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BLOOD COMPONENTS

TRANSFUSION

Characterization and first-in-human clinical dose-escalation safety evaluation of a next-gen human freeze-dried plasma

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Abstract

Background: Early plasma transfusion is life-saving for bleeding trauma patients. Freeze-dried plasma (FDP) provides unique formulation advantages for infusion in the prehospital setting. We describe characterization and clinical safety data of the first, next-generation FDP stored in plastic bags with rapid reconstitution.

Study design and methods: Coagulation and chemistry parameters on 155 pairs of fresh frozen plasma (FFP) and their derivative FDP units were compared. Next, a first-in-human, dose-escalation safety evaluation of FDP, involving 24 healthy volunteers who donated either whole blood or apheresis plasma to create autologous FDP, was performed in three dose cohorts (270, 540, and 810 ml) and adverse events (AEs) were monitored. Cohort 3 was randomized, double-blind with a cross-over arm that compared FDP versus FFP using descriptive analysis for AEs, coagulation, hematology, and chemistry parameters.

Results: FDP coagulation factors, clotting times, and product quality (pH, total protein, and osmolality) post-lyophilization were preserved. FDP infusions, of up to 810 ml per subject, were found to be safe and with no serious

List of abbreviations: ACD-A, anticoagulant citrate dextrose solution A; ACL, automated coagulation analyzer; AE, adverse event; ANOVA, analysis of variance; aPTT, activated partial thromboplastin time; AST, asparte aminotransferase; ALT, alanine aminotransferase; CPD, citrate phosphate dextrose solution; DAT, direct antiglobulin test; FDA, Food and Drug Administration; FDP, freeze dried plasma; FFP, fresh frozen plasma; FII, factor II or prothrombin; FV, factor V; FVII, factor VII; FVIIa, factor VII activated; FVIII:c, factor VIII coagulant; FIX, factor IX; FX, factor X; FXI, factor XI; FXII, factor XII; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, international normalized ratio; NAPTT, non-activated partial thromboplastin time; PF1.2, prothrombin fragment 1.2; PT, prothrombin time; SAE, serious adverse event; SUSAR, suspected unexpected serious adverse reaction; SWFI, sterile water for infusion; TAT, thrombin-antithrombin complexes; TEAE, treatment-emergent adverse events; TT, thrombin time.

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AEs (SAEs) related to FDP. The average time to reconstitute FDP was 67 s (range: 43–106). No differences in coagulation parameters or thrombin activation were detected in subjects infused with 810 ml of FDP compared with FFP.

Conclusion: This first next-generation FDP product preserves the potency and safety of FFP in a novel rugged, compressible, plastic container, for rapid transfusion, allowing rapid access to plasma in resuscitation protocols for therapy in acute traumatic hemorrhage.

KEYWORDS
coagulation, freeze-dried plasma, hemorrhage, next generation, safety

1 | INTRODUCTION

Hemorrhage is the leading cause of potentially preventable deaths on the battlefield and the second leading cause of death in civilian trauma.[1,2] Survival of traumatic hemorrhage is improved when damage control resuscitation efforts include transfusion of blood products in a ratio approaching 1:1 (plasma: red cells) within the first 6 h of injury.[3–6] Consequently, early transfusion of plasma in a prehospital environment has the potential to reduce downstream complications attributable to hemorrhage and is an important component in ensuring optimal survival of patients with traumatic hemorrhage.[7] Approximately 10% reduction in 30-day mortality was associated with the prehospital transfusion of thawed plasma during transport of patients at risk for hemorrhagic shock relative to standard of care, which included infusion of a crystalloid solution as the primary resuscitative fluid.[8] The reduction in mortality is dependent on transport time to hospital (>20 min), as prehospital infusion within an urban area failed to demonstrate differences in survival.[9] Since availability and time to infusion are of essence, current plasma preparations (FFP or liquid, thawed plasma) that require cold storage (as well as a thawing process for FFP) are impractical for successful prehospital use.[10]

Lyophilizing techniques for producing FDP were invented as early as the 1930s.[11] FDP was widely used for hemorrhage control during World War II; however, it was subsequently halted for several decades due to the identification of disease transmission risk in the FDP product that was pooled.[7,12] With improvements in donor infectious disease testing and/or application of pathogen reduction technologies, FDP manufacturing has been able to safely resume.[7,13,14] Several countries, outside of the United States, have licensed FDP products for clinical use,[7,15–17] while use and access to these products are limited in the United States. Currently, the US Food and Drug Administration (FDA) has granted emergency use authorization of a French manufactured FDP for US military use only.[18] Further, the current available FDP products pose some limitations for effectively treating trauma patients in the prehospital setting, including the use of glass bottles for storage, several minutes to complete reconstitution, and dependence on gravity flow.

Here we present the preclinical and first-in-human clinical safety evaluation of a next-generation FDP product, stored in plastic IV bag containers with rapid reconstitution for immediate infusion, that is moving forward through the regulatory approval process in the United States.

2 | MATERIALS AND METHODS

2.1 | Preclinical in vitro characterization of FDP

Each FPD unit was manufactured from a single-donor FFP unit (the starting material) using pilot scale lyophilization methods. For the initial comparative in vitro characterization, a total of 54 apheresis-derived FFPs (ACD-A anticoagulant) and 101 whole blood-derived FFPs (CPD anticoagulant) were purchased from Memorial Blood Centers (Minneapolis, MN) and processed for FDP manufacturing and in vitro analysis that included type A, B, O, and AB blood groups. FFP units were maintained in their frozen state (freezer set at ≤−18°C) in calibrated freezers. The lyophilization of the FFP into FDP was performed using a Magnum shelf freeze dryer (Millrock Technology). Sample analysis of each unit took place on the day of thaw using an aliquot of FFP and followed approximately 2–3 weeks later on the day of reconstitution using an aliquot of the resulting FDP. Each FDP unit was reconstituted with approximately 260–270 ml (250 ml bag with overage) of Sterile Water for Injection (SWFI). All samples were characterized pre- and post-lyophilization utilizing a fully automated coagulation analyzer (ACL Top 700, Instrumentation Laboratories, Bedford MA) to measure clotting times

(prothrombin time [PT], activated partial thromboplastin time [aPTT], non-activated partial thromboplastin time [NAPTT], thrombin time [TT], and international normalized ratio [INR]), and pro- and anticoagulation factor activities/levels (Fibrinogen; Clauss method), FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, vWF, Protein C, Protein S, Plasminogen, Antithrombin III (Antithrombin) and plasmin inhibitor per manufacturer's instructions. FVIIa levels (Stago STA Compact Max Diagnostica Stago, Inc., Parsippany, NJ), pH (Orion StarA211 Benchtop pH Meter, Thermo Fisher Scientific, Waltham MA), pCO_2 (ABL805 Flex, Radiometer), and Osmolality (Osmette A Automatic Osmometer, Precision Systems, Natick MA) were also measured. Total protein was measured using a modified biuret reaction method (Dimension Xpand Plus Clinic Chemistry System, Siemens, Newark, DE). Moisture content using a Karl Fisher titration method (C30 Coulometer with Stromboli Oven Autosampler, Mettler Toledo, Columbus OH) was determined for FDP units only. Results from pre- and post-lyophilization samples were separately pooled by anticoagulant to determine mean, standard deviation, and percentage change for each analytical test. In addition, statistical analysis of the pre- and post-lyophilization results was performed using a paired t-test (Minitab version 19.2 Software, State College, PA) to determine if the mean results differed. A p value $< .05$ was considered significant.

2.2 | Clinical safety trial study design

This trial was a single-site, Phase 1 dose-escalation study to evaluate the safety of FDP after autologous transfusion in normal healthy volunteer subjects. The study was reviewed and approved by the US FDA, the University of Cincinnati, and US Army Human Research Protection Institutional Review Boards. The study was registered in ClinicalTrials.gov (identifier NCT02930226). The safety clinical trial was performed at Hoxworth Blood Center, University of Cincinnati (Cincinnati, OH). The FFP units of autologous plasma (using both CPD and ACD FFP starting material) were shipped to Vascular Solutions LLC (Minneapolis, MN), manufactured into FDP, and returned to Hoxworth for subject FDP infusions. Three sequential cohorts of subjects received escalating doses of autologous FDP transfusions consisting of 270 (1 unit, Cohort 1), 540 (2 units, Cohort 2), and 810 ml (3 units, Cohort 3).

Cohort 3 infusions were administered within a partial double-blind, randomized study design. In this cohort, subjects, principal investigator, and nurses in charge of the infusions were blinded while the laboratory group in charge of plasma preparations and data management, located at the same institution where the procedures took place, was unblinded. The unblinded data management and laboratory groups were in charge of randomization and blinding, respectively. Subjects were randomized to an infusion schedule of 3 units of FDP infusion followed by a 14-day washout period and another infusion of 3 units of FFP infusion (sequence A), or the reverse sequence starting with FFP infusion and followed by FDP infusion (sequence B). Permuted-block randomization with varying block sizes was used to determine subject treatment sequence assignment. Randomization took place no more than 48 h prior to the subject's first infusion (in a 1:1 ratio), which determined the order in which the two plasma treatment products were to be infused. Unblinded site data management and laboratory personnel who managed data and prepared plasma products completed a randomization questionnaire worksheet to verify subject eligibility and submitted through a password protected (with limited access) study website. A total of 40 subjects were screened, 10 subjects did not fulfill eligibility criteria (Table S1), and 30 subjects were enrolled. For the first five subjects of each cohort, no staggering of infusions was allowed. Upon review of the safety data 72 h after infusion, both the principal investigator and clinical monitoring physician had to agree to proceed with the next subject's infusion under strict stopping rules (Figure 1). A data and safety monitoring board reviewed the safety data after the conclusion of each cohort and a recommendation for proceeding was required to allow the continuation of the study. All subjects screened were given a unique identifier that was qualified by the cohort number and in the Cohort 3 by the infusion order.

The primary safety endpoint was enumeration and description of treatment-emergent adverse events (TEAEs), which are AEs that emerge during or post-infusion having been absent pre-infusion. In Cohort 1 and Cohort 2, of 20 subjects that were enrolled, four discontinued participation (one for PI decision in relation with compliance and three due to problems of vein access to collect enough plasma). A total of 16 subjects were infused and evaluated in Cohort 1 and Cohort 2. In Cohort 1, six subjects were males and two were females. The mean age was 34.5 years (range: 27–48 years). In Cohort 2, all subjects were males with a mean age of 33.6 years (range: 18–53 years). All subjects in Cohorts 1 and 2 were White, non-Hispanic. The design of Cohorts 1 and 2 included the use of whole blood-derived citrate-phosphate-dextrose (CPD) anticoagulated FFP (4 subjects per arm; arms 1 and 3) or apheresis-derived acid citric-citrate-dextrose (ACD) anticoagulated FFP (4 subjects per arm, arms 2 and 4) as starting material for the manufacture of FDP, for a total of eight subjects in each cohort.

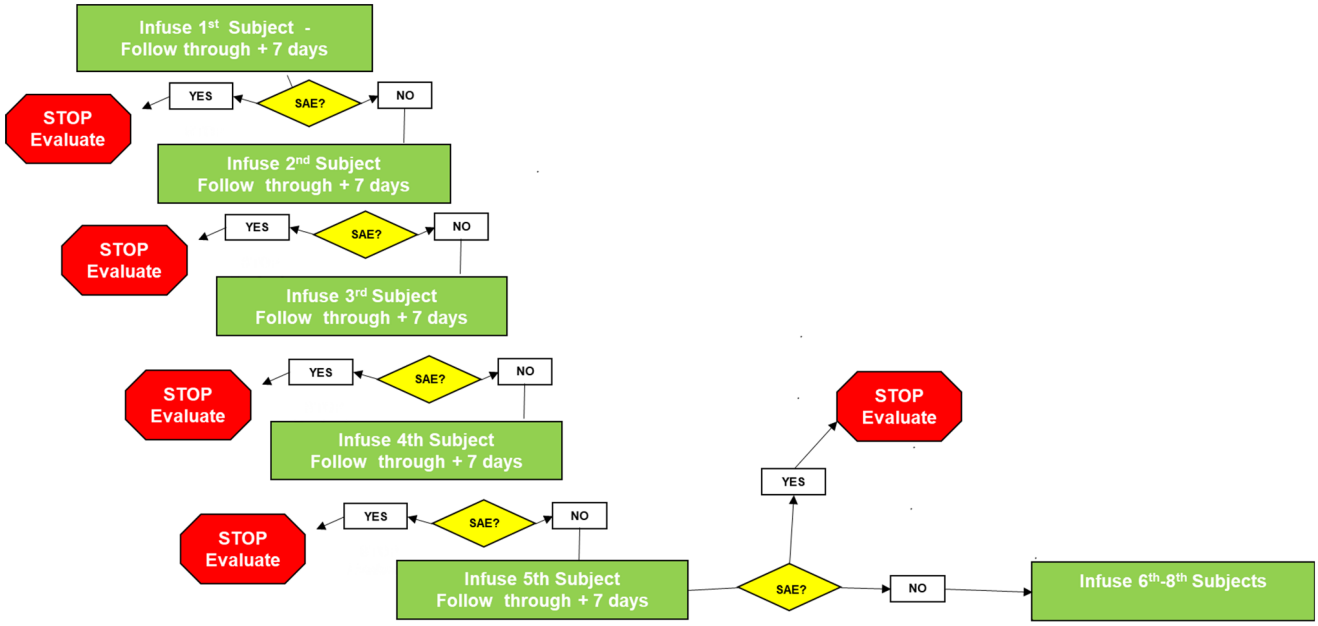

FIGURE 1 Stopping rules of the dose-escalation safety clinical trial in Cohorts 1 and 2

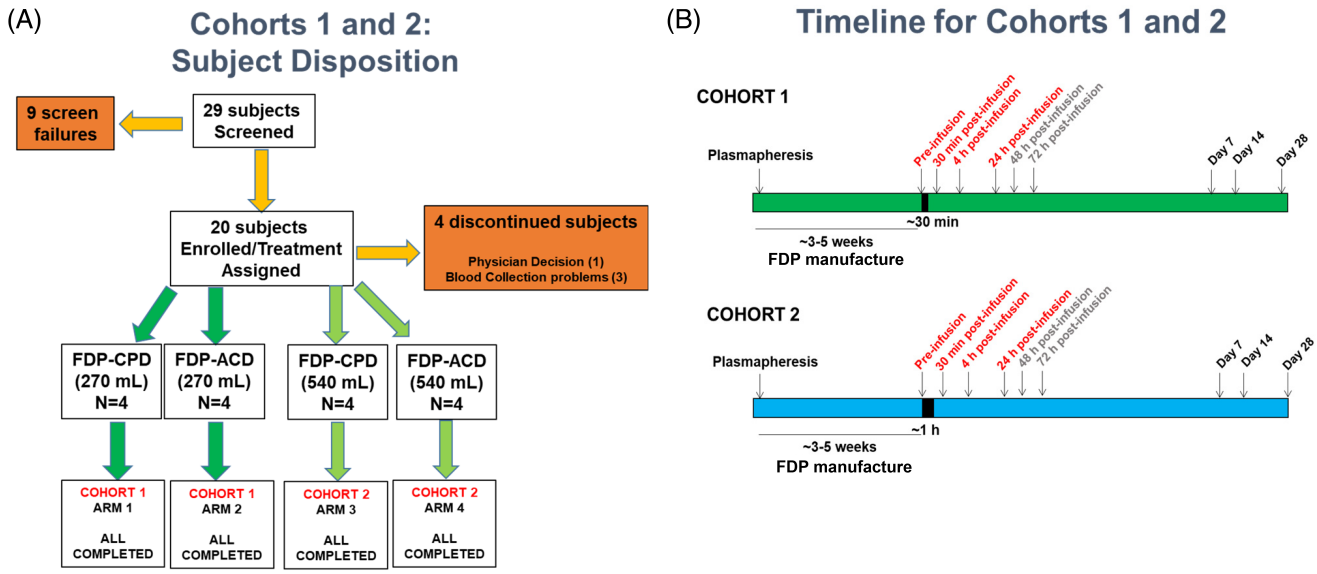

FIGURE 2 Subject disposition and timelines for Cohorts 1 and 2. (A) Subject disposition in Cohorts 1 and 2. (B) Timelines for Cohorts 1 and 2 enrolled subjects. Infusion rates were kept identical between Cohorts and therefore the difference in time to end the infusion depended on the larger volume infused in Cohort 2

Subjects in Cohort 2 donated two whole blood units with a minimum interval of 4 weeks between donations. Disposition for subjects in Cohorts 1 and 2 is seen in Figure 2A and timelines for Cohorts 1 and 2 are presented in Figure 2B. The subject disposition (infusion flowchart) for subjects enrolled in Cohort 3 is presented in Figure 3A, and the timeline for Cohort 3 is presented in Figure 3B.

For Cohort 3, autologous units of apheresis (ACD-anticoagulated) FFP were randomly assigned for storage on site or shipment for manufacturing into FDP. Secondary endpoints in the Cohort 3 analysis included the change in pre- and post-infusion vital signs and (in subject blood samples) of coagulation parameters PT, INR, aPTT, fibrinogen, Factors II, V, VII, VIII, IX, X, XI, D-Dimer, von Willebrand factor activity, Protein S

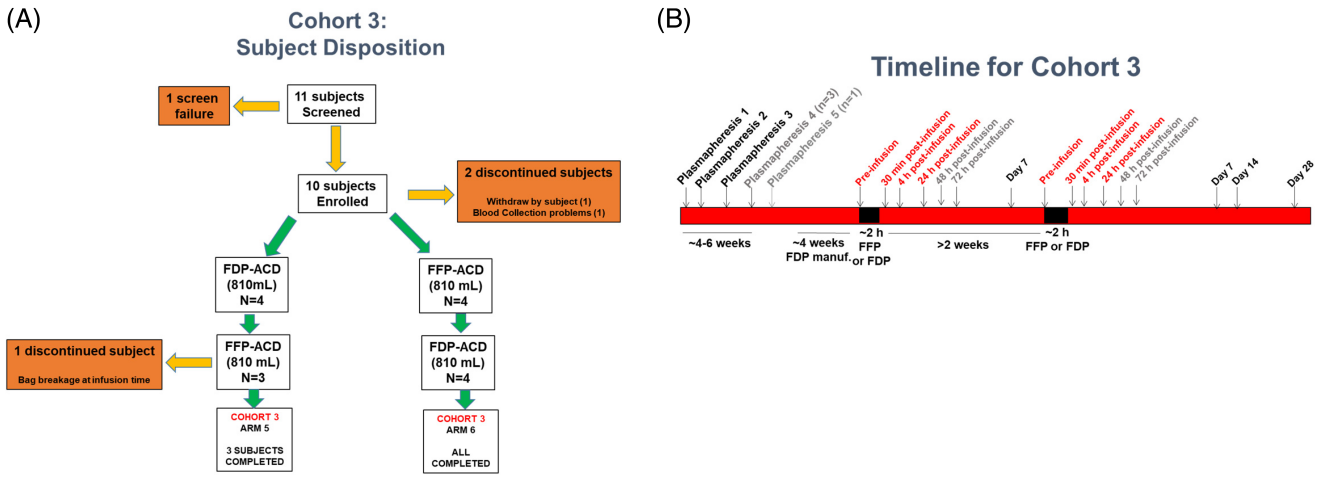

FIGURE 3 Subject disposition and timelines in Cohort 3. (A) Subject disposition in Cohort 3. (B) Timeline for Cohort 3. Infusion rate was kept identical as in Cohorts 1 and 2, and therefore the difference in time to end the infusion depended on the larger volume infused in Cohort 3

activity, Protein C activity, PF 1 + 2, TAT, Antithrombin III, Alpha-2 Antiplasmin, and C3a; and hematology, chemistry, urinalysis, and direct antiglobulin test (DAT) values. Post-infusion measurements included analysis after infusion of either 3 units of FDP or 3 units of FFP. Eleven individuals were screened for Cohort 3 and one individual failed screening. Six of the enrolled subjects were White, non-Hispanic males, one subject was a Black, non-Hispanic male, and one subject was a White, non-Hispanic female. The mean age was 33.9 years (range: 22–47 years). The design of Cohort 3 infusions included a cross-over analysis of comparative transfusion effects of autologous FDP with autologous FFP. One Cohort 3 subject did not receive one of the infusion arms because of breakage of one unit. Post hoc unblinding unveiled that the broken bag intended for this subject was an FFP unit. Because the FFP infusion could not take place, no paired analysis could be performed for this subject, and therefore, seven subjects were evaluated by paired analysis for the effects of FFP–FDP infusions.

2.3 | Clinical FFP plasma collections

All subjects' screen samples were analyzed with the HTLV antibody screening test; HIV-1/2, hepatitis B (HBV), hepatitis C (HCV), and West Nile nucleic acid tests, as required by AABB and FDA guidance and regulations, by Indiana Blood Center (Versiti, Indianapolis, IN) following their standard operating procedures.

For arms 1 and 3 of Cohorts 1 and 2, respectively, whole blood (WB) donation (500 ± 50 ml) was performed in CPD-containing blood bags (Fresenius-Kabi, Lake Zurich, IL). WB units were centrifuged with a validated hard centrifugation protocol (5000g for 10 min), and plasma was manually expressed after collection. For arms 2 and 4 of Cohorts 1 and 2, respectively, apheresis plasma collections of approximately 700 ml (Trima Accel 7, TerumoBCT, Lakewood, CO) were collected. All FFP units were collected approximately 3–5 weeks before infusion. For Cohort 3, three to five plasmapheresis procedures were necessary to collect in excess of 1620 ml of plasma for manufacturing FFP ($n = 3$) and FDP ($n = 3$) units required per subject. Four subjects needed either four ($n = 3$) or five ($n = 1$) collections due to bag breakage during the lyophilization process. Freezing of ACD or CPD plasma was performed within 8 h of collection in a XBF40-MD blast freezer (Forma Thermo-Fisher Scientific, Waltham, MA) and stored at ≤−18°C.

2.4 | Clinical FDP manufacturing

FFP units of autologous plasma were shipped to Vascular Solutions LLC in temperature-controlled (≤−18°C), dry shippers that included data loggers. The individual FFP units were then manufactured into FDP units using a Magnum shelf freeze dryer (Millrock Technology), with unit traceability maintained throughout the manufacturing process. FDP units were then returned to the Hoxworth clinical site in temperature-controlled (2–8°C), dry shippers that included data loggers. The FDP units were then stored refrigerated (1–6°C) until the time of planned subject infusion.

2.5 | Clinical plasma infusions

Prior to subject infusion, each required autologous FDP unit was individually reconstituted with 250 ml of SWFI from the transfusion kit supplied by Vascular Solutions

LLC (Figure S1, note that in the clinical trial the FDP unit was not co-packaged, as pictured, with the other components of the transfusion kit). In Cohort 3, each autologous FFP unit was thawed in a water bath at 37°C. Each FDP or FFP unit was: (1) tested for reverse blood group type, (2) cross-matched with a freshly collected red cell specimen from the subject (per Hoxworth SOPs using CLIA certified tests), and (3) transfused (after data review and approval by the principal investigator) within 2 h after preparation.

Cohort 3 was partial double-blinded, where the subject and the clinical team remained unaware of whether FFP or FDP was administered to the subject at a given infusion visit (unit[s] covered by a black fabric bag). All infusions were performed at a starting rate of 3 ml/min and then increased to 10 ml/min after 10 min of clinical observation, if indicated. Vital signs were measured every 15 ± 5 min during infusion and every 30 ± 5 min for up to 4 h post-infusion. Subjects were observed during and after infusion for AEs including evidence of acute transfusion reactions. Full physical examination included Wells' score criteria for assessment of thrombosis (performed before infusion, at 30 min, and 4 h after the infusion ended). Once a 4-hour post-infusion D-dimer plasma level result was reviewed, subjects were discharged (typically around 5 h after the infusion).

2.6 | Clinical laboratory subject sample tests

Pre- and post-infusion laboratory studies were performed including complete blood count, chemistry panel (including bilirubin, aspartate and alanine aminotransferases, alkaline phosphatase, creatinine, ionized calcium, magnesium and blood urea nitrogen), DAT [immunoglobulin G (IgG) and complement factor 3 (C3)]; and D-dimer test. Frozen samples were analyzed for coagulation tests fibrinogen, FII, FV, FVII, FVIII, FIX, FX, FXI, vWF activity, and protein C and S activities and enzyme-linked immunoassays for alpha-2-antiplasmin, thrombin–antithrombin complexes (TAT, Enzygnost TAT, Siemens Healthcare), prothrombin fragment 1.2 (PF1.2, Enzygnost, Siemens Healthcare), and C3a (Microvue C3a des-Arg Quidel, San Diego, CA) as previously described.[19]

2.7 | Clinical safety assessments

Safety was assessed, at increasing fixed doses, by evaluating for AEs, vital signs, and laboratory tests during and after infusion. No formal statistical hypothesis was tested. The primary safety analysis assessed the frequency and proportion of adverse events (AEs), treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), suspected unexpected serious adverse reactions (SUSARs) as defined by 21 US CFR 314.80 and 312.32; and deaths in each cohort and treatment group. Subjects were monitored for development of AEs during observation for 4 h after ending the infusion, and then at 24 h, 7, and 28 days. Phone follow-up for evaluation of possible AEs was also performed at 2, 3, and 14 days post-infusion. The secondary safety analysis was limited to Cohort 3 and compared by period treatment groups by summarizing the change from baseline to each time point for vital signs, hematology, clinical chemistry, and coagulation factor tests using the same descriptive statistics used for the baseline demographic characteristics. Urine analysis and DAT were also assessed for abnormalities post-infusion.

2.8 | Clinical statistical analysis

The sample size for this single-site study was set to 24: eight subjects in each cohort. A safety outcome occurring at a rate of 10% incidence in 24 subjects would have a 95% confidence interval from 1.6% to 29.2% and a corresponding width of 27.6 at a two-sided alpha error of .05.

A 1:1 ratio of Cohort 3 subjects was randomized to one of two treatment arms that determined whether subjects received FFP at the first infusion visit followed by FDP at the second infusion visit or, alternatively, received FDP at the first infusion visit followed by FFP at the second. Randomization was performed less than 48 h prior to the subjects' first scheduled infusion visit. The randomization process assigned the product infusion sequence, indicating whether FFP or FDP was infused at the first infusion visit.

Descriptive statistics are used to present the study data. Discrete variables are presented as number of observations and percentages. Continuous variables are given as means and standard deviations (SDs) or range. For summary descriptive statistics, missing data are represented by counts and were treated as missing at random, and no adjustments were made. A post hoc data analysis was done using paired Student's t and two-way ANOVA tests (Graphpad Prism 9 Software, San Diego, CA). For all statistical tests, p values $< .05$ were considered significant.

3 | RESULTS

3.1 | Preclinical in vitro characterization of FDP

As outlined in Table 1, the coagulation factor activities and clotting times for samples of FFP (pre-lyophilization)

TABLE 1 Coagulation factor activities and clotting times comparison of ACD FFP and CPD FFP to FDP

		ACD			CPD		
		$n = 54$			$n = 101$		
Test parameter	**Clinical ref range per test instrument**	**FFP Mean ± SD**	**FDP Mean ± SD**	**% Change**	**FFP Mean ± SD**	**FDP Mean ± SD**	**% Change**
PT	9.4–12.5 s	12.1 (±0.9)	12.9 (±1.0)*	7.19	11.6 (±0.8)	12.6 (±0.9)*	8.52
INR	0.9–1.1	1.11 (±0.1)	1.18 (±0.0)*	7.06	1.1 (±0.1)	1.1 (±0.1)*	8.33
aPTT	25.1–36.5 s	29.6 (±1.9)	31.1 (±2.1)*	5.15	33.2 (±2.7)[a]	35.3 (±3.2)*	6.41
NAPTT	>60 s	171.6 (±39.9)	192.4 (±34.3)*	12.13	165.7 (±24.1)	178.0 (±23.8)[a,*]	7.42
TT	15.8–24.9 s	22.0 (±1.5)	24.2 (±2.0)*	9.60	20.0 (±2.3)	22.3 (±2.5)*	11.90
Fibrinogen	200–393 mg/dl	254.6 (±44.1)	237.2 (±40.8)*	−6.83	263.5 (±61.7)	250.9 (±55.7)*	−4.81
Factor II activity	79%–131%	93.2 (±10.6)	82.2 ± (9.6)*	−11.87	86.5 (±9.3)	77.7 (±7.9)*	−10.19
Factor V activity	62%–139%	82.1 (±15.0)	73.8 (±13.7)*	−10.05	92.6 (±16.4)	79.0 (±12.9)*	−14.67
Factor VII activity	50%–129%	81.2 (20.5)	75.4 (±18.5)*	−7.15	85.8 (±19.3)	77.1 (±18.0)*	−10.12
Factor VIII activity	50%–150%	144.4 (±32.7)	129.8 (±28.9)*	−10.11	115.8 (±37.2)	107.9 (±29.1)*	−6.85
Factor IX activity	65%–150%	106.8 (±14.5)	92.8(±13.4)*	−13.08	110.1 (±17.6)[b]	95.3 (±17.2)*	−13.50
Factor X activity	77%–131%	83.1 (±12.3)	73.4 (±11.7)*	−11.65	94.9 (±16.4)	81.2 (±13.2)*	−14.35
Factor XI activity	65%–150%	109.9 (±23.3)	97.9 (±24.0)*	−10.94	107.9 (±18.7)	102.9 (±17.8)*	−4.63
Factor XII activity	50%–150%	91.3 (±18.8)	80.9 (±17.0)*	−11.37	98.7 (±24.8)[c]	83.1 (±21.2)*	−15.73
Factor VIIa	10.0–105.0 mU/ml	58.0 (±25.0)	49.2 (±20.4)*	−15.12	42.1 (±20.9)[d]	39.8 (±18.6)[b]	−5.42
Protein C activity	70%–140%	95.2 (±17.3)	89.2 (±15.5)*	−6.33	99.8 (±17.3)	90.5 (±15.6)*	−9.28
Protein S activity	54.7%–146.1%	91.9 (±17.0)	90.8 (±15.4)	−1.17	100.2 (±14.8)	92.7 (±13.5)*	−7.48
Plasmin inhibitor	98%–122%	93.0 (±9.5)	86.9 (±9.9)*	−6.53	96.8 (±7.2)	92.5 (±6.8)*	−4.48
Plasminogen activity	80.2%–132.5%	86.5 (±15.0)	82.3 (±13.5)*	−4.92	94.8 (±11.1)	86.8 (±17.4)*	−8.49
Antithrombin	83%–128%	90.0 (±10.2)	85.0 (±9.6)*	−5.49	97.6 (±9.9)	90.7 (±9.8)*	−7.07
vWF activity	40.3%–163.4%	130.7 (±38.9)	121.4 (±35.5)*	−7.13	124.5 (±46.6)[e]	111.4 (±40.0)*	−10.52
vWF antigen	42.0%–176.3%	158.4 (±42.2)	142.8 (±40.1)*	−9.87	135.3 (±47.7)	123.0 (±44.0)*	−9.13

[a]100 samples.
[b]96 samples.
[c]90 samples.
[d]99 samples.
[e]95 samples.
*p value < .05, FFP versus FDP.

TABLE 2 pH, pCO_2, osmolality, total protein, and moisture comparison of FFP and FDP

	ACD			CPD		
	***n* = 54**			***n* = 101**		
Test parameter	**FFP**	**FDP**	**Difference**	**FFP**	**FDP**	**Difference**
pH Mean ± SD Range	7.24 (±0.0) 7.16–7.31	6.88 (±0.1)* 6.60–7.21	−0.4[d]	7.3 (±0.0) 7.3–7.5	6.9 (±0.2)* 6.6–7.7	−0.4[d]
pCO_2 (mm Hg) Mean ± SD	58.6 (±7.7)[c]	119.6* (±28.3)	98.75%	53.8 (±5.6)[b]	119.5 (±30.7)*	122.3%
Osmolality (mOsm/kg) Mean ± SD	293.5 (±3.2)	280.8* (±12.8)	−4.32%	308 (±3.9)	298 (±7.2)*	−3.2%
Total protein (g/dl) Mean ± SD	5.6 (±0.3)	5.3 (±0.3)*[,c]	−5.37%	5.9 (±0.3)	5.7 (±0.3)*	−3.98%
Moisture content (%) Mean ± SD	N/A	1.0 (±0.2)[a]	N/A	N/A	1.1 (±0.0)[c]	N/A

[a]7 samples.
[b]99 samples.
[c]49 samples.
[d]Absolute difference.
*p value < .05, FFP versus FDP.

and FDP (post-lyophilization and reconstitution) derived from apheresis ACD or whole blood CPD collection are presented (along with the clinical reference ranges). The effect of the lyophilization process on either ACD or CPD plasma was found to be generally similar with preservation of coagulation factor activities and clotting times in the post-lyophilized product (based on the means and standard deviation of the data). Both CPD FDP and ACD FDP had a slight prolongation of mean clotting times (PT, INR, aPTT, and TT) and minimal or modest reductions in the mean content of fibrinogen, factors II, V, VII, VIII, IX, X, XI, XII, Protein C, Protein S, plasmin inhibitor, plasminogen, antithrombin III, vWF activity, and vWF antigen, compared with the FFP control (Table 1). The mild changes in clotting times are demonstrated by the fact that the changes in mean PT and INR (for both CPD and ACD FDP) result in values that are just at, or slightly above, the clinical reference range (PT reference range up to 12.5 s: FDP values were 12.9 and 12.6 s, ACD FDP and CPD FDP, respectively, and INR reference range up to 1.1: FDP values were 1.18 and 1.1, ACD FDP and CPD FDP, respectively; Table 1), while the mean aPTT was within the clinical reference range (up to 36.5 s) for ACD FDP (31.1 s) and CPD FDP (35.3 s) (Table 1). The TT was within the reference range (up to 24.9 s) for both ACD and CPD products (24.2 s vs 22.3 s, respectively; Table 1).

Individual coagulation factors in both CPD FDP and ACD FDP were found to be within approximately ±15% agreement (mean percent change) of the respective starting materials and within clinical reference range for all coagulation assays except for Factor II (CPD FDP) and Factor X (ACD FDP). The magnitude of coagulation factor values that were below clinical reference range was inconsequential: mean Factor II in CPD FDP was 77.7% versus 79% cutoff for the clinical reference range, and mean Factor X in ACD FDP was 73.4% versus 77% cutoff for clinical reference range (Table 1). While there was a maximum of approximately 15% change in mean individual coagulation factors in comparison to FFP starting material, the magnitude of these differences is very similar to the ones reported to the licensed PF24 and PF24RT24 plasma products,[20] used in trauma centers for resuscitation. The NAPTT was always higher than 60 s (>60 s) and FVIIa was always lower than 100 mU/ml (≤105.0 mU/ml) for CPD FDP and ACD FDP, indicating that the FDP manufacturing process did not result in relevant clotting activation of either the intrinsic or extrinsic pathways.

Additional analysis of FDP showed a decrease in pH, increase in pCO_2, and a modest decrease in the osmolality and total protein (Table 2). The moisture content of FDP products was approximately 1% (Table 2). The preclinical in vitro characterization data of FDP displayed sufficient preservation of plasma parameters, and therefore supported advancing to first-in-man clinical safety testing.

3.2 | Clinical trial safety evaluation

There were no dose-limiting toxicities (no SAEs, no triggering of stopping rules, and no deaths) throughout the dose-escalation study. None of the TEAEs observed were

TABLE 3 Overview of subject incidence of AEs-safety population S1 ($N = 24$)

Parameter	Cohort 1		Cohort 2		Cohort 3[a]	
	FDP-CPD ($N = 4$) *n* (%)	FDP-ACD ($N = 4$) *n* (%)	FDP-CPD ($N = 4$) *n* (%)	FDP-ACD ($N = 4$) *n* (%)	FDP-ACDxFFP ($N = 4$) *n* (%)	FFPxFDP-ACD ($N = 4$) *n* (%)
AEs	2 (50.0)	2 (50.0)	0 (0.0)	0 (0.0)	2 (50.0)	4 (100.0)
TEAEs	2 (50.0)	2 (50.0)	0 (0.0)	0 (0.0)	2 (50.0)	3 (75.0)
TEAEs related to study treatment	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SAEs	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AE leading to discontinuation of investigational product	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fatal adverse events	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note: Subjects may be counted in more than one row. Denominator for each % is the treatment group *N*.

Abbreviations: ACD, acid phosphate dextrose; AE, adverse event; CPD, citrate phosphate dextrose; FDP, freeze-dried plasma; FFP, fresh frozen plasma; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

[a]This table includes data from the Cohort 3 subject who successfully completed the first infusion with FDP but was unable to complete the second planned infusion with FFP.

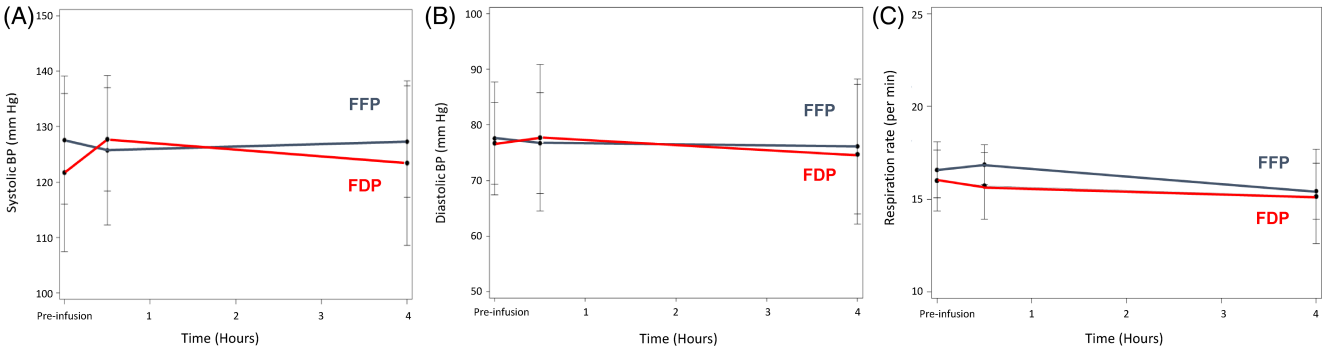

FIGURE 4 Blood pressure and respiratory rate of subjects before and after infusion in Cohort 3. (A) Systolic blood pressure (BP). (B) Diastolic blood pressure. (C) Respiration rate. Data are presented as average ± one standard deviation (SD)

considered to be related to the FDP infusion, even at the highest dose (810 ml), which utilized a blinded, comparative, nonstatistical AE analysis with the FFP control arm.

A summary of overall safety events showed 9 of 24 subjects (37.5%) reported 17 TEAEs (Table 3 and Table S2). With the exception of one TEAE that was considered to be of moderate severity (bursitis), all other TEAEs were of mild severity (Table S2), none required stopping or changes in treatment dose, and all resolved. Two TEAEs were considered possibly related to FFP (increased blood thrombin and increased thrombin–antithrombin III complex) and none were considered related to FDP (Table S2). In Cohort 1, four of the eight subjects experienced a total of six TEAEs of including: one subject with one TEAE (weakly positive DAT that had no effect on hematologic indices or other evidence of hemolysis up to 29 days after infusion of FDP), one subject with one TEAE ("transaminitis" with AST/ALT modestly increased above the upper limit of normal on Day 7 that returned to normal on testing performed on Days 12 and 28, in the context of acetaminophen use for back pain, and found to be negative for viral markers on Day 28), one subject with two TEAEs (transient effects,1 time point, postprandial hyperglycemia and glycosuria 6 h post-FDP infusion), and one subject with two TEAEs, of nasal congestion (at 48-hour follow-up) and temperature changes (7 days post-infusion). In Cohort 2, there were no TEAEs or other safety signals. In Cohort 3, five of the eight subjects experienced 11 TEAEs, of which one subject had 2 TEAEs (increased blood thrombin and increased thrombin–antithrombin III complex), which were classified as possibly related to treatment (FFP). One subject had one TEAE of

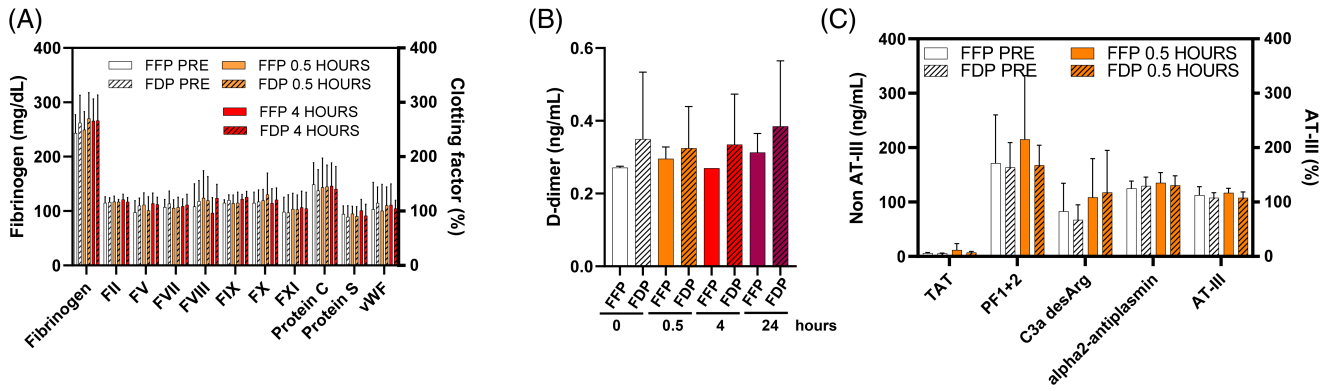

FIGURE 5 Time kinetics of clotting factor levels and pro-thrombotic indicators in plasma before and after infusions in Cohort 3. (A) Fibrinogen, prothrombin (FII), factor V (FV), factor VII (FVII), factor VIII:Coagulant (FVIII), factor IX (FIX), factor X (FX), factor XI (FXI), protein C, protein S, and von Willebrand factor antigen (vWF). Protein C levels higher than 200% were found outside the linearity range and used as of 200% for quantitative calculations. (B) D-dimer. Level of sensitivity was 0.27 ng/ml. For specimens with levels lower than 0.27 ng/ml, a level of 0.27 ng/ml was used for quantitative calculations. (C) Thrombin–antithrombin (TAT) complexes, prothrombin fragment 1 + 2 (PF1 + 2), complement C3a desArg fragment, alpha2-antiplasmin, and antithrombin III (AT-III). Data are presented as average + one SD. No statistically significant differences between the levels of any of the parameters analyzed before or after the infusions of FFP or FDP were found

elevated D-dimer (prior to FDP infusion), one subject presented with three TEAEs (cold symptoms: runny nose, chest congestion, and sore throat) during the 24-hour follow-up period after the first infusion of FDP, and one subject had what was described as a "metallic taste" immediately after FFP infusion. The remainder of the TEAEs are of the type found in a normal healthy volunteer study, headache ($n = 2$), bursitis ($n = 1$), and seasonal allergy ($n = 1$), see Table S2). No effects on blood pressure or respiratory rate between FDP and FFP arms were observed in subjects enrolled in Cohort 3 (Figure 4). The average time to reconstitute clinical FDP units was 67 ± 15 s (range: 43–106 s).

3.3 | Clinical laboratory evaluation of Cohort 3 infusions

Analysis of clotting factors and anticoagulation factors were analyzed before infusion and at 30 min and 4 h post-infusion of FDP or FFP in Cohort 3 (Table S3). We found no differences in the kinetics of these factors between subjects receiving FFP and FDP (Figure 5A and Table S3). D-dimer levels were analyzed before infusion and at 30 min, 4, and 24 h post-infusion, showing no differences either (Figure 5B and Table S3). Byproducts dependent on thrombin activation PF1 + 2 and TAT as well as levels of the inflammatory marker C3a desArg, antifibrinolytic alpha2-antiplasmin, and antithrombotic antithrombin III were analyzed before and at 30 min post-infusion (Figure 5C and Table S3). None of these parameters showed differences between subjects receiving FDP or FFP except in one subject upon infusion of FFP in which the one subject (identifier 101,135) had high levels of TAT and PF1 + 2 after infusion of FFP, which were not found increased after infusion of FDP (Table S3).

4 | DISCUSSION

Over the last few years, the use of blood products has been proven to reduce morbidity and mortality of the severely injured casualties,[3–5] where the early transfusion of blood products, especially plasma[6,8] has been found to be important to ensure optimal survival of patients with traumatic hemorrhage.

Plasma is a more effective resuscitative fluid than currently available clear fluids, mainly by virtue of its high oncotic pressure and coagulation factor content.[21] FDP with similar characteristics to FFP, both in terms of its coagulation factor content and its effect on plasma oncotic pressure, has been argued to be a product that can also result in decreased morbidity and mortality in bleeding trauma patients.[22,23] A plasma product that can be used without certain restrictive logistical requirements including cold storage (frozen storage and transport or thawing) is needed to enable plasma transfusion where medically needed, instead of occurring only in centralized locations where freezers and other required pieces of equipment are available.[24,25] All currently available FDP products are stored in glass bottles, require several minutes to reconstitute, and depend on gravity flow. As such, the ability of current FDP products to quickly treat bleeding trauma patients, including those with severe

bleeding in austere military settings, or prehospital civilian settings with prolonged field time, may have limitations. In addition, no FDP product is currently licensed by the US FDA.[26] Recent efforts to manufacture FDP in plastic bags for transfusion and their in vitro characterization have been reported.[27,28] However, none of these modern FDP products have been tested in humans.

We present the first-in-human use of a next-generation FDP product stored in a plastic IV bag container, which permits rapid reconstitution and immediate infusion by simply squeezing the reconstituted unit. Further, the rugged kit design permits transport to far forward military settings (with minimal breakage) and use within previously inaccessible prehospital environments. All of these enhancements could allow earlier access to plasma in the treatment of acute traumatic hemorrhage and attenuation of trauma-induced coagulopathy (prehospital and in-hospital).

The preclinical and clinical safety data as reported support that FDP is safe to be infused and the levels of quantitatively and/or functionally assessed clotting, antithrombotic, antifibrinolytic, and pro-inflammatory content are comparable with alternative licensed products[20] with clinically acceptable post-lyophilization preservation of coagulation factor activities and clotting times. No significant differences in clotting factor content or outcome of subjects undergoing autologous transfusion of up to 810 ml of FDP were identified, and no SAEs were found after the infusions of any dose of FDP on study in healthy volunteers. While the clinical study was small in scale, and not designed for efficacy analysis, the high level of similarities (including coagulation factor content, chemistry measures, and adverse event profile) between FDP and FFP are suggestive that its efficacy should be similar to the one found in the prehospital administration of FFP or PF24,[6,8] and has an improved utility over frozen plasma or current FDP products based on its rugged packaging, rapid reconstitution, immediate infusion capability by simply squeezing the IV plastic bag, and potential to demonstrate storage stability without cold chain requirements.

This single-donor FDP product represents the first example of next-generation FDP products under development[29] that the US Government is supporting. This investigational FDP product is a candidate for biologics license approval by the US FDA. Additional studies to determine ultimate shelf life and storage conditions for FDP include those at refrigerated or room temperature, or a combination of temperatures. A potential FDP product with the capacity of storage at room temperature for extended periods of time could enhance the effective therapy of massive bleeding from trauma and the prevention of trauma-induced coagulopathy. Future developments of next-generation FDP products like this one may include the use of pathogen-reduced plasma.

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CONFLICT OF INTEREST

The authors declare that AE, MLC, LM, JM, SP are employees of Vascular Solutions LLC, a wholly-owned subsidiary of Teleflex Incorporated. JCP and VWM are paid consultants for Teleflex/Vascular Solutions LLC. AJA and MKV are employees of the US Federal Government. The opinions presented in this publication do not represent those of the US Army or the Department of Defense. No other relevant conflicts of interest.

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SUPPORTING INFORMATION

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