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Freeze-Dried Platelets Are a Promising Alternative in Bleeding Thrombocytopenic Patients with Hematological Malignancies

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Key Points:

- Freeze dried platelets are safe as shown in a phase 1 study of bleeding thrombocytopenic patients with hematologic malignancies.
- Potential efficacy will be evaluated in the ongoing Phase 2 trial (ClinicalTrials.gov#NCT04631211).

ABSTRACT

Thrombosomes are trehalose-stabilized, freeze-dried group O platelets with a 3-year shelf life. They can be stockpiled, rapidly reconstituted, and infused regardless of the recipient's blood type. Thrombosomes thus represent a potential alternative platelet transfusion strategy. The present study assessed the safety and potential early signals of efficacy of Thrombosomes in bleeding thrombocytopenic patients.

We performed an open-label, phase 1 study of single doses of allogeneic Thrombosomes at 3 dose levels in 3 cohorts, each consisting of 8 patients who had hematologic malignancies, thrombocytopenia, and bleeding. Adverse events, dose-limiting toxicities (DLTs), World Health Organization (WHO) bleeding scores, and hematology values were assessed.

No DLTs were reported. The median age was 59 years (24-71). Most patients had AML (58%) or ALL (29%), followed by MDS (8%) and myeloproliferative neoplasm (4%). The WHO scores of 22 patients who were actively bleeding at a total of 27 sites at baseline either improved (n=17 [63%]) or stabilized (n=10 [37%]) through day 6. Twenty-four hours after infusion, 12 patients (50%) had a clinically significant platelet count increase. Of 8 patients who received no platelet transfusions for 6 days after Thrombosomes infusion, 5 had a clinically significant increase in platelet count of ≥5000 platelets/μL and 2 had platelet count normalization.

Thrombosomes doses up to 3.78 x 10^8 particles/kg demonstrated safety in 24 bleeding, thrombocytopenic patients with hematological malignancies. Thrombosomes may represent an alternative to conventional platelets to treat bleeding. A phase 2 clinical trial in a similar patient population is underway.

INTRODUCTION

Thrombosomes are freeze-dried group O platelets that are loaded with trehalose to stabilize the platelet membrane. Platelet transfusions are critical treatments for bleeding associated with thrombocytopenia due to hematologic malignancies. However, the availability of liquid stored platelets (LSPs) is limited owing to their shelf-life of 5-7 days at room temperature (RT; 20-24℃) and their need for constant agitation.[1] In addition, the hemostatic properties of LSPs decrease during storage.[2,3] Furthermore, LSPs are associated with safety risks, including allergic reactions, transfusion-associated circulatory overload, febrile nonhemolytic reactions, transfusion-associated lung injury, sepsis from bacterial contamination, [4-10] and thrombosis risk.[11]

Blood product inventories are increasingly vulnerable to supply chain disruptions.[12-14] The COVID-19 pandemic exemplifies the impact that unexpected or catastrophic events can have on blood product supply chains.[15] Thus, alternative platelet products are needed that are readily available, have a longer shelf life, broader storage parameters, and lower risk of reactions and infections.

Once proven safe and efficacious, Thrombosomes should have several advantages over LSPs, including a shelf life of up to 3 years at RT without agitation, lower risks of immunogenicity,[16,17] transfusion reaction,[16] and viral and bacterial infection (Supplemental Table 1). Thrombosomes are produced from a pool of up to 10 group O universal donors to increase availability and eliminate major ABO incompatibility. They also have a significantly lower plasma content compared with LSPs, can be reconstituted within 2 to 3 minutes and easily administered. The 3-year shelf life of Thrombosomes also offers the opportunity to avoid wasting platelets. Of

2,338,000 apheresis platelet units collected in the United States in 2017, 344,000 (14.7%) were outdated (Figure 1). The median cost of these units was $517, resulting in a loss of approximately $178,000,000.[18]

Thrombosomes' safety and lack of thrombogenicity and immunogenicity were demonstrated previously in mice, New Zealand White Rabbits (NZWR), swine, canines, and non-human primates as well as in a phase 1 clinical trial in healthy volunteers.[16,17] Patients with hematologic malignancies are at risk of thrombosis. Thrombosomes may offer reduced risk of thrombogenicty given the lack of thrombogenicity observed in: preclinical animal models, phase 1 clinical trial in healthy volunteers[16], and the present study in patients with hematologic malignancies.[16]

The primary objective of the present study was to assess the safety of increasing dose levels of Thrombosomes in bleeding patients with thrombocytopenia. The secondary objective was to explore early signals of clinical efficacy of Thrombosomes in this population. The secondary objectives included: 1) evaluation of the impact on WHO bleeding scores at various timepoints; 2) number and type of blood products infused through day 6 follow up period; and 3) post-hoc analysis of hematology, coagulation and chemistry. This Phase 1 study was not designed to evaluate the Thrombosomes' effect on immunomodulatory events or the endothelium as compared to LSP.

METHODS

Manufacture of Thrombosomes

The manufacture of Thrombosomes was described previously.[17] Briefly, after concentration and plasma removal by tangential flow filtration, group O leukoreduced

apheresis platelets are loaded with trehalose, suspended in buffer, and lyophilized.[17] This process removes 90% or more of the donors' plasma, resulting in less than 1 mL of plasma in each 10 mL of Thrombosomes, reducing the risk of minor incompatibility associated hemolysis and increasing survival of group O platelets in universal transfusion settings. Following lyophilization, a thermal treatment is applied for pathogen reduction, resulting in a 3- to 6-log reduction in viral load (Supplemental Table 1). During thermal treatment dry heat is applied to Thrombosomes in an incubator for 24 hours at 80 degrees centigrade. Before they are released for use, the manufactured Thrombosomes are cultured to detect any aerobic or anaerobic bacteria.

Trial Oversight and Design

We performed a multi-center, open-label, phase 1 single dose dose-escalation study of Thrombosomes in 3 dose-level cohorts. The clinical protocol was approved by the Western Institutional Review Board (IRB) and the IRBs of the 6 participating centers. The trial was performed in accordance with the principles of the Declaration of Helsinki and in accordance with U.S. Food and Drug Administration (FDA) Good Clinical Practice guidelines. All patients provided written informed consent before screening and enrollment (Supplemental Figure 1).

Patients

At the initial screening, patients' vital signs were measured and their WHO bleeding scores assessed by clinical investigators on the study, using standard WHO Bleeding Criteria Guidelines (Supplemental Figure 2). Patients also underwent 12-lead

electrocardiography (ECG), and samples for complete blood counts (CBC), blood chemistry, blood typing, and coagulation profiles were collected and evaluated.

Eligible patients were men and women age 18-74 years who had thrombocytopenia (platelet count 5,000–70,000 platelets/μL) and acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), myelodysplasia, aplasia, and/or chemotherapy- or radiation-induced bone marrow aplasia or hypoplasia with thrombocytopenia lasting ≥2 days. Eligible patients also had WHO grade 1 bleeding at screening restricted to epistaxis, hematuria, oral petechiae, oropharyngeal bleeding, or bleeding at invasive or other wound sites or had WHO grade 2 bleeding of any type.

Patients were excluded if they had a coagulopathy (disseminated intravascular coagulation or prothrombin time [PT] or activated partial thromboplastin time [aPTT] value greater than 1.3 times the upper limit of normal); persistent headache (including migraines); active acute infection or fever; graft-versus-host disease; hyper- or hypotension; known inherited coagulation disorder, including familial thrombotic thrombocytopenic purpura and hemolytic uremic syndrome; or a history of arterial or venous thromboembolism. Patients who received anticoagulation or platelet-inhibiting therapy or who had used antifibrinolytics, nonsteroidal anti-inflammatory drugs, aspirin, or COX-2 inhibitors within the preceding 2-5 days were excluded. Pregnant or breastfeeding women were excluded (Supplemental Figure 1).

Thrombosomes Infusion

Thrombosomes were stored in the pharmacy or blood bank at RT. Thrombosomes were reconstituted by the addition of sterile water and infused intravenously through a Hemo-

Nate 18-μm filter at a rate of approximately 1 mL/minute. Patients in cohorts 1, 2, and 3 received a single infusion of approximately 5, 10, or 20 mL of Thrombosomes. The dose was calculated on a particles/TGPU per kg basis of 9.45 x 10^7 particles/kg (165 thrombin generation potency units [TGPUs]/kg), 1.89 x 10^8 particles/kg (330 TGPUs/kg), and 3.78 x 10^8 particles/kg (660 TGPUs/kg), respectively. The starting dose received by cohort 1 was approximately 5 times the highest dose used in a previous dose-escalation study in healthy volunteers.[16] The doses were chosen based on safety and preliminary efficacy data from preclinical studies. Efficacy studies were conducted in a thrombocytopenic New Zealand White Rabbit (NZWR) ear bleed model. The lowest observed efficacious dose (LOED) was (1.89 x 10^8 particles per kg or 330 TGPU per kg). The low dose cohort was ½ the LOED; the mid dose, cohort 2, was equal to the LOED; and the high dose, cohort 3, was 2x the LOED. Safety studies in NZWR and canine models were conducted at 52x, 26x, and 13x the low, medium and high doses, respectively.[17]

Thrombin generation potency units (TGPU)

The Calibrated Automated Thrombogram (CAT) by Diagnostica Stago assay was modified to determine the Thrombin Generation Potency Units (TGPU) as a potency measure of Thrombosomes. Cellphire engaged with the FDA to develop and establish an arbitrary unit of TGPU based on 1,000,000 Thrombosomes when compared to World Health Organization International Thrombin Standard and an internal reference batch of Thrombosomes. One million Thrombosomes generate approximately 1.3-1.9 TGPUs. Each lot of Thrombosomes released for clinical use must meet acceptance criteria

indicating that the thrombin generation potential is consistent between lots. The relationship of Thrombosomes to produce thrombin in this assay and clinical effect has not been established. There is a direct and proportional relationship to the number of Thrombosomes, thrombin production and TGPU.

Safety and WHO Bleeding Score Assessments

Patients underwent physical examinations 1 hour, 6 hours, 24 hours, and 6 days after Thrombosomes infusion. Coagulation testing (aPTT, D-dimer, Prothrombin Fragment 1+2, Thrombin Generation Assay (TGA), Thrombin Antithrombin (TAT) was performed at screening and baseline and 1 hour, 6 hours, and 24 hours after infusion. Blood chemistry tests were performed at baseline, 6 hours and 24 hours after infusion.

Patients were monitored for treatment-emergent adverse events (TEAEs) for 6 days and for serious TEAEs for 30 days. Safety data were reviewed by a Data Monitoring Committee (DMC) (an independent committee of adjudication) prior to initiating each dose cohort. The serious adverse events (SAEs) considered as stopping rules were thrombotic or embolic events including myocardial infarction, pulmonary embolism, non-catheter-related venous thromboembolism, stroke, and transient ischemic attack; acute lung injury occurring within 48 hours of infusion; and anaphylaxis occurring within 4 hours. The standard definitions of SAEs were the following: death; life-threatening situation; in-patient hospitalization (excluding those for study therapy or placement of an indwelling catheter, unless associated with other SAEs); prolongation of existing hospitalization; persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions; congenital anomaly; important medical

events that may jeopardize the patient or may require medical or surgical intervention to prevent one of the outcomes listed above. Electrocardiogram assessments were performed at initial screening, baseline, and 6 hours post infusion. Troponins (I or T) were evaluated at baseline, 6 hours, and 24 hours post infusion.

Bleeding was assessed by recording ordinal changes in the WHO bleeding scores for each bleeding site 1 hour, 6 hours, 24 hours, and 6 days after infusion in comparison to screening and baseline observations. The active bleeding site with the highest WHO score at screening was designated the primary bleeding site. Blood product usage and laboratory test parameters were recorded at the same timepoints and daily thereafter through day 6. This study allowed the administration of LSPs as clinically indicated up to 6 hours before and more than 1 hour after Thrombosomes infusion. If at any time the patient's clinical bleeding remained unchanged or worsened, the patient was eligible to receive blood products, hemostatic agents, or coagulation factors without limitation.Thrombosomes were administered to patients meeting the inclusion criteria with modified WHO Grade 1 or 2 bleeding due to hematology/oncology diseases or bone marrow aplasia secondary to cancer chemo/radiotherapy**.**

Statistical Analysis

A sample size of 8 patients per cohort (24 patients total) was selected to ensure a clinically meaningful assessment of safety while minimizing the number of patients potentially at risk.

For descriptive analyses, continuous variables were summarized by number of patients, median, minimum and maximum (range). Categorical variables were summarized as frequencies, using numbers and percentages of patients (or events).

For quantitative variables, summaries of observed responses and changes from baseline relative to each post-infusion time included the sample size, median, minimum, and maximum. The statistical significance of changes from baseline to the post-infusion times was determined using a paired t-test using GraphPad Prism version 7.01 (San Diego, CA) and reported as individual data points with medians and ranges. For all statistical tests, P values <0.05 were considered significant.

Role of the funding source

Cellphire, the clinical investigators on the study, and subject matter experts designed the study. Manuscript development, data collection, and clinical data analysis and interpretation were completed with the contributions of all authors. The funders (Department of Health and Human Services; Biomedical Advanced Research and Development Authority, under Contract No. HHSO100201300022C) had no role in data analysis or data collection. The lead authors and clinical investigators together with Cellphire analyzed and interpreted the results. The corresponding author [MO] in collaboration with the co-lead author [JC] had final responsibility for the content of the manuscript and the decision to submit for publication.

RESULTS

Thirty-six patients were screened to enroll the 24 patients needed for participation. (Twelve screened patients [33%] were found to be ineligible, Supplemental Figure 6.) In this study, first patient was enrolled on 17 May 2018 and last patient was enrolled on 01 August 2019; therefore, duration of study was approximately 15 months. The overall median age was 59.0 years; the youngest subject was 24 years old, and the oldest was 71 years old. The median age in Cohort 1 was slightly lower (55.5 years) compared to Cohort 2 (59.5 years) and Cohort 3 (60.5 years). About 75% of the patients were white with slightly more men than women enrolled in the study (58.3% versus 41.7%). Patients with blood groups O and A were the most frequently reported (45.8% and 50.0%, respectively), and most patients (87.5%) were positive for Rh factor. Most of the patients were diagnosed with either AML (58%) or ALL (29%); 2 patients were diagnosed with myelodysplastic syndrome (MDS, 8%) and 1 with myeloproliferative neoplasm (MPN, 4%). Of the 24 patients enrolled, 8 (33%) had WHO grade 1 bleeding and 16 (67%) had WHO grade 2 bleeding at the primary bleeding sites at screening or baseline. Five patients developed new bleeding after initial screening prior to infusion, and 22 patients were actively bleeding at baseline. Among the 22 patients who had bleeding at baseline, 8 (37%) had WHO grade 1 bleeding and 14 (63%) had WHO grade 2 bleeding (Table 1). Median platelet counts at baseline were 20.5, 18.5 and 14.5 $x10^3/\mu L$ for cohorts 1, 2, and 3 respectively, while the median across all patients was 18.5 with a range of 2 to 51 $x10^3/\mu L$.

Safety

Infusions of Thrombosomes were performed per protocol with no reported deviations. We found no DLTs throughout the study. One clinically insignificant instance of a grade 1 systolic murmur that was possibly related to Thrombosomes infusion was noted 1 day after infusion but resolved by day 7 with no subsequent cardiac sequela. Treatment-emergent adverse events (TEAEs) are summarized by grade (Table 2) and by cohort (Supplemental Table 2). Eight patients (33%) had infection complications owing to the immune impairment caused by their hematologic malignancies.

Of 111 TEAEs, 18 (16%) were serious and occurred in 10 patients, 5 of whom died of complications related to their underlying disease during the 30-day follow-up period (Supplemental Table 5). One additional patient with refractory AML died of septic shock 14 days after the 30-day follow-up period had ended. Of the 6 patients who died, 3 were in cohort 2, and 3 were in cohort 3. All deaths were attributed to infection complications in the setting of refractory/relapsed hematologic malignancies (Supplemental Table 5).

The 111 TEAEs reported on study were comparable across cohorts. The majority of patients, 18/24 (75%) overall experienced at least one TEAE through day 6 [+4 days]. TEAEs considered related to the underlying disease were reported in the majority of patients, with comparable results across cohorts: 7 (87.5%) patients in Cohort 1, 6 (75.0%) patients in Cohort 2, and 5 (62.5%) patients in Cohort 3. No patients were discontinued from the study due to adverse events (AEs). There were no patients who met study stopping rules or suspension rules. Possible neurologic system TEAEs were infrequent and evenly distributed across cohorts (1 patient each per cohort). There was

1 patient in Cohort 3 with a TESAE of pituitary hemorrhage on Day 32 after Thrombosomes infusion considered unlikely related to IP.

Comparative coagulation parameter analysis performed before and after Thrombosomes infusion revealed no evidence of increased risk of thrombosis (Supplemental Table 3). Across all cohorts, there was no significant change from baseline in levels of markers of coagulation including TAT, TGA, and D-dimer (Supplemental Figure 4A, 4B, 4C).

Bleeding Responses

Two patients who were bleeding at the time of screening but not actively bleeding at the time of Thrombosomes infusion were excluded from the WHO bleeding score analysis. The 22 patients included in the WHO bleeding score analysis had a total of 27 bleeding sites pre-infusion. All 22 patients' WHO scores for their primary bleeding sites either improved (n=13 [59%]) or remained stable (n=9 [41%]) 6 days after Thrombosomes infusion. Five patients had primary and secondary bleeding sites at baseline prior to infusion. Among the 5 patients with secondary bleeding sites at baseline, 4 (80%) had improved and 1 (20%) had stabilized for those sites 6 days after Thrombosomes infusion (Figure 2A). Through day 6, the number of sites with a WHO score of 2 gradually decreased, and concomitantly, the number of sites with a WHO score of 0 increased (Figure 2B). Of the 27 bleeding sites (22 primary and 5 secondary) documented at baseline for the 22 patients included in the WHO bleeding score evaluation, the ordinal changes in the WHO bleeding score from baseline at all time points are as follows. At 1 hour post infusion, 0 sites worsened, 24 sites had no change

and 3 improved. At 6 hours, 0 sites worsened, 21 had no change, and 6 had improved. At 24 hours, 0 sites worsened, 17 had no change, and 10 sites improved. At 6 days post infusion, 0 sites worsened, 10 had no change and 17 improved (Figure 2C). Changes in WHO scores from baseline demonstrated a similar trend among all patients, even those who did not receive LSPs, showing an increased rate of improved WHO scores by day 6 (Figure 2C–D). Three patients (1 from cohort 2 at 6 hours and 2 from cohort 3 at 6 and 24 hours) developed secondary bleeding sites post infusion.

Hematological Response and Blood Product Transfusion Requirements

All 24 patients were evaluable for assessments of hematology laboratory parameters and blood product usage. Twenty-four hours after infusion, 12 patients (50%) had a clinically significant platelet count increase (predefined as ≥5,000 platelets/μL) from baseline. All patients had a statistically significant increase in mean platelet count through day 6 compared to baseline ($p=0.04$, Figure 3A).

A post hoc analysis of 8 patients who received no platelet transfusions for 6 days after Thrombosomes infusion revealed that 5 patients' platelet counts consistently increased by ≥5000 platelets/μL by day 1 or day 6 and that 2 patients' platelet counts normalized by day 6 (Figure 3C; Supplemental Figure 5). Only 1 out of the 8 patients received platelets within 24 hours prior to Thrombosomes (at 15 hours prior to infusion). Consistent with the stable or improved WHO bleeding scores, there were no significant changes in hemoglobin or hematocrit levels (Supplemental Figure 3) or in blood product usage among all 24 patients across all time points during the study (Supplemental Figure 4). A spaghetti plot of platelet counts by dose cohort for all patients through 24

hours post-infusion is shown in Supplemental Figure 7 (dotted lines represent high and low reference ranges).

None of the 24 patients received platelets or RBC at 1 hr post Thrombosomes and only 2/24 patients received platelets within 24 hours prior to baseline CBC and Thrombosomes infusion (at 11 and 15 hours prior). For platelet products, 16 units of platelet products were transfused through day 6 post Thrombosomes infusion; 5 in cohort 1, 5 in cohort 2, and 6 in cohort 3. Among the 24 patients, 16 patients received platelet products in addition to Thrombosomes. Thirteen patients received an average of 1.38 units of platelets per patient between 2 hours and 24 hours. Thirteen patients received an average of 3.46 units of platelets per patient between 24 hours and day 6. Overall, post-Thrombosomes infusion through day 6, 17 units of red cell products were transfused; 7 in cohort 1, 6 in cohort 2, and 4 in cohort 3. Among the 24 patients, 16 patients received red cell products in addition to Thrombosomes. Eight patients received an average of 1.625 units between 2 hours and 24 hours after Thrombosomes. Fourteen patients received an average of 2.86 units between 24 hours and day 6 (Supplemental Table 4). A comparison between cohorts resulted in no statistical significance for either LSP or red cell transfusion.

DISCUSSION

This study provides the first clinical data supporting the safety and potential impact of allogeneic Thrombosomes in treating acute or ongoing bleeding in humans.

This study is also the second of two phase 1 trials demonstrating the safety of Thrombosomes.[16] There were no DLTs. The number of TEAEs observed in this trial were consistent with an acutely ill population of patients with myelosuppression and immune impairment due to advanced hematologic malignancies. None of the adverse events or serious TEAEs observed was considered to be related to Thrombosomes infusion. Its results also suggest that Thrombosomes are simple to use in patients with hematological malignancies who have thrombocytopenia. Given the fragility of blood product supply chains, as observed during the current COVID-19 pandemic, this off-the-shelf, freeze-dried platelet product could help enhance and stabilize the platelet supply.

Although the reported study was not powered to assess efficacy, and it lacked a control group, secondary endpoints were collected and are reported as observations that could indicate early signals of effect. All patients, including 8 patients who received no additional platelet transfusions through day 6, had stabilized or improved WHO bleeding scores from one hour post treatment through day 6 after Thrombosomes infusion. Sixteen patients received transfusions of blood products, including LSPs, through day 6 after Thrombosomes infusion. Patients were at different stages of treatment and the increase in platelet count in this subset of patients cannot be definitively ascribed to the infusion of Thrombosomes. Acknowledging that these patients routinely receive platelet transfusions as standard of care due to their underlying disease, all patients in this study were expected to require routine platelet transfusions.

Of the 8 patients who did not require LSP transfusions through day 6, 5 demonstrated a consistent, clinically significant, platelet count increase, and 2 had

normalization of their platelet counts. This unexpected observation requires further evaluation in a larger, yet similar, patient population. As an activated platelet product, Thrombosomes have a short circulation time (20-30 min) and are subsequently sequestered and cleared through the liver.[19] As a result, Thrombosomes are not expected to elevate the count immediately after infusion. One potential mechanism of action of Thrombosomes is to treat bleeding by coating the endothelium, preserving the glycocalyx and thereby allowing the endogenous platelets to recover the patient's counts, as recently demonstrated in a small animal model.[20] An ongoing phase 2 trial has been designed to evaluate this potential effect.

Thrombosomes have several potential advantages over LSPs. High variability among platelet donors leads to unpredictable clinical effects from the transfusion of a unit of LSP from a single donor.[21] As a pooled product with in-process manufacturing controls, Thrombosomes exhibit reduced variability, which is ensured by meeting strict release criteria and should provide a consistent product with a predictable effect. Since LSPs have long circulation kinetics, they are routinely used prophylactically to increase the patient's platelet count; however, their low levels of activation may result in suboptimal hemostasis in bleeding patients. In contrast, Thrombosomes are activated upon their rehydration, prior to infusion, as evidenced by their expression of P-selectin, phosphatidylserine, GPIIb-IIIa, and short circulation time in vivo.[17,20] Rehydrated Thrombosomes retain the essential hemostatic properties of platelets, including adhesion to exposed collagen, thrombin production, aggregation in response to thrombin, and participation in clot formation as assessed by Thromboelastography (TEG).[17] Thrombosomes have decreased aggregation responses to arachidonic acid,

collagen, ristocetin, and ADP. Thrombosomes contain significantly less plasma than LSPs. Ten ml of Thrombosomes contains less than 1 ml of plasma. The high dose of Thrombosomes in the ongoing Phase 2 study is expected to be about 90ml in a 100kg patient, corresponding to about 9ml of plasma, whereas each unit of LSPs contains 200-250 ml of plasma.

Notably, there were no cases of immediate or delayed transfusion reactions following infusion of Thrombosomes made from pooled allogeneic platelets in this trial or using autologous platelets as a starting material in the previous trial in healthy volunteers.[16] This promising observation indicates potentially lower rates of platelet- or plasma-related transfusion reactions.

The risk of platelet transfusions transferring pathogens to patients is not trivial, and historically about 1 of every 1,000-2,500 platelet units could be bacterially contaminated[7]; conversely, the LSP that are used to manufacture Thrombosomes are cultured according to FDA and AABB requirements at the collection center prior to Thrombosomes productions. After production, Thrombosomes are also heat-treated to prevent viral infection (Supplemental Table 1) and manufactured according to Good Manufacturing Practice (GMP), which includes submitting samples from the final product to detect bacterial contamination. Thrombosomes are cultured to detect any aerobic or anaerobic bacteria in accordance with the methods outlined in the current USP <71 > (Harmonized Pharmacopeial Method) / 21 CFR 610.12 for sterility testing).[22] Thus, cultures for bacterial contamination are performed twice during production.

On Oct 1st 2021, all blood-banking centers were required to implement a new method to ameliorate bacterial contamination of platelets, through either large volume

delayed sampling or pathogen reduction technology; however, this has not been universally adopted.[23] It is difficult to predict the impact of this change. The estimated frequency of bacterial contamination is expected to decrease to less than 1 in 1,000 platelet transfusions once it is fully implemented. However, the risk persists even with pathogen reduction technology and the need for bacterial detection methods at the time of transfusion has been advocated as a way to further prevent contaminated platelets.[24] Alternative methods of platelet preparation including Thrombosomes, refrigerated and cryopreserved platelets have the potential to improve patient safety by decreasing the risk of bacterial contamination, while concurrently allowing for a longer shelf-life and improved hemostatic effectiveness in actively bleeding patients.

Given the significant risk of thrombosis in patients with hematologic malignancies,[25] the increased thrombosis risk of LSP's,[11] the lack of thrombogenicity observed both in the Phase 1 clinical trial of healthy volunteers[16] and in the present study in patients with advanced hematologic malignancies demonstrates the safety and potential advantages of Thrombosomes and supports the continuation of larger studies.

Thrombosomes were simple to store and easy to use, indicating that they can be stockpiled and rapidly utilized in emergency situations in hospitals, and in a variety of situations that pose logistical challenges in platelet collection and storage. Multiple doses of Thrombosomes may be generated from one unit of LSPs, which could increase supply. Determining the number of doses that can be obtained from a single LSP unit is dependent on the outcome of the ongoing Phase 2 dose ranging study.

This is critical, given that fewer donors are available during pandemics and natural disasters. Thrombosomes could significantly ameliorate the seasonal shortages

of platelet products during disaster times and eliminate wastage during seasons of reduced usage, thereby decreasing the more than $178M wasted annually from outdated platelets.[26]

The potential of Thrombosomes is further supported by a prospective, randomized study of StablePlate® Canine, a trehalose-stabilized lyophilized canine platelet approved by the FDA Center for Veterinary Medicine for use in veterinary practice. In this study in 88 thrombocytopenic bleeding dogs, including 50 that received StablePlate® and 38 that received dimethyl sulfoxide (DMSO) cryopreserved canine platelets (CPP), StablePlate® was non-inferior to DMSO CPP in terms of safety and efficacy.[27]

In conclusion, our findings suggest that Thrombosomes are safe to use and represent a potential alternative to LSPs in the treatment of severely thrombocytopenic, bleeding patients with hematologic malignancies. As an activated platelet product, Thrombosomes require further evaluation for multiple indications to determine their potential to treat hemorrhage in all types of patients. Encouraging results from future studies could support the use of Thrombosomes in situations in which platelets would likely be inadequate and/or scarce. A phase 2 clinical trial of Thrombosomes is underway, and a single-patient expanded access policy is in place (ClinicalTrials.gov #NCT03394755).

Table 1. Clinical Characteristics by Treatment Cohort				
Characteristic	**Cohort 1 (N = 8)**	**Cohort 2 (N = 8)**	**Cohort 3 (N = 8)**	**Total (N = 24)**
Particles/kg	9.45×10^7	1.89×10^8	3.78×10^8	
Median age (range), years	55.5 (27-64)	59.5 (24-66)	60.5 (26-71)	59.0 (24-71)
Sex				
Female	3 (37.5)	4 (50.0)	3 (37.5)	10 (41.7)
Male	5 (62.5)	4 (50.0)	5 (62.5)	14 (58.3)
Ethnicity				
Hispanic or Latino	0	0	1 (12.5)	1 (4.2)
Not Hispanic or Latino	8 (100)	6 (75.0)	7 (87.5)	21 (87.5)
Unknown	0	2 (25.0)	0	2 (8.3)
Race				
Black or African American	1 (12.5)	0	3 (37.5)	4 (16.7)
White	7 (87.5)	7 (87.5)	4 (50.0)	18 (75.0)
Unknown	0	1 (12.5)	1 (12.5)	2 (8.3)
Blood type				
O	4 (50.0)	5 (62.5)	2 (25.0)	11 (45.8)
A	4 (50.0)	3 (37.5)	5 (62.5)	12 (50.0)
B	0	0	1 (12.5)	1 (4.2)
Rh factor				
Positive	7 (87.5)	7 (87.5)	7 (87.5)	21 (87.5)
Negative	1 (12.5)	1 (12.5)	1 (12.5)	3 (12.5)
Hematologic parameters at baseline (range)				
Median hemoglobin, g/dL	8.7 (7.9-10.4)	8.7 (7.2-8.9)	8.9 (6.9-9.6)	8.7 (6.9-10.4)
Median hematocrit, %	26.4 (22.9-29.8)	25.6 (21.0-26.1)	25.9 (19.5-29.4)	25.6 (21.0-29.8)
Median platelet count, $x10^3/\mu L$	20.5 (12-37)	18.5 (3-4)	14.5 (2-51)	18.5 (2-51)
Screening WHO bleeding score, all sites of bleeding (n=24)				
Grade 1	4 (40.0)	7 (58.3)	3 (25.0)	14 (41.2)
Grade 2	6 (60.0)	5 (41.7)	9 (75.0)	20 (58.8)
Screening WHO bleeding score, primary site of bleeding (n=24)				
Grade 1	2 (25.0)	4 (50.0)	2 (25.0)	8 (33.3)
Grade 2	6 (75.0)	4 (50.0)	6 (75.0)	16 (66.7)
Baseline WHO bleeding score, all sites of bleeding (n=22)*				
Grade 1	3 (37.5)	5 (50.0)	3 (25.0)	11 (36.7)
Grade 2	5 (62.5)	5 (50.0)	9 (75.0)	19 (63.3)
Baseline WHO bleeding score, primary site of bleeding (n=22)*				
Grade 1	2 (28.6)	3 (42.9)	2 (25.0)	7 (31.8)
Grade 2	5 (71.4)	4 (57.1)	6 (75.0)	15 (68.2)
Hematologic malignancy diagnosis				

Acute myeloid leukemia	2 (25.0)	4 (50)	8 (100.0)	14 (58.3)
Acute lymphoblastic leukemia	4 (50.0)	3 (37.5)	0	7 (29.2)
Myelodysplastic syndrome	1 (12.5)	1 (12.5)	0	2 (8.3)
Myeloproliferative neoplasm	1 (12.5)	0	0	1 (4.1)
Note: All data are no. of patients (%) unless otherwise noted. *Two patients were not bleeding at baseline (prior to Thrombosomes infusion).				

Table 2. Summary of Serious Treatment-Emergent Adverse Events by Grade				
	Grade 3	**Grade 4**	**Grade 5**	**Total**
Infections and infestations				
Sepsis		1	3	4
Bacteremia			1	1
Cholecystitis infective	1			1
Encephalitis viral			1	1
Pathogen resistance	1			1
Septic shock			1	1
General disorders and administration site conditions				
Fatigue	1			1
Multiple organ dysfunction syndrome	1			1
Necrosis*	1			1
Blood and lymphatic system disorders				
Febrile neutropenia	2			2
Anemia	1			1
Endocrine disorders				
Pituitary hemorrhage	1			1
Gastrointestinal disorders				
Upper gastrointestinal hemorrhage		1		1
Immune system disorders				
Acute GVHD	1			1
Total	**10**	**2**	**6**	**18**

GVHD, graft-versus-host disease.
*One patient had necrosis secondary to a bitten lip after Thrombosomes infusion.

Supplemental Table 1. Results of Heat Treatment for Enhancing the Safety of Thrombosomes Through Viral Reduction		
Virus	**Sample Designation**	**Log_{10} Reduction**
A-MuLV	Dry Heat- $T_{24\ hours}$- Run 1	> 5.80 ± 0.12*
	Dry Heat- $T_{24\ hours}$- Run 2	> 5.83 ± 0.18
BVDV	Dry Heat- $T_{24\ hours}$- Run 1	3.25 ± 0.11
	Dry Heat- $T_{24\ hours}$- Run 2	3.02 ± 0.21
PPV	Dry Heat- $T_{24\ hours}$- Run 1	2.86 ± 0.07
	Dry Heat- $T_{24\ hours}$- Run 2	2.64 ± 0.20
PrV	Dry Heat- $T_{24\ hours}$- Run 1	> 6.44 ± 0.09
	Dry Heat- $T_{24\ hours}$- Run 2	> 6.63 ± 0.08
HIV	Dry Heat- $T_{24\ hours}$- Run 1	> 3.92 ± 0.06*
	Dry Heat- $T_{24\ hours}$- Run 2	> 3.99 ± 0.17*
A-MuLV, amphotropic murine leukemia virus (Retroviridae); BVDV, bovine viral diarrhea virus (Flaviviridae); PPV, porcine parvovirus (Parvoviridae); PrV, pseudorabies virus (Suid Herpesvirus); HIV, human immunodeficiency virus (Retroviridae). *Virus reduced to non-detectable levels.		

Supplemental Table 2. Summary of Treatment-Emergent Adverse Events (TEAEs) – Safety Population				
Summary Category	**Cohort 1 (N = 8)**	**Cohort 2 (N = 8)**	**Cohort 3 (N = 8)**	**Total (N = 24)**
No. of events				
AEs (non-serious or serious)	42	37	40	119
TEAEs	40	34	37	111
TESAEs	4	6	8	18
No. of patients with events (%)				
TEAEs	7 (87.5)	6 (75.0)	5 (62.5)	18 (75.0)
TEAEs related to the study treatment[1]	1 (12.5)	0	0	1 (4.2)
TEAEs leading to study withdrawal	0	0	0	0
Stroke events reported as TEAEs	0	0	1 (12.5)	1 (4.2)
Neurological results reported as TEAEs	1 (12.5)	1 (12.5)	1 (12.5)	3 (12.5)
TESAEs	1 (12.5)	5 (62.5)	4 (50.0)	10 (41.7)
TESAEs related to the study treatment[1]	0	0	0	0
TEAEs of severity grade 3 or 4	3 (37.5)	4 (50.0)	4 (50.0)	11 (45.8)
TEAEs with outcome of death (severity grade 5)	0	3* (37.5)	3 (37.5)	6 (25.0)

Note: Patients are counted only once per summary category.
AE, adverse event; TESAE, treatment-emergent serious adverse event.
[1]Includes one AE considered to be possibly related to the study treatment.
*One patient in cohort 2 died 14 days after study completion.

Supplemental Table 3. Changes From Baseline* in Coagulation Measures Over Time – Safety Population

Analyte	CFB to visit	Statistics	Cohort 1	Cohort 2	Cohort 3	Total
aPTT, sec	CFB to 1 hour after infusion	N	7	6	7	20
		Median	-0.5	0.9	2.2	0.85
		Min, Max	-1.9, 1.6	-1.4, 3.2	-1.1, 6.9	-1.9, 6.9
PT, sec	CFB to 1 hour after infusion	N	8	6	8	22
		Median	0	-0.2	0.35	0
		Min, Max	-0.7, 0.4	-0.7, 0.2	-0.8, 0.9	-0.8, 0.9
D-dimer, µg/mL FEU	CFB to 6 hours after infusion	N	7	7	8	22
		Median	-0.04	-0.1	-0.1	-0.06
		Min, Max	-0.9, 0.3	-0.8, 0.6	-0.7, 0.4	-0.85, 0.57
PF 1+2, pmol/L	CFB to 1 hour after infusion	N	8	8	8	24
		Median	2.5	-0.5	-64.0	-20.00
		Min, Max	-153, 78	-57, 62	-783, -21	-783, 78
	CFB to 6 hours after infusion	N	8	8	8	24
		Median	-48.5	-18.0	-30.5	-41.00
		Min, Max	-158, 0	-61, 61	-197, 20	-197, 61
	CFB to 24 hours after infusion	N	8	7	8	23
		Median	47.5	32.0	-67.5	12.0
		Min, Max	-92, 444	-11, 78	-708, 103	-708, 444
TGA-TPH (thrombin peak height), nM	CFB to 6 hours after infusion	N	7	7	8	22
		Median	-6.8	24.0	0	9.0
		Min, Max	-59.4, 183	-11, 194	-23, 148	-59.4, 194
TGA (ETP), nM*min	CFB to 6 hours after infusion	N	7	7	8	22
		Median	20.4	87.0	-24.0	44.0
		Min, Max	-87, 345	-22, 330	-129, 706	-129, 706

aPTT, activated partial thromboplastin time; CFB, change from baseline, PT, prothrombin time; ETP, endogenous thrombin potential; Max, maximum; Min, minimum; PF, prothrombin fragment; SD, standard deviation; TGA-TPH (thrombin generation assay-thrombin peak height) *Baseline values were assessed immediately before infusion on day 1.

Supplemental Table 4. Summary of Patients Who Received Transfusions Post Thrombosomes Infusion

	Visit	Number (%) of Patients			
		Treatment Cohort			
		Cohort 1 (N = 8)	Cohort 2 (N = 8)	Cohort 3 (N = 8)	Total (N = 24)
Patients transfused	1h post infusion	0	0	0	0
	6h post infusion	1 (12.5)	2 (25.0)	1 (12.5)	4 (16.7)
	24h post infusion	4 (50.0)	5 (62.5)	5 (62.5)	14 (58.3)
	Day 3	3 (37.5)	4 (50.0)	4 (50.0)	11 (45.8)
	Day 4	3 (37.5)	3 (37.5)	2 (25.0)	8 (33.3)
	Day 5	2 (25.0)	4 (50.0)	3 (37.5)	9 (37.5)
	Day 6	4 (50.0)	3 (37.5)	5 (62.5)	12 (50.0)
	Total	7 (87.5)	6 (75.0)	6 (75.0)	19 (79.2)
Reason for Transfusion					
Low platelet count		5 (62.5)	5 (62.5)	7 (87.5)	17 (70.8)
Anemia, low RBC, low Hgb		7 (87.5)	4 (50.0)	4 (50.0)	15 (62.5)
Active bleeding non-surgical		2 (25.0)	1 (12.5)	1 (12.5)	4 (16.7)
Marrow hypoplasia or aplasia		0	1 (12.5)	1 (12.5)	2 (8.3)
Chronic disease		0	1 (12.5)	0	1 (4.2)
Low fibrinogen		1 (12.5)	0	0	1 (4.2)
Combined transfusion type from post infusion through Day 6					
Combined pRBCs and Whole blood		7 (87.5)	6 (75.0)	4 (50.0)	17 (70.8)
Average units per patient		2.29	2.50	5.50	3.12
Combined Pooled WBD platelets and Apheresis platelets		5 (62.5)	5 (62.5)	6 (75.0)	16 (66.7)
Average units per patient		3.60	3.60	4.50	3.94
Cryoprecipitate		1 (12.5)	0	0	1 (4.2)
Combined transfusion type from post infusion through 24 hours					
Combined pRBCs and Whole Blood		3 (37.5)	4 (50.0)	1 (12.5)	8 (33.3)
Average units per patient		1.67	1.5	2	1.625
Combined pooled WBD and Apheresis Platelets		4 (50.0)	3 (37.5)	6 (75.0)	13 (54.2)
Average units per patient		1.5	1.33	1.33	1.38
Combined transfusion type from 24 hours through Day 6					
Combined pRBCs and Whole Blood		6 (75.0)	5 (62.5)	3 (37.5)	14 (58.3)
Average units per patient		1.83	1.80	6.67	2.86
Combined pooled WBD and Apheresis Platelets		3 (37.5)	5 (62.5)	5 (62.5)	13 (54.2)
Average units per patient		4	2.8	3.8	3.46

Hgb = hemoglobin; pRBC = packed red blood cell; RBC = red blood cell; WBD = whole blood derived
Baseline = assessment pre-infusion on Day 1.
Denominators for percentages equal the number of patients in the Safety Population for the given dose cohort.

Supplemental Table 5. Cause-of-Death Characteristics of the 6 Patients Who Died Following Thrombosomes Infusion*

Age, years	Primary leukemia diagnosis	Past medical history of infectious diseases	Serious adverse events leading to death	Cause of death	Relationship to Thrombosomes Infusion
60	B-cell ALL	Lung infection (no organism specified), E. faecalis urinary tract infection	Sepsis	Sepsis and multiorgan failure in the setting of refractory B-cell ALL	Onset 15 days post, death 30 days post
51	CLL; AML	Pneumonia (no organism identified), Enterobacter bacteremia, multidrug-resistant E. coli bacteremia, and positive bronchoalveolar lavage with Aspergillus/Achromobacter denitrificans/Xylosoxidans	Infections and infestations – other, bacteremia	Bacteremia with sepsis in the setting of refractory AML and CLL	Onset 14 days post, death 43 days post
71	AML	Progressive refractory AML. Chemotherapy was not received by the patient. No infections noted.	Infections and infestations – other, central nervous infection (viral encephalitis)	Viral encephalitis in the setting of refractory AML	Onset 8 days post, death 25 days post
59	AML	Pneumonia (no organism specified), cytomegaloviral pneumonia, mycobacterial pneumonia, pulmonary infiltrate, and nail infection (fungal – no organism specified)	Sepsis	Sepsis, pneumonia, and multiorgan failure in the setting of relapsed/refractory AML	Onset 5 days post, death 26 days post
57	AML	Cellulitis, fungal pneumonia (no organism specified), sepsis (no organism specified)	Sepsis	Sepsis with multiorgan failure in the setting of refractory AML	Onset sepsis 5 days post, GI hemorrhage 6 days post, death 10 days post
65	AML	Invasive pulmonary Aspergillosis	Septic shock	Septic shock in the setting of refractory AML	Onset 21 days post, death 26 days post

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphoblastic leukemia. *One patient developed refractory AML and died 43 days after Thrombosomes infusion, after the completion of the 30-day follow-up period.

Figure Legends

Figure 1. Thrombosomes manufacturing process and comparison to Liquid Stored Platelets (LSPs).

Apheresis platelets are collected from group O donors (A). The platelets are loaded with trehalose, a sugar molecule (B). The trehalose-loaded platelets are dehydrated to form a powder (C). The Thrombosomes are stored at room temperature for up to 3 years (D). The Thrombosomes can be rapidly rehydrated with sterile water (E). Within 2-3 minutes of rehydration, Thrombosomes can be rapidly administered to stop bleeding (F).

Figure 2. Changes in WHO bleeding scores from baseline through day 6 after Thrombosomes infusion.

Changes in WHO bleeding scores from baseline through day 6 after Thrombosomes infusion. Change in WHO bleeding score status distribution per bleeding sites (A). Distribution of all patients' WHO scores by timepoint (B). Changes in WHO bleeding scores for all patients bleeding at baseline (C). Changes in WHO bleeding scores from baseline for patients who did not receive platelets through day 6 after Thrombosomes infusion. Through 1 hour, no patients received platelet transfusion; through 6 hours, 1 patient received platelet transfusion; through 24 hours, 12 patients received platelet transfusion; and through day 6, 14 patients received platelet transfusion (D). For panels C and D, no patients experienced worsening WHO bleeding scores at the indicated times; n represents the number of patients (P=not significant).

Figure 3. Platelet counts through day 6 after Thrombosomes infusion.

Platelet counts through day 6 after Thrombosomes infusion for all patients (P=0.04) (A); for 16 patients who received both Thrombosomes and platelets transfusions (P=not significant)(B); and for 8 patients who did not receive standard platelet transfusion following Thrombosomes infusion (P= not significant)(C) Individual data points are presented with medians (denoted in red) and ranges. Platelet counts at the different time points after baseline are presented as changes from baseline and evaluated using a paired t-test.

Author Contributions:

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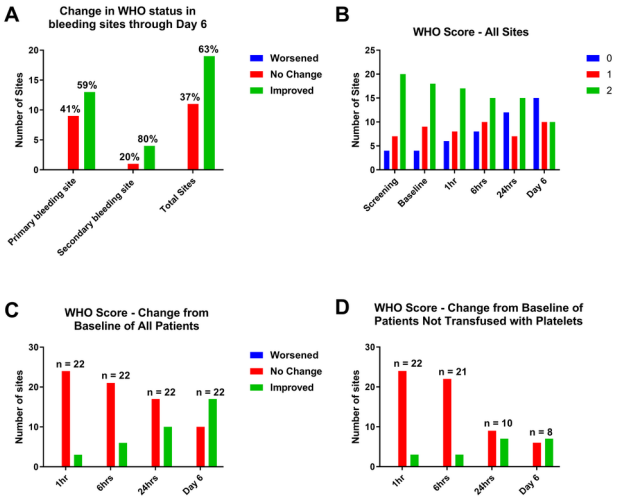

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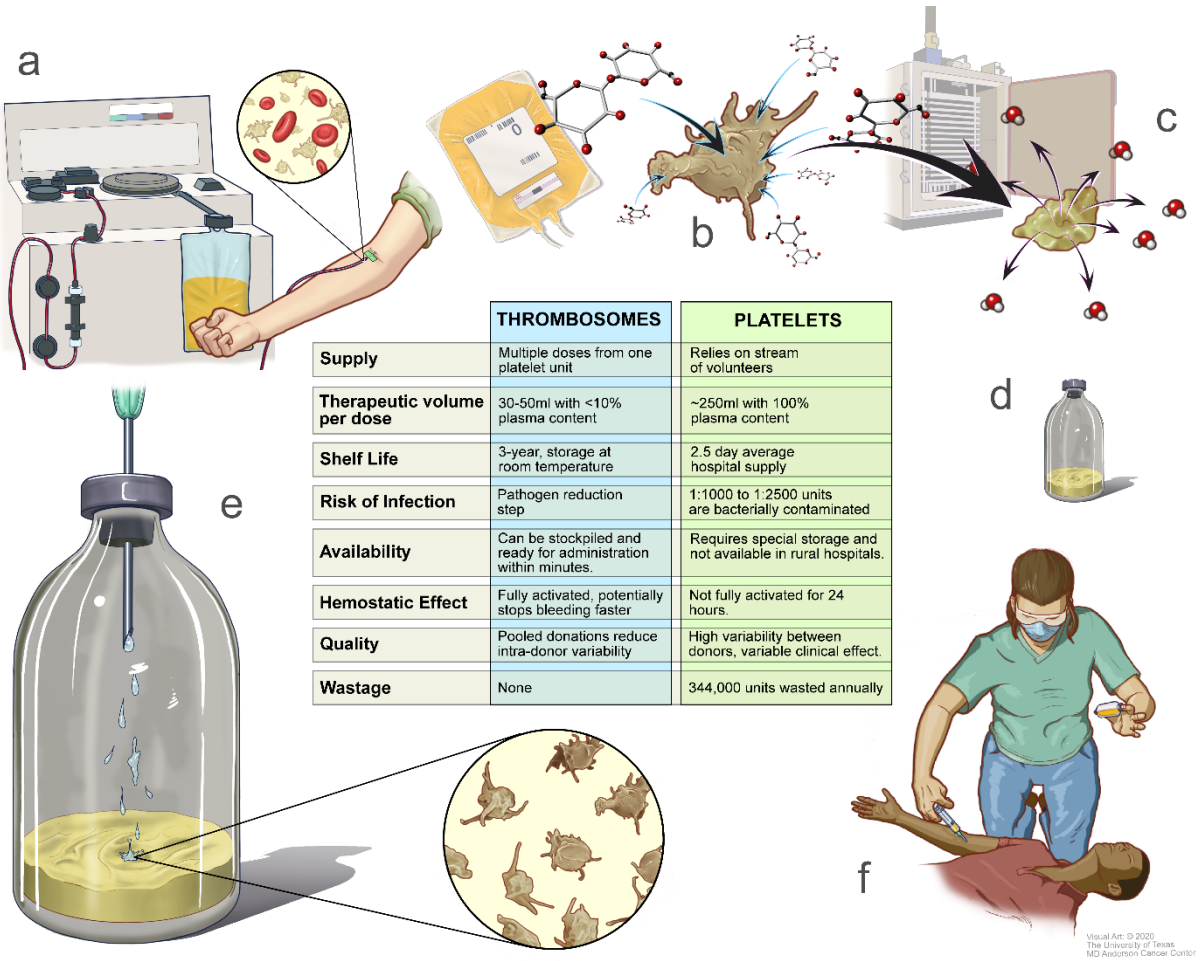

	THROMBOSOMES	PLATELETS
Supply	Multiple doses from one platelet unit	Relies on stream of volunteers
Therapeutic volume per dose	30-50ml with <10% plasma content	~250ml with 100% plasma content
Shelf Life	3-year, storage at room temperature	2.5 day average hospital supply
Risk of Infection	Pathogen reduction step	1:1000 to 1:2500 units are bacterially contaminated
Availability	Can be stockpiled and ready for administration within minutes.	Requires special storage and not available in rural hospitals.
Hemostatic Effect	Fully activated, potentially stops bleeding faster	Not fully activated for 24 hours.
Quality	Pooled donations reduce intra-donor variability	High variability between donors, variable clinical effect.
Wastage	None	344,000 units wasted annually

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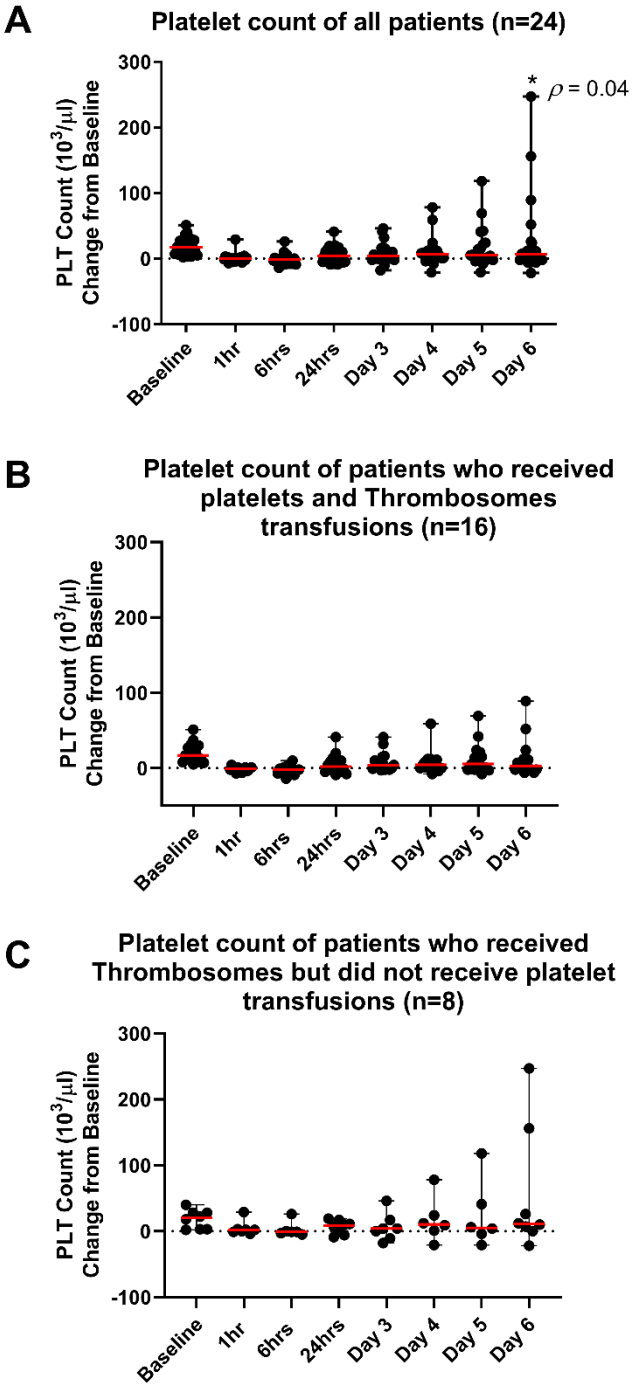

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