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SAFETY EVALUATION OF A LYOPHILIZED PLATELET DERIVED HEMOSTATIC PRODUCT

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Abstract

Background: Hemorrhage causes significant morbidity and mortality in persons under 65. 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 5 control subjects received a buffer solution. There were 5 Cohorts, 3 subjects/Cohort (2 Thrombosomes, 1 Control). 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, ½ the dose was infused two hours apart. Cohorts 1–3 were monitored for 42 days, Cohorts 4–5 for 60 days using hematology, coagulation and chemistry assays, and antibody testing.

Results: There were no serious adverse events (SAEs) and no subject withdrawals. There were 8 treatment related adverse events (TRAEs) in 5/15 subjects (33%) [4 Thrombosomes (40%), 1 control]. Three of 4 subjects receiving the highest doses had TRAEs. One had elevated D-dimer, Prothrombin Fragment 1+2, and WBC (subject had concurrent upper respiratory infection), one had T-wave inversions in pre-cordial 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: As there were no SAEs in this small study. Thrombosomes were considered safe at the doses assessed. Future, larger trials will be needed to further assess safety and efficacy.

Keywords

platelets

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INTRODUCTION

Traumatic injury resulting in uncontrolled hemorrhage is a worldwide public health concern in individuals under 46 and is considered a major cause of preventable death.⁽¹⁻⁴⁾ A platelet-derived hemostatic agent (PDHA), Thrombosomes, has been developed by freeze drying platelets. This agent has the potential to treat uncontrolled, non-compressible bleeding (such as internal hemorrhage), diffuse vascular bleeding, or bleeding associated with platelet dysfunction.⁽⁵⁾

In thrombocytopenic New Zealand white rabbits (NZWRs) subjected to an 'ear cut through injury' model, human Thrombosomes have reduced blood loss by 80% or more.⁽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 non-immunogenic and demonstrated a circulating half-life of about 24 hours.⁽⁷⁾ In a non-human primate (*Macaca mulatta*) involving partial hepatectomy, human Thrombosomes demonstrated a strong trend to reduce blood loss when administered 15 – 20 minutes after the onset of bleeding, supporting the hemostatic efficacy seen in the rabbit model.⁽⁵⁾

The Phase 1 dose escalation trial reported here 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 (IND) application, and, in compliance with FDA Guidance, the starting dose was approximately 1/1000th up to 1/10th of the estimated hemostatically effective dose based on the NZW rabbit studies.

MATERIALS AND METHODS

Study Design

This was a single-site, single-blind, randomized, controlled safety study of ascending microdoses of Thrombosomes divided into 5 Cohorts with 3 subjects per Cohort. The study was reviewed and approved by the Western Institutional Review Board (WIRB #20141084). Each Thrombosomes infusion contained platelet-derived particles manufactured from autologous apheresis platelets collected within approximately 12 weeks prior to transfusion. In Cohorts 1 – 4, two subjects received ascending microdoses of either autologous Thrombosomes, or one control subject received the buffering solution used to stabilize/re-suspend the Thrombosomes. In Cohort 5, two subjects received the same Thrombosomes dose as in Cohort 4, but 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 determined the severity and causality of the AEs on study 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: 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; not take nonsteroidal anti-inflammatory or aspirin-containing drugs. Subjects had a negative serum pregnancy test if a pre-menopausal woman and agreed to practice medically-acceptable contraception.

Exclusion Criteria—Subjects were excluded if: less than 18 or greater than 45-years old; pregnant or previously pregnant; received prior blood products; exhibited abnormal vital signs; anti-platelet or anti-HLA antibodies; smoked within the prior 6 months; family or personal history of venous or arterial thrombosis before age 50; abnormal laboratory assays or electrocardiogram (EKG); any medical illness; splenectomy/splenomegaly; history of taking anti-platelet agents, any anticoagulant, or platelet function inhibitors.

Apheresis Collection

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

Thrombosomes Manufacturing

The units were stored at 22°C in a Helmer Platelet Incubator (Noblesville, IN) 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; Brea, CA); size distribution, surface markers (CD41a, CD42b, and CD62P), and Annexin V binding by flow cytometry (Beckman Coulter Gallios flow cytometer). Following pH adjustment and removal of supernatant plasma, the platelets were resuspended in a trehalose containing proprietary buffer. After incubation, platelets were diluted to $\sim 2.0 \times 10^6$ 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.; Kingston, NY). 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 Aptec; Marietta, GA). Thrombosomes were also tested for aggregation to thrombin, collagen, and arachidonic acid (AggRAM, Helena Laboratories; Beaumont, TX); clot strength by Thromboelastograph (Hemostasis Analyzer Model 5000, Haemonetics Corporation; Braintree, MA); thrombin generation using either the Technoclone method (Technoclone GmbH; Vienna, Austria) or the Calibrated Automated Thrombogram (CAT) (Stago Diagnostica Inc.; Parsippany, NJ).

Release criteria for each assay were established for both the apheresis platelets and the finished product based on 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 FITC labelled F(ab')₂ goat anti-human IgG (Fc) and PE labelled F(ab')₂ donkey anti-human IgM (Fc). Platelets were prepared by differential centrifugation of whole blood (365g) in a Sorvall RC5 (Thermo Fisher Scientific; Waltham, MA) drawn in EDTA. The platelet-rich plasma (PRP) was concentrated (1548g) to pellet the platelets, re-suspended in buffer (. 1MTris pH 7.5; EDTA; NaCl), and adjusted to a concentration of $300 \times 10^6/\mu\text{L}$. Then, 300 μL of platelets and 300 μL of citrated plasma were incubated in a 37°C H₂O bath for 30 minutes. A Sepharose 2B column equilibrated in 1.6% BSA –.015 tromethamine (TRIS) buffer was used to separate platelets from unbound plasma proteins. These column purified platelets were incubated with the fluorescent mixture, washed 1X with the 1.6% BSA buffer and analyzed on a FACSCanto (BD Biosciences; San Jose, CA). A sample was positive if the log fluorescence was at least 3 SD above the calculated cutoff for the run using 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 $2 \times 10^5/\mu\text{L}$, and 100 μL were incubated with 100 μL of plasma. Thrombosomes-plasma samples were washed 2X with buffer; 20 μL s of FITC goat anti-human IgG (Fc) were incubated with the Thrombosomes suspended in 100 μL of buffer, and washed with buffer. Analysis was performed on the FACSCanto. The cutoff was established using Thrombosomes and individual normal negative control plasmas. If the mean fluorescent intensity (MFI) exceeded the negative plasma MFI by 3SD, 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 specific assay to detect anti-GP IIb IIIa and -GP Ib IX antibodies was considered for further evaluation if a clinically significant Ab was detected by the standard method described above.

Human Leukocyte Antigen (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.; Canoga Park CA). Test serum was incubated for 30 minutes with microparticles coated with purified HLA antigens. Unbound antibodies were washed off the beads, and PE-labelled goat anti-human-IgG was added to the microparticles, and 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 is coated with a different HLA antigen) was above the local cutoff values, compared to 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 ID number prior to transferring it to the IV administration

staff for infusion. In addition, all doses were diluted in 10ml 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 $1/1,000^{\text{th}}$ of the equivalent projected human therapeutic dose (2.06×10^8 Thrombosomes per Kg). Ten subjects received ascending doses of Thrombosomes from $1/1,000^{\text{th}}$ to $1/10^{\text{th}}$ the projected minimum effective dose, and 5 received the control buffering solution. All doses were diluted in 10ml of the control buffer.

CRC Admission

Subjects were admitted to the Clinical Research Center (CRC) at the University of Washington Medical Center (Seattle, Washington) for pre- and 24 hour post-infusion monitoring. Pre-infusion, the following evaluations were performed: complete history and physical exam, baseline global neurological 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 (PF1+2), complete blood count (CBC) [which was performed (at select times) at two different laboratories in order to provide a comparison of results over time from CBC testing performed at two locations], urinalysis, serum pregnancy test, hs Troponin I, Chem 12 panel, Gamma-Glutamyl Transferase (GGT), LDH, total cholesterol, 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 and 60 days for Cohorts 1–3 and for 4 and 5, respectively (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 transfused within one 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, and significance was set at 0.05.

RESULTS

Subjects

We screened 90 healthy subjects, 35 were enrolled, 2 failed to complete apheresis, and 18 withdrawn because: Thrombosomes did not meet acceptance criteria (11); subject withdrew (3); not eligible (2); abnormal baseline EKG (1); and study was completed before infusion (1). The remaining 15 subjects were infused.

More subjects were male (60%), mean age was 27.7 years (range 20 to 42), and mean weight was 208 pounds, 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 (1x) and increased incrementally as 1x, 10x, 30x, and 100x 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 based on actual subject weight and estimated blood volume. The mean weight in Cohort 5 (176 lbs, 80 kg) was lower than in Cohort 4 (229 lbs, 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 Exam—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 adverse event, possibly related to investigational product and moderate in severity (detailed information below).

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

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

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 below).

One subject in Cohort 2 receiving Control developed an elevated PF 1+2. One other subject in Cohort 4 receiving Thrombosomes had an increased PF 1+2 value at baseline that showed further elevations, peaking at 24 hours after infusion (detailed information below).

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 statistical significant differences were detected after analyses; given the small sample size, this was not unexpected.

Adverse Events (AEs)

All of the AEs were mild or moderate in severity with no serious adverse events, no deaths, and no subject discontinued study participation. Forty AEs were considered treatment emergent (TEAEs) in 12 of 15 (80%) subjects (3 Control and 9 Thrombosomes subjects) (Table 3). The most frequently reported TEAEs (occurring in 2 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 (33%) subjects (1 Control and 4 Thrombosomes subjects; i.e., these were considered TRAEs and 3 subjects had their treatment unblinded) (Table 4). Overall, 3 of 4 subjects (75%) who received the highest level of Thrombosomes in Cohorts 4 and 5 had TRAEs of either a positive platelet autoantibody, abnormal EKG T-wave inversion, or elevated PF 1+2, D-dimer and WBCs.

Treatment-Related Adverse Events Description by Subject—One subject (#05) in Cohort 1 receiving Thrombosomes experienced moderate dizziness that was possibly-related, which started approximately 4 hours following infusion and resolved within 1 minute. The principal investigator (PI) designated this as moderate severity because the site increased vital sign monitoring and provided oral hydration (Table 4).

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

One subject (#83) in Cohort 5 receiving Thrombosomes, was a 28 year old female who had a normal EKGs at screening and also right before infusion (baseline) (Figure 1). The subject did not have any known risks for thromboembolic disease. At 1 and 2 hours post her second Thrombosomes infusion, the subject had T-wave inversions in leads V2 and V3, which resolved by Day 21 (Figure 1). The baseline, 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 (2 were blinded to study group treatment) who suggested these changes were possibly a normal variant. In addition, a stress echocardiogram performed at 5 months post-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 post-infusion that was considered unrelated.

One subject (#90) in Cohort 5 receiving Thrombosomes, had a TRAE of a low level positive platelet IgG autoantibody test (35x 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, Figure 2). There were no changes in platelet counts. Tests for autoantibody against autologous Thrombosomes were positive at baseline, Days 7, 14, and Day 21, and became negative on Days 42 and 60. Positive control MFI values were 140 to 300 while the subject's highest MFI was 7. There were no alloantibodies against donor platelets or pooled random donor Thrombosomes. HLA antibody tests done on Days 21, 42, and 62, and tests for platelet-specific antibodies and red cell allo- or autoantibodies were done at Day 14 were all negative. This adverse event was designated as moderate in severity because additional laboratory testing was performed.

Additional TEAEs in this subject (#90) considered to be either unlikely or not related to treatment included urticaria on Day 13 following the use of topical sun screen, menorrhagia and dysmenorrhea (there was no previous history of menorrhagia or dysmenorrhea), and 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 (#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 post-infusion (Supplemental Data 2). These changes in coagulation tests were deemed related and mild in severity. WBC counts also tended to rise and fall in the same time frames as the coagulation assays but were never outside the normal range. Approximately 5 weeks following infusion, the subject experienced left facial numbness that lasted about 1 hour concurrent with a headache similar to previous headaches (prior to enrollment in 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 5 Thrombosomes Cohorts given at ascending doses of 1/1,000th to 1/10th of the minimum hemostatically-effective dose based on 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 O₂ saturation and Holter monitoring. Other monitoring for the first 24 hours included frequent EKGs, a large panel of laboratory assays (coagulation, chemistries, cardiac, and antibody testing platelet, Thrombosomes, platelet-specific, red cell, and HLA), and neurologic assessments. Follow-up evaluations 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 neo-antigen was formed during manufacturing. Transfusion of allogeneic Thrombosomes manufactured from platelet pools could induce anti-platelet 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 less than 45 years of age with no personal or family history of thrombotic events prior to age 50, 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 transfused subjects completed the study without serious adverse events, or deaths. Pre- and post-infusion 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 8 of these AEs (in 5 subjects) were considered possibly related to the transfusion (Table 4). However, 3 of the 4 Thrombosomes subjects, in Cohorts 4 and 5 given the highest dose, had TRAEs following infusion; i.e., one subject (#63) (at the time of a URI) had elevated PF 1+2, D-dimer and WBC at 1 to 8 hours post-infusion that resolved by Day 7 without other changes in coagulation factors (Supplemental Data 2); one subject (#90) had T-wave inversions in EKG leads V-2 and V-3 at 1 hour post-infusion (Figure 1) that resolved by Day 21 with no changes in Troponin levels and complete cardiac work-up showed no cardiac damage; and another subject developed a low level platelet autoantibody at Day 7 that resolved by Day 42 without evidence of platelet-specific or HLA antibodies (either auto or allo) and no decrease in her platelet count (Supplemental Data 3, Figure 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 3 of the 4 subjects given the highest Thrombosomes dose had treatment-related adverse events, 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 non-compressible 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biography

Arthur Palfrey Bode, Ph.D.

October 12, 1953 – November 11, 2016

The authors wish to acknowledge the contributions of Dr. 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 more than 25 years in both an academic setting full professorship in Pathology at East Carolina University, and 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, and was 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 friend; he is missed.

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FIGURE 1: EKG Treatment Emergent Adverse Events - Subject 83.

The subject had two normal EKGs prior to infusion. At one-hour post her second Thrombosomes infusion in Cohort 5, she demonstrated T-wave inversions in Leads V2 and V3 which resolved by Day 21.

FIGURE 2: Autoplatelet Antibody Test Results Through Day 60 - Subject 90.

The subject's platelets exhibited mean fluorescent intensity (MFI) results that were positive (MFI > the mean of the Neg. control plus 3 SD on days 7, 14, and 21 and were negative on screen, baseline, and days 42 and 60 post infusion. Positive control MFI values were 140 to 300 while the subject's highest MFI was 7 and the mean negative control plus 3SD selected as the cutoff to indicate a positive result ranged from was 4.0 to 5.6

TABLE 1.**BASELINE DATA.**

	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
Min, Max	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 greater than 100%.

TABLE 2.

THROMBOSOMES INFUSED

	THROMBOSOMES				
	Cohort 1 (N=2)	Cohort 2 (N=2)	Cohort 3 (N=2)	Cohort 4 (N=2)	Cohort 5 (N=2)
Total Volume Infused (mL) * (SD)	10.5 (2.1)	10.5 (0.7)	10.0 (0.0)	10.0 (0.0)	20.0 (0.0)
Total Thrombosomes Infused (particles $\times 10^6$)	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 Kilogram Body Weight (particles $\times 10^6/\text{kg}$)	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 $\times 10^6/\text{mL}$)	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 $\pm$ 1 standard deviation.

* All doses were prepared to a standard volume of 10 mL and for subjects in Cohort 5, the dose was evenly split and administered during two infusions 2 hours apart.

† Blood volume calculated as 70 mL/Kg.

Note: Dose of Thrombosomes/kg was determined by the Total Particles Infused (Thrombosomes concentration per mL) $\times$ Dose Thrombosomes administered (Cohort 1 = 0.01 mL, Cohort 2 = 0.1 mL, Cohort 3 = 0.333 mL, Cohorts 4 and 5 = 1.0 mL) divided by Body Weight in kg.

TABLE 3.**ADVERSE EVENTS**

	Thrombosomes (N=10)	Control (N=5)	All Subjects (N=15)
Number Adverse Events (AEs) Reported			
• Treatment-Emergent [*] AEs (TEAEs)	29	11	40
• Treatment-Related [†] AEs (TRAEs)	6	2	8
At Least One Treatment-Emergent AE [‡]	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)

Note: 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 Thrombosomes or Control groups.

^{*} Treatment-emergent adverse event (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).

[†] Treatment-related AE is a treatment-emergent adverse event considered related or possibly related to the study drug by the investigator.

[‡] Number of subjects (%).

TABLE 4.

TREATMENT-RELATED ADVERSE EVENTS (TRAES).

Subject ID	Cohort	Randomization	AE-Preferred Term (Verbatim Term)	Causality	Reasons for Moderate Severity	Unblinding*
05	1	Thrombosomies	Dizziness	Possibly Related	Increased vital sign monitoring and oral hydration	No
63	4	Thrombosomies	D-dimer Increased	Possibly Related	Additional laboratory testing	Yes to determine what additional testing should be performed, if any
63	4	Thrombosomies	Elevated Prothrombin Fragment 1+2	Possibly Related	Additional laboratory testing	See above
63	4	Thrombosomies	White Blood Cell Count Increased	Possibly Related	Additional laboratory testing	See above
83	5	Thrombosomies	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	Thrombosomies	Autoantibody Positive (Platelet Autoantibody)	Related	Additional laboratory testing	No
11	2	Control	Thrombin-antithrombin III Complex Increased	Related	N/A-mild severity	No
11	2	Control	Prothrombin Level Increased (PF 1+2)	Related	N/A-mild severity	No

N/A = Not applicable.

* One other subject (not in table) with a headache and numbness in maxillary area of face on D42 after infusion had his treatment unblinded. Headache was similar to almost daily headaches, AE determined not related.