A total of 40 AEs were observed, but they were all considered either mild or moderate in severity (Table 3). Only eight of these AEs (in five subjects) were considered possibly related to the transfusion (Table 4). However, three of the four subjects in the Thrombosomes group, in Cohorts 4 and 5, given the highest dose, had TRAEs after infusion. One subject (Subject 63) (at the time of a URI) had elevated PF 1 + 2 level, D-dimer level, and WBC count at 1 to 8 hours after infusion that resolved by Day 7 without other changes in coagulation factors (Supplemental Data 2). One subject (Subject 90) had T-wave inversions in EKG leads V2 and V3 at 1 hour after infusion (Fig. 1) that resolved by Day 21, with no changes in troponin levels; complete cardiac workup showed no cardiac damage. One subject (Subject 90) developed a low-level platelet autoantibody at Day 7 that resolved by Day 42, without evidence of platelet-specific or HLA antibodies (either autoantibodies or alloantibodies) and no decrease in platelet count (Supplemental Data 3, Fig. 2). This subject, interestingly, also had an antibody against her autologous Thrombosomes before infusion that became negative on Day 42. The subject had no platelet or Thrombosomes alloantibodies.

Although three of the four subjects given the highest Thrombosomes dose had treatment-related AEs, they were without sequelae; and they were all different, making it difficult to ascertain their clinical significance. Because Thrombosomes are being developed as a hemostatic agent for noncompressible bleeding when conventional platelets are not available and because of the small sample size of the current study that was performed in normal healthy subjects, additional studies in bleeding patients are clearly warranted to determine the safety and efficacy of the product at clinically relevant doses in patients with disease-related risk factors. The next Thrombosomes study will involve hematology/oncology patients with hypoproliferative thrombocytopenia, an important progression away from studies in normal subjects.

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Arthur Palfrey Bode, Ph.D.
October 12, 1953–November 11, 2016

The authors want to acknowledge the contributions of Dr. Arthur Bode (Art), not only to this clinical trial and the advancement of Thrombosomes to this stage, but we also recognize his lifelong dedication to bringing lyophilized platelets to fruition. Art's background included the scientific investigation of platelet function and transfusion for >25 years in an academic setting, full professorship in pathology at East Carolina University (ECU). His background also included several years at a start-up company as Chief Science Officer for projects involving spray-dried plasma or lyophilized platelets. He authored six patents on stabilization of blood cells for long-term storage, stemming from work funded primarily by the Department of Defense, and had numerous publications in scientific journals and textbooks. While at ECU, Dr. Bode was the Scientific Director of the Clinical Coagulation Laboratory and Director of the Platelet Research Laboratory as well as Director of the Core Facility in Flow Cytometry and Confocal Microscopy; he was also a fellow of the American Heart Association and the Society in Clinical and Applied Thrombosis/Hemostasis. He joined Cellphire in early 2014. Art was eminent in the field of platelet research, a joy with whom to work, a gentleman, a scholar, and a friend; he is missed.

CONFLICT OF INTEREST

JB, BO, GT, EP, and SJS have disclosed no conflicts of interest.

MF, RB, and AY are employees of Cellphire. JP is a paid consultant for Cellphire.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Supplemental Data 1: Schedule of tests/evaluations.
Supplemental Data 2: Laboratory TRAEs in Subjects 11 and 63.
Supplemental Data 3: Autoantibody TRAEs in Subject 90.