Innovative Blood Products
Lyophilized Platelets
Why do we need “Innovative” platelets?

- Advance hemostatic blood products into the 21st century
- Reduce the currently high outdate 8 - 9.5% nationally; Transfusion 2017 June;57 (Suppl 2):1588-1598 doi:10.1111/trf.14165
- Expand availability to the military, prehospital, rural and other hospitals
- **Support “STOP the BLEED”**

Whole blood is making a comeback - first used routinely in World War I 1913 (red cells, plasma, and platelets) [Kendrick DB: Blood Program in World War II. Washington DC Office of the Surgeon General, Dept. of the Army 1964]

Freeze-dried plasma first used in World War II. [Kendrick DB: Blood Program in World War II. Washington DC Office of the Surgeon General, Dept. of the Army 1964]


Freeze-dried platelets 1959.
EFFECTIVENESS OF TRANSFUSIONS OF FRESH AND LYOPHILIZED PLATELETS IN CONTROLLING BLEEDING DUE TO THROMBOCYTOPENIA *

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(Submitted for publication April 15, 1959; accepted June 19, 1959)

Infusions of platelets preserved by freezing (4) and lyophilization (5) have not resulted in increase of recipient’s platelet levels although such infusions have been reported to control bleeding due to thrombocytopenia.
Support Zero Preventable Deaths

Platelet transfusions improve hemostasis and survival in a substudy of the prospective, randomized PROPR trial

Jessica C. Cardenas,1,2 Xu Zhang,3 Erin E. Fox,1-3 Bryan A. Cotton,1-3 John R. Hess,4 Martin A. Schreiber,5 Charles E. Wade,1-3 and John B. Holcomb,1-3 on behalf of the PROPR Study Group

1Division of Acute Care Surgery, Department of Surgery, McGovern School of Medicine, 2Center for Translational Injury Research, and 3Center for Translational and Clinical Studies, University of Texas Health Science Center, Houston, TX; 4Department of Laboratory Medicine, Harborview Medical Center, University of Washington, Seattle, WA; and 5Division of Trauma, Critical Care and Acute Care Surgery, Department of Surgery, Oregon Health and Science University, Portland, OR

Figure 1. Kaplan-Meier curves. Curves demonstrate cumulative incidence of death during the first 6 hours (A) and 30 days (B).
Need for Innovative Products

- Platelet supply challenges (platelet gap)
  - Shelf-life
    - RT 5-7 days
    - Cold-stored 7-14 days when approved
  - Seasonal and regional shortages

- Currently only one *extended storage* product in use, Netherlands
  - DMSO cryopreserved platelets (CPP)
    - Storage < -65C, cold chain storage
    - 2 – 4 year expiration (country dependent)
    - Used routinely in Netherlands Armed Forces
    - Several countries exploring civilian use
    - Phase 1 trial in US completed
    - Phase 2 pending
Lyophilized Platelets

- Two approaches to preparing lyophilized platelets
  - Paraformaldehyde treatment
    - Entegrion
    - Development suspended
  - Trehalose treatment
    - Cellphire
    - Autologus phase 1 safety trial completed
    - Currently in Phase 1 clinical trial
  - Advantages
    - Standard
    - 2+ year shelf life
    - RT storage
    - Could be suitable for Prehospital use
Thrombosomes Employ Nature’s Solution

- A shrimp larva produces Trehalose for protection during dehydration (Trehalose, green as determined by FTIR)
- When exposed to water the brine shrimp comes to “life”
- Loading platelets with Trehalose preserves hemostatic function, resulting in a PDHA
Thrombosomes®

• Manufactured from a pool of FDA licensed apheresis platelet units (up to 10 Group O donors)

• Composed of physiologic buffers and sugars used as stabilizers, cryoprotectants, and excipients, Trehalose, and Polysucrose.

• Once rehydrated Thrombosomes® demonstrate retention of essential hemostatic properties of platelets
  • Adhesion
  • Thrombin Production
  • Aggregation
  • Clot consolidation
  • Reduction of infectious agents documented (unpublished data), suggesting a lower risk of disease transmission
# Physical Characterization

*Fitzpatrick GM, Cliff R, Tandon N. Thrombosomes: a platelet-derived agent for control of noncompressible hemorrhage. Transfusion 2013; S3*

<table>
<thead>
<tr>
<th>Surface Markers</th>
<th>Stored Platelets</th>
<th>Thrombosomes®</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPIb</td>
<td>√</td>
<td>Reduced Expression</td>
</tr>
<tr>
<td>GPIIbIIIa</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Annexin V Binding</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Total Phospholipid</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Function</th>
<th>Stored Platelets</th>
<th>Thrombosomes®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion</td>
<td>√</td>
<td>Collagen</td>
</tr>
<tr>
<td>Aggregation</td>
<td>Ristocetin, Arachidonic Acid (AA), Collagen, ADP, Thrombin</td>
<td>Thrombin - Normal AA, Collagen - Reduced</td>
</tr>
<tr>
<td>Thrombin Generation</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Thromboelastograph (TEG)</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Microparticle Content</td>
<td>5 – 10%</td>
<td>&lt; 2%</td>
</tr>
</tbody>
</table>
Preclinical Studies

- Normal Healthy – NZWR, Canine, Human, Mouse
- Immunogenicity – IV and Intradermal Sensitization of NZWR
- Pathologic Conditions – Pre-existing DVT (NZWR), Pre-existing AT (Canine), Hemorrhage (NHP), Acute Radiation Sickness (NZWR, NOD-SCID Mouse)

- Ear Bleed in Thrombocytopenic (Drug Induced) NZWR
  - >80% reduction in bleeding, spontaneous clot
- LD 50/30 Radiation Sickness in NZWR
  - 2-day increase in time to death (10 days to 12), significant reduction in organ microhemorrhage scores
- 4-Gy Total Body Irradiation NOD-SCID Mouse
  - 52% improvement in bleeding in treated mice
- Hemorrhagic Shock Induced by Partial Hepatectomy in NHP
  - Trend of about 56% less bleeding in treated animals vs, saline, non-inferiority to RT PLTs
- CABG in Canine Model
  - Significant reduction in blood loss at medium and high doses

- NZWR Radiation Study (LD50/30) and NOD-SCID Mouse Radiation Study (4-Gy)
  - provided safety and efficacy data in clinically relevant model of thrombocytopenia
- NHP Liver Injury and Canine CABG Study
  - Provided safety and efficacy data in clinically relevant surgical model
- Clinical Thrombocytopenic Canine Study
  - Demonstrated efficacy in thrombocytopenic bleeding dogs
NHP Hemorrhage Model, Liver Injury

Uncontrolled Hemorrhage

- **Time 0** Liver Laceration

- **15 min START** Infusion of Thrombosomes® or saline (control)

- **30 min STOP** Infusion of Thrombosomes® or saline (control)

- **120 min** Open Laparotomy, blood and clot collection from the peritoneum

- **480 min** Euthanize

Resuscitation with fluids, blood products or other interventions

- **6 hour simulated hospitalization phase**

Conduct necropsy, collect samples

- **2 hour pre-hospital phase**
NHP Hemorrhage Model, Liver Injury
Blood Loss

Thrombosomes® reduced the Blood Loss Index (BLI)

56.9% less than mean - 59.1% less than median
Canine CABG

Objective:

• Evaluate the safety of canine lyophilized platelets in comparison to liquid stored platelets in a model of on-pump cardiopulmonary bypass in the canine.

• Secondary endpoints include evaluation of blood loss and coagulation parameters as an indication of the potential efficacy of the test article.

Test Groups:

• Five groups, 8 animals per group
  ▪ Buffer
  ▪ Liquid Stored Canine Platelets
  ▪ High, Medium and Low Dose Lyophilized Canine Platelets
Canine CABG Study
Total Blood Loss (gms/kg 4 Hours Post Infusion)

Blood Loss Post Treatment (g)

- Vehicle
- LSP
- 3.30%
- 10%
- 33%

10/23/2018
Canine CABG
Blood Flow Rate through the Anastomosis Site

No difference observed in flow rates between groups
Clinical Studies
Clinical Development as a Hemostatic Agent

- Early Phase 1 – Dose escalation (microdose) safety study of autologous Thrombosomes® in normal healthy subjects completed
- Phase 1 – Dose escalation safety study of pooled allogeneic Thrombosomes® in bleeding thrombocytopenic patients, 2018
- Potential Phase 2
  - Bleeding thrombocytopenic patients, 2019-20
  - Appropriate surgical indication, 2019-20
- Determine optimal pathway for general hemostatic agent approval
- Prepare Phase 3 (possible) adaptive trial design
Clinical Studies- exploratory IND Study

Early Phase 1 Clinical Trial Completed in 2016

• A small autologous, single unit, single and multiple dose, microdose escalation, safety study of Thrombosomes in normal healthy human subjects, completed in 2016.

• 15 subjects were randomized, (Thrombosomes, n=10 and Control, n=5) and treated with doses of about 1/1000th to 1/10th of the lowest observed dose with biologic activity.

• The primary endpoint of the study was to evaluate the safety and tolerability of Thrombosomes, compared to a placebo control, based on physical exam, global neurological assessment, EKG, antibody formation and clinical and laboratory signs and symptoms.

• The highest dose administered 1.55 X 10⁷ particles per kg was used to calculate the associated TGPU per µl blood volume.

• There were no test article-related safety concerns identified.
Other Innovative Agents

- **Platelet/Cell Modification**
  - Pegylation of stored platelets
    - Numerous investigators
  - Fibrinogen peptide coated RBC
  - Thromboerythrocytes

- **Hemostatic Drugs**
  - GPIbα conjugated to albumin microspheres
  - Liposome formulations
    - Reybak M, Biomater Artif Cells Immob Biotechnol. 1993
  - RVIIa
  - Tranexamic acid

- **Increased shelf life**
  - Platelet Additive Solutions (PAS)

- **Platelet Pharming**
  - Megakaryon
  - Platelet Biogenesis
THANK YOU

FUNDING PROVIDED THROUGH THE BIOMEDICAL ADVANCED RESEARCH AUTHORITY
DEPARTMENT OF HEALTH AND HUMAN SERVICES CONTRACT HHS0100201300021C
T-TAS

• T-TAS® (Total Thrombus-formation Analysis System) is an automated microchip flow chamber system developed for the quantitative assessment of the thrombus formation process under variable flow conditions.

• AR Chip – coated with collagen and tissue thromboplastin for quantitative evaluation of white thrombus formation mediated by the activation of both coagulation and platelets.

• GPRP is a synthetic peptide that inhibits fibrin polymerization and thus thrombin induced plasma coagulation by binding to the carboxy-terminal region of the γ-chain located in the D domain of fibrin [23], [24]. GPRP also inhibits ADP-evoked platelet aggregation by inhibiting fibrinogen binding to αIIbβ3 [25], [26].
# AR Chip: Blood Tests

## T-TAS Thrombosome Dose Response

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Actual Tsome Concentration (x10^3/µL)</th>
<th>Occlusion Time (hh:mm:ss)</th>
<th>Occlusion Speed (kPa/min)</th>
<th>Area Under Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrated Whole Blood</td>
<td>0</td>
<td>0:14:03</td>
<td>25.6</td>
<td>1393.9</td>
</tr>
<tr>
<td>Platelet Reduced Citrated Whole Blood</td>
<td>0</td>
<td>0:16:57</td>
<td>16.4</td>
<td>1180.6</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>0:13:47</td>
<td>26.9</td>
<td>1380.9</td>
</tr>
<tr>
<td></td>
<td>173</td>
<td>0:13:22</td>
<td>18.7</td>
<td>1498.5</td>
</tr>
<tr>
<td></td>
<td>255</td>
<td>0:10:40</td>
<td>33.9</td>
<td>1653.1</td>
</tr>
</tbody>
</table>

## AR Chip: GPRP Comparison

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Actual Tsome Concentration (x10^3/µL)</th>
<th>Base Pressure (kPa)</th>
<th>Occlusion Start Time (hh:mm:ss)</th>
<th>Occlusion Time (hh:mm:ss)</th>
<th>Occlusion Speed (kPa/min)</th>
<th>Area Under Curve</th>
<th>Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>GK PPP (No Tsomes)</td>
<td>0</td>
<td>2.7</td>
<td>0:25:34</td>
<td>0:00:00</td>
<td>0</td>
<td>138.8</td>
<td>Test Timed out</td>
</tr>
<tr>
<td>GK PPP + 1mM GPRP (No Tsomes)</td>
<td>0</td>
<td>3.5</td>
<td>0:00:00</td>
<td>0:00:00</td>
<td>0</td>
<td>52.43</td>
<td>Test Timed Out</td>
</tr>
<tr>
<td>GK PPP + 375k Tsomes</td>
<td>384</td>
<td>2.8</td>
<td>0:10:54</td>
<td>0:12:20</td>
<td>48.8</td>
<td>1479.8</td>
<td></td>
</tr>
<tr>
<td>GK PPP + 375k Tsomes with 1mM GPRP</td>
<td>380</td>
<td>3.2</td>
<td>0:10:09</td>
<td>0:14:32</td>
<td>16</td>
<td>1426.9</td>
<td></td>
</tr>
</tbody>
</table>
Cold Storage of Platelets:  
The future is now!

U.S. Army Institute of Surgical Research
COL Andrew P. Cap, MS, MD, PhD, FACP
Coagulation & Blood Research Department
Disclaimer:
The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Disclosures:
I have no relevant conflicts of interest.
I am an active duty officer in the U.S. Army.
Current Platelet Product Standard-of-Care: 
Primary use = *prophylaxis*

- **Room temperature** (RT, 22°C) with gentle agitation for up to 5-7 days (7 days if using point-of-release bacterial detection)

**Advantages**
- *Longer in vivo platelet circulation times* suggest benefit for prophylactic transfusion

**Disadvantages**
- **Increased risk of microbial contamination**
- **Platelet storage lesion** → Loss of functionality
- **Short shelf life** → Limitation in supply
- May be sub-optimal for bleeding patients (especially trauma) as reflected by poor *in vitro* function

Shelf life driven by bacterial risk…
Recovery and survival of labeled platelets in healthy volunteers drives current storage practices

Optimized for prophylactic transfusion?

Refrigerated stored platelets are cleared from circulation within 2 days


**BUT, for treatment of hemorrhage, platelet activity may be more important than recovery and survival.**
Platelets are the critical effectors of hemostasis: best metrics of function?

1. Adhesion $\rightarrow$ primary hemostatic plug:
   PLT + VWF on exposed collagen

2. Aggregation $\rightarrow$ recruitment of PLT, binding to and organization of fibrin into bundles

3. Clot retraction $\rightarrow$ mechanical hemostasis

4. Thrombin generation $\rightarrow$ PLT surface (and microparticle) phosphatidyl serine + FVa = catalytic surface (cell-based model of coagulation)

5. Secretion $\rightarrow$ PLT recruitment, PAI-1/A2AP (anti-fibrinolysis), sCD40L (immune activation), serotonin (vasoconstriction), etc.

6. Circulation time $\rightarrow$ recovery & survival

Prophylaxis vs. Bleeding?
Platelet Dose Study (PLADO): dose-dependent increase in Transfusion-related Adverse Events (TRAEs)

Any TRAE

Randomized treatment group (2 d.f. p=0.02) Reference group: Medium dose
Low dose (p=0.89) High dose (p=0.01)

Fever

Randomized treatment group (2 d.f. p=0.05) Reference group: Medium dose
Low dose (p=0.20) High dose (p=0.01)

Nov/Dec 2017 BPAC topic: platelet bacterial safety

Platelet Dose Study (PLADO): no dose-response effect on bleeding, transfusions

### Table 3. Primary and Key Secondary End Points, According to Treatment Group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low Dose (N=417)</th>
<th>P Value, Low vs. Medium Dose</th>
<th>Medium Dose (N=423)</th>
<th>P Value, Medium vs. High Dose</th>
<th>High Dose (N=432)</th>
<th>P Value, High vs. Low Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary end point</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1 Episode of bleeding of grade 2 or higher — % of patients</td>
<td>71</td>
<td>0.60</td>
<td>69</td>
<td>0.71</td>
<td>70</td>
<td>0.94</td>
</tr>
<tr>
<td>Red-cell transfusions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1 Transfusion — % of patients</td>
<td>95</td>
<td>0.12</td>
<td>92</td>
<td>1.00</td>
<td>92</td>
<td>0.09</td>
</tr>
<tr>
<td>Total units per patient†</td>
<td>4.62</td>
<td>0.70</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>2–8</td>
<td>2–8</td>
<td>2–8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet transfusions‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. per patient</td>
<td>&lt;0.001</td>
<td>0.09</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>3–9</td>
<td>2–6</td>
<td>2–6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. of platelets transfused, based on at-iss...</td>
<td>9.25</td>
<td>11.25</td>
<td>19.63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Double the PLT transfused… no change in bleeding or transfusions!

Similar bleeding risk from 10K-80K
Table 3. Time to grade 2 or higher bleeding

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>% censored</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized treatment group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD</td>
<td>247</td>
<td>51.0</td>
<td>1.00</td>
<td>0.77-1.30</td>
<td>.77</td>
</tr>
<tr>
<td>MD</td>
<td>251</td>
<td>54.2</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>280</td>
<td>47.5</td>
<td>1.08</td>
<td>0.84-1.39</td>
<td>.54</td>
</tr>
<tr>
<td>Platelet source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apheresis</td>
<td>552</td>
<td>54.5</td>
<td>Referent</td>
<td></td>
<td>.44</td>
</tr>
<tr>
<td>WBP</td>
<td>220</td>
<td>53.2</td>
<td>1.15</td>
<td>0.81-1.65</td>
<td>.44</td>
</tr>
<tr>
<td>ABO matching status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABO-identical</td>
<td>467</td>
<td>64.2</td>
<td>Referent</td>
<td></td>
<td>.33</td>
</tr>
<tr>
<td>Minor mismatch</td>
<td>75</td>
<td>76.0</td>
<td>0.85</td>
<td>0.52-1.40</td>
<td>.52</td>
</tr>
<tr>
<td>Major mismatch</td>
<td>198</td>
<td>75.8</td>
<td>0.78</td>
<td>0.56-1.09</td>
<td>.15</td>
</tr>
<tr>
<td>Storage duration, d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>48</td>
<td>83.3</td>
<td>0.86</td>
<td>0.39-1.87</td>
<td>.70</td>
</tr>
<tr>
<td>3</td>
<td>158</td>
<td>79.8</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>223</td>
<td>81.2</td>
<td>1.03</td>
<td>0.64-1.64</td>
<td>.91</td>
</tr>
<tr>
<td>5</td>
<td>221</td>
<td>77.8</td>
<td>1.13</td>
<td>0.72-1.79</td>
<td>.59</td>
</tr>
</tbody>
</table>

Adjusted frailty models of days from first platelet transfusion to ≥ grade 2 bleeding. Hazard ratios > 1.00 indicate higher risk of bleeding in patients receiving platelets with the specified characteristic compared with patients receiving platelets with the reference characteristic. Each model includes one platelet characteristic (randomized dose assignment, source, matching status, or storage duration) and is also adjusted for patient sex, patient age group, patient treatment category, and site (with site treated as a random effect). Data on time to bleeding were censored if any of the following occurred before the patient experienced grade 2 or higher bleeding: a transfusion that differed from the patient's first transfusion in the characteristic being analyzed or had missing data on that characteristic; a day with missing data on whether grade 2 or higher bleeding had occurred; or end of study.

N indicates number of evaluable patients; LD, low dose; MD, medium dose; HD, high dose; and WBP, whole blood platelet pools.

Effect of storage duration:

Low numbers of “fresh” platelets… PSL trend?

(Blood. 2012;119(23):5553-5562)
PROMMTT: aged, RT stored platelet associated w/ higher risk AEs, sepsis

Older platelet unit → higher risk of complications in trauma patients – especially sepsis

Also: 5-7 day shelf life causes shortages

Inaba J Trauma 2011
Presence of live platelets at RT increases bacterial growth in plasma over plasma alone by 4 logs!!! (clinical isolates, *Acinetobacter baumanii*)

Driven by lactate...

Current Platelet Product Standard-of-Care: Increased risk for DoD (and trauma patients)

- Room temperature (RT, 22°C) drives shelf life of 5-7 days → unable to maintain inventories
- Impossible to ship from US to deployed units
- Unavailable at forward locations
- Forces down-range collections → untested units
- Limited bacterial testing
- Limited donor pools
- Loss of hemostatic function (PSL) may increase bleeding risk
- No evidence that R&S matters in acute hemostasis

It is (and has been) impossible for DoD to provide standard-of-care platelets to combat casualties.
50% of US population lives >1hr from a trauma center (i.e., no platelets)

From: National Inventory of Hospital Trauma Centers

Level 1&2 trauma centers

Level 3, 4, 5 trauma centers (mostly rural)

No platelets → higher rural trauma mortality
Platelet dysfunction in trauma is associated with mortality

- 46% of patients on admission
- 91% during ICU stay


**Figure 2.** Platelet ADP (A), TRAP (B), AA (C), and collagen (D) responsiveness as area under the aggregation curve in units (U) over time. Platelet count measurements (E) are shown for comparison. Data points are mean values, with capped bars indicating 95% confidence intervals; dotted lines indicate the lower bound (fifth percentile) of normal values for each measurement.

**Figure 3.** Kaplan-Meier 30-day survival curves showing survival differences between patients with below-normal admission platelet responsiveness to ADP (A), TRAP (B), AA (C), and collagen (D). Survival curves for patient admission platelet counts below the 25th percentile (E) are shown for comparison. *p < 0.05 by log-rank test.
PROPPR as a study of giving early platelets – reduced early exsanguination

Transfusion of Plasma, Platelets, and Red Blood Cells in a 1:1:1 vs a 1:1:2 Ratio and Mortality in Patients With Severe Trauma
The PROPPR Randomized Clinical Trial

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CONCLUSIONS AND RELEVANCE Among patients with severe trauma and major bleeding, early administration of plasma, platelets, and red blood cells in a 1:1:1 ratio compared with a 1:1:2 ratio did not result in significant differences in mortality at 24 hours or at 30 days. However, more patients in the 1:1:1 group achieved hemostasis and fewer experienced death due to exsanguination by 24 hours. Even though there was an increased use of plasma and platelets transfused in the 1:1:1 group, no other safety differences were identified between the 2 groups.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01545232

Figure 2. Kaplan-Meier Failure Curves for Mortality at 24 Hours and 30 Days

The colored areas indicate 95% confidence bands, which were calculated using the Hall-Wellner method. The Hall-Wellner bands extend to the last event (death) in each group. For 24-hour mortality, the Cox proportional hazards regression model, adjusted for site as a random effect, produced a hazard ratio (HR) of 0.72 (95% CI, 0.49-1.07). There were no patients lost to follow-up during the first 24 hours from randomization. For 30-day mortality, the Cox proportional hazards regression model, adjusted for site as a random effect, produced an HR of 0.83 (95% CI, 0.61-1.12). Between 24 hours and 30 days, 4 patients were lost to follow-up and were censored when they withdrew consent or were last known to be alive (3 in the 1:1:1 group and 1 in the 1:1:2 group).
Code of Federal Regulation Title 21 Sec. 640.20 Platelets. (b) Source. The source material for Platelets is plasma which may be obtained by whole blood collection or by plateletpheresis.

Sec. 640.24 Processing. c)...a count of not less than $5.5 \times 10^{10}$ platelets per unit in at least 75 percent of the units tested.

(d) The volume of original plasma used for resuspension of the platelets shall be determined by the maintenance of a pH of not less than 6.2 during the storage period... One of the following storage temperatures shall be used continuously:

(1) 20 to 24 °C. (2) 1 to 6 °C.

Code of Federal Regulation Title 21 Sec. 640.25 General requirements

a) Storage. Immediately after resuspension, Platelets shall be placed in storage at the selected temperature range. If stored at 20 to 24 deg. C, a continuous gentle agitation of the platelet concentrate shall be maintained throughout the storage period.

Agitation is optional if stored at a temperature between 1 and 6 deg. C.
Hemostatic function is more important than platelet survival for bleeding patients

Shorter bleeding time *in vivo* (aspirin & thrombocytopenia)

Cold platelets work and are legal.

84% response rate to bleeding for cold-stored but only 39% RT stored

21 CFR 606.65(e) & 610.53(c)
To store apheresis platelets at refrigerator temperature (1-6°C) without agitation for up to 3 days. The cold stored platelets will only be used in the resuscitation of actively bleeding patients. The new storage conditions will be reflected in Circular of Information.

DoD needs:
• 1-6C storage
• apheresis or WB-derived platelets
• without agitation
• In PAS or plasma
→ For up to 21 days

- Already done, thanks!
- Supported by data
- Supported by data and needed for practical benefit
21-day Cold Stored Platelets (CSP): Not as radical as it sounds...

- Bacterial safety clearly better than RT
- Would allow deployment of fully tested product (true standard of care for TTDs)
- CSP contained in licensed product stored to 35 days (in CPDA WB)
- CSP contained in licensed product stored to 40 days (liquid plasma)
- Efficacy is PRESUMED for RT-PLT and no proof of efficacy required for extension of shelf life from 3 → 5 → 7 days (only bacterial safety)

At worst, assuming minimal CSP efficacy, you are transfusing liquid plasma +/- PAS... and in any case, downrange and in most of US, you don’t have platelets anyway!
How about a platelet product that really works?

**Which platelets would you want if you were bleeding?**

- 5d room temp stored clots are weak
- 4C storage maintains clot strength

* Compared to Fresh; n=4, p < 0.05

Nair BJH 2017 in press
Aggregation response well preserved in PAS at 4°C

Collagen 5 µg/mL

TRAP 20 µM

EPI 2 µM

Getz Transfusion 2016
Why would you use RT platelets in bleeding patients?

**Collagen 2.5 μg/mL + EPI 2 μM**

- Baseline
- 22°C PAS
- 4°C PAS

**ADP 5 μM + EPI 2 μM**

- Baseline
- 22°C PAS
- 4°C PAS

Getz Transfusion 2016
Mitochondrial respiration better conserved in 4C storage (Also, mitochondrial gene expression…)

A

RT mitochondrial failure

B

Day of Storage
Apoptosis in RT storage
PAS is better than plasma

RT caspase activation

RT loss of membrane integrity
Cold stored platelets sometimes form aggregates in plasma?

<table>
<thead>
<tr>
<th></th>
<th>Cold Stored</th>
<th>Room Temp Stored</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>4°C</td>
<td>22°C</td>
</tr>
<tr>
<td><strong>Day 3</strong></td>
<td>4°C</td>
<td>22°C</td>
</tr>
<tr>
<td><strong>Day 5</strong></td>
<td>4°C</td>
<td>22°C</td>
</tr>
</tbody>
</table>

**PLATELET COUNT per μL**

<table>
<thead>
<tr>
<th></th>
<th>4°C Baseline</th>
<th>4°C Day 3</th>
<th>4°C Day 5</th>
<th>22°C Baseline</th>
<th>22°C Day 3</th>
<th>22°C Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cold Stored</strong></td>
<td>1145 X 10^3</td>
<td>827 X 10^3</td>
<td>854 X 10^3</td>
<td>1150 X 10^3</td>
<td>1155 X 10^3</td>
<td>1155 X 10^3</td>
</tr>
<tr>
<td>+/- 36 SEM</td>
<td>+/- 118 SEM</td>
<td>+/- 30 SEM</td>
<td></td>
<td>+/- 26 SEM</td>
<td>+/- 28 SEM</td>
<td>+/- 28 SEM</td>
</tr>
</tbody>
</table>

Getz et al. Transfusion, 2016
Plasma vs. PAS cold storage: Reduced aggregates in PAS – who cares?

Cold Stored in 100% Plasma

4°C Day 5

Visible Aggregates

Cold Stored in 65% PAS

4°C Day 15

No Visible Aggregates

Getz et al. Transfusion, 2016
PAS looks better visually, plasma R&S is better plus has fibrinogen – it’s a wash!
Cold Platelets: aggregation & count after platelet transfusion in cardiac surgery

- Cold platelet transfusion: better aggregation recovery
- ROTEM MCF similar in both cold and RT.

*Results reported as mean ± SEM. Results include first transfusion episode. Storage to 7 days.
Blood product utilization

*Results reported as mean ± SEM. Observation time: from start of surgery until 7 o’clock day 1 after surgery.
Blood loss lower in Cold Platelet arm (correlates with better aggregation)

*Results reported as mean ± SEM. Observation time: from chest closure until 7 o’clock day 1 after surgery.

No difference in:
- Mortality
- Thromboembolism
- ICU days

<table>
<thead>
<tr>
<th>Chest drain output (ml)</th>
<th>4 °C (N=20)</th>
<th>22 °C (N=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>576 ± 53</td>
<td>838 ± 112</td>
</tr>
</tbody>
</table>

*Results reported as mean ± SEM. Independent Samples T-Test. SPSS version 24.0. p<0.05 considered significant.
How about 14 day CSP?!  

Strandenes and Hervig. AABB 2018.
How about 14 day CSP?! Improved aggregation response!

Strandenes and Hervig. AABB 2018.

Going in the right direction! Even after 14 days!
Whole Blood Hemostatic Function: 4C>RT, +/- Mirasol PRT → LTOWB as universal (RDCR)

Fig. 4. Multiple electrode PLT aggregometry. A repeated measures analysis demon-

Fig. 5. TEG. Storage at 4°C preserved TEG R time, K, α-angle, MA, clot strength, and
What about CSP + Intercept?

Preliminary data looks promising...

ADP

Collagen

TRAP

Epinephrine (EPI)

ADP+EPI

Chronolog LTA
Cold stored platelets vs. Frozen platelets?

CPP: minimal aggregation but lots of thrombin generation due to PS-pos microparticles.
USE PLATELETS THAT WORK -- NOW!
STOP BLEEDING COLD!

Code of Federal Regulation Title 21 Sec. 640.20 Platelets. (b) Source. The source material for Platelets is plasma which may be obtained by whole blood collection or by plateletpheresis.

(d) The volume of original plasma used for resuspension of the platelets shall be determined by the maintenance of a pH of not less than 6.2 during the storage period. One of the following storage temperatures shall be used continuously:

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2. 1 to 6 °C.

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Agitation is optional if stored at a temperature between 1 and 6 deg. C.

Cold platelets legal in US; used by: DoD, Mayo Clinic.
Cold-stored WB used by: DoD, Norwegian Military, Mayo Clinic, U Pitt, UT-Houston, Cooper U Hospital/NJ, others…
Questions?

LTOWB

Cold Platelets
Dried Plasmas for Rapid Reconstitution and Deployment in Underserved Areas

Jose A. Cancelas, MD, PhD
AABB 2018
Educational Session
Boston, Oct 14th 2018
Relevant Disclosures/Disclaimers

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Fresenius-Kabi Inc.
Cellphire Inc.
Cytosorbents Inc.
Cincinnati Children’s Hospital Medical Center
Hoxworth Blood Center
University of Cincinnati Academic Health Center (CTSA)

Scientific Advisory Board
Hemanext Inc.
Objectives

1. Why we need dried plasma products
2. Description of current FDP products
3. Safety of FDP in plastic bags
Early plasma infusion correlates with better survival in distant pre-hospital settings

- Increasing the ratio of plasma to RBCs from 1:3 to 1:1 demonstrated a 40% decrease in mortality (Borgman, et al., J Trauma 2007;63: 805-813).

- Positive correlation between FFP:PRBC ratio and survival (Teixeira P.G. et al., J. Trauma 2009).

- Ratio of 1 Plasma:1 Platelet:1 RBC equally safe and achieved hemostasis by 24 hours vs. 1:1:2 product ratio (PROPPR study, Holcomb JB, JAMA 2015).

- Prehospital administration of plasma results in lower 30-day mortality and lower median PT-time ratio than standard-care resuscitation (Sperry JL, et al., NEJM, 2018).
Dried plasma preparations have most often been prepared by lyophilization, a process in which plasma is frozen and dehydrated by sublimation under vacuum for several days.

Dried plasmas are expected to have reduced cold chain requirements, rapid reconstitution, and other advantages over frozen plasma.

During WWII, US and Britain used freeze dried plasma. Sweden manufactured SDP.
Spray Dried Plasmas

Atomization of liquid plasma to droplets and brief exposure to hot (up to 150°C) gas in the drying chamber, followed by rapid evaporative cooling.

Removes water with minimal alteration of plasma protein levels. This method can dry a unit (250 mL) of plasma in approximately 25 minutes.

This process may result in some loss of factor activity.

Pathogen reduced SDP

Spray dried plasma (n = 20) was rehydrated and compared to paired FFP control plasmas (n = 20). Data are shown as mean ± standard deviation. All values for SpDP were within 20% of paired controls except as noted.

* >20% change
# Available dried plasma products for transfusion

<table>
<thead>
<tr>
<th></th>
<th>FLYP</th>
<th>LyoPlas N-w</th>
<th>Bioplasma FDP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Use</strong></td>
<td>1994-present—French Military 2011-present—Civilian (austere)</td>
<td>General population—Germany</td>
<td>General population—South Africa &amp; neighboring countries</td>
</tr>
<tr>
<td></td>
<td>- Pooled apheresis FFP &lt;11 donors</td>
<td>- Hemovigilance Program Frozen &gt;/= 4 mos for donor retest Leukoreduced No HLA Ab+ women</td>
<td>- Comprehensive testing Hemovigilance program S/D treatment for PR</td>
</tr>
<tr>
<td></td>
<td>- All volunteer donors</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Donor screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Testing—disease &amp; factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Hemovigilance program</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 2003—Leukoreduced</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 2010—No HLA Ab+ women</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 2010—Amotosalen PR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Characteristics</strong></td>
<td>- Normal factor levels</td>
<td>- Normal factor levels ABO type specific</td>
<td>- Factor levels: &gt;/= 0.40 IU ABO-universal plasma Store below 25°C</td>
</tr>
<tr>
<td></td>
<td>- ABO-universal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shelf-life</strong></td>
<td>2 years at room temperature</td>
<td>15 months at 2°C-25°C</td>
<td>&lt;10 minutes Where plasma and/or coagulation factors are required</td>
</tr>
<tr>
<td><strong>Reconstitution</strong></td>
<td>&lt;6 minutes</td>
<td>A few minutes</td>
<td></td>
</tr>
<tr>
<td><strong>Indication</strong></td>
<td>As sole source of plasma where used</td>
<td>Same as frozen plasma</td>
<td></td>
</tr>
<tr>
<td><strong>Safety</strong></td>
<td>No adverse events reported (including TRALI) since 1994 start of hemovigilance program</td>
<td></td>
<td>Contraindicated: Severe Protein S deficiency</td>
</tr>
<tr>
<td><strong>Efficacy</strong></td>
<td>Clinical use reports support efficacy as part of a 1:1 DCR approach⁷</td>
<td>No restrictions related to clinical efficacy have been identified</td>
<td>No restrictions related to clinical efficacy have been identified</td>
</tr>
</tbody>
</table>
FLyP: Freeze-dried Plasma in the Initial Management of Coagulopathy in Trauma Patients (TRAUCC) study

1. Open-label, phase 3, randomized trial
2. Adult trauma patients requiring an emergency pack of 4 plasma units within 6 h of injury.
3. Randomly assigned patients to receive 4-FLyP units or 4-FFP units.
4. 48 patients were randomized (FLyP, n = 24; FFP, n = 24).
5. FLyP reduced the time from randomization to transfusion of first plasma unit compared with FFP (14 vs. 77 min).
6. FLyP achieved a higher fibrinogen concentration 45 min after randomization compared with FFP (baseline-adjusted mean difference, 0.29 g/L) and a greater improvement in prothrombin time ratio, factor V and factor II.
7. The between-group differences in coagulation parameters remained significant at 6 h. FLyP reduced fibrinogen concentrate requirements.
8. 30-day in-hospital mortality rate was 22% with FLyP and 29% with FFP.

Garrigue D et al., J. Thromb. Hemost., 2018
**US FDP: HemCon LyP characterization**

<table>
<thead>
<tr>
<th>Test</th>
<th>Ref Range</th>
<th>FFP (n = 144)</th>
<th>LyP (n = 144)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>14.0-21.0 seconds</td>
<td>17.4 ± 0.8</td>
<td>20.0 ± 1.0</td>
<td>14.9*</td>
</tr>
<tr>
<td>PT</td>
<td>11.0-14.5 seconds</td>
<td>13.8 ± 1.0</td>
<td>15.2 ± 0.8</td>
<td>10.1*</td>
</tr>
<tr>
<td>INR</td>
<td>0.9-1.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>9.1*</td>
</tr>
<tr>
<td>aPTT</td>
<td>28.0-40.0 seconds</td>
<td>32.4 ± 3.0</td>
<td>34.6 ± 3.3</td>
<td>6.8*</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>200-400 mg/dL</td>
<td>313 ± 54</td>
<td>302 ± 65</td>
<td>-3.5*</td>
</tr>
<tr>
<td>vWF activity</td>
<td>50%-150%</td>
<td>99 ± 48</td>
<td>88 ± 37</td>
<td>-11.1*</td>
</tr>
<tr>
<td>Factor II</td>
<td>70%-130%</td>
<td>88 ± 12</td>
<td>83 ± 12</td>
<td>-5.7*</td>
</tr>
<tr>
<td>Factor V</td>
<td>70%-130%</td>
<td>94 ± 19</td>
<td>86 ± 15</td>
<td>-8.5*</td>
</tr>
<tr>
<td>Factor VII</td>
<td>70%-130%</td>
<td>96 ± 20</td>
<td>90 ± 16</td>
<td>-6.3*</td>
</tr>
<tr>
<td>Factor VIIa</td>
<td>0.5-8.4 ng/mL</td>
<td>2.7 ± 1.5</td>
<td>2.6 ± 1.1</td>
<td>-3.7</td>
</tr>
<tr>
<td>Factor III</td>
<td>50%-150%</td>
<td>102 ± 25</td>
<td>92 ± 23</td>
<td>-9.8*</td>
</tr>
<tr>
<td>Factor IX</td>
<td>50%-150%</td>
<td>93 ± 15</td>
<td>86 ± 18</td>
<td>-7.5*</td>
</tr>
<tr>
<td>Factor X</td>
<td>70%-130%</td>
<td>95 ± 15</td>
<td>95 ± 16</td>
<td>0.0</td>
</tr>
<tr>
<td>Factor XI</td>
<td>70%-130%</td>
<td>90 ± 18</td>
<td>88 ± 18</td>
<td>-2.2*</td>
</tr>
<tr>
<td>Factor XII</td>
<td>70%-130%</td>
<td>100 ± 22</td>
<td>99 ± 24</td>
<td>-1.0</td>
</tr>
<tr>
<td>vWF antigen</td>
<td>50%-150%</td>
<td>113 ± 44</td>
<td>99 ± 33</td>
<td>-12.4*</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>&gt;75%</td>
<td>93 ± 11</td>
<td>93 ± 12</td>
<td>0.0</td>
</tr>
<tr>
<td>Protein C</td>
<td>50%-150%</td>
<td>106 ± 24</td>
<td>94 ± 18</td>
<td>-11.3*</td>
</tr>
<tr>
<td>Protein S</td>
<td>70%-140%</td>
<td>94 ± 20</td>
<td>89 ± 17</td>
<td>-5.3*</td>
</tr>
<tr>
<td>Alpha-2 Antiplasmin</td>
<td>80%-120%</td>
<td>103 ± 8</td>
<td>108 ± 9</td>
<td>4.9*</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>80%-120%</td>
<td>97 ± 21</td>
<td>91 ± 12</td>
<td>-6.2*</td>
</tr>
<tr>
<td>Total Protein</td>
<td>6.0-8.5 g/dL</td>
<td>6.0 ± 0.3</td>
<td>5.9 ± 0.3</td>
<td>-1.4*</td>
</tr>
</tbody>
</table>

Comparisons of hemostatic parameters pre- versus post-lyophilization are shown, with values listed as mean ± standard deviation.

* Significant difference p < 0.05.
LyP Safety (Phase 1) Clinical Trial (2010-11)

Primary:
• To assess the safety and tolerability of increasing doses of infused autologous units of LyP in healthy volunteers and to define possible LyP adverse reactions.

Secondary:
• To demonstrate that coagulation function assays and specific coagulation factors are similar within clinically meaningful levels for post-infusion autologous LyP and FFP (Cohort 3 only).
Plasmapheresis Trima, Caridian BCT

~3-5 weeks
LyP manufacture
1 batch

$\text{COHORT 1}$

Pre-infusion
30 min post-infusion
4 h post-infusion
24 h post-infusion

Day 7
Day 14
Day 28

~30 min

Infusion: 7 mL/min

Plasmapheresis Trima, Caridian BCT

$\text{COHORT 2}$

Pre-infusion
30 min post-infusion
4 h post-infusion
24 h post-infusion

Day 7
Day 14
Day 28

~1 h

~3-5 weeks
LyP manufacture
2 batches
Subject Infusion Flowchart
(Cohorts 1 and 2)

Infuse 1st Subject in Cohort - Follow-Up through Visit #7 (7-Day Post Infusion)

YES SADR? NO

STOP & Evaluate Proceed to Infuse 2nd Subject in Cohort Follow-Up through Visit #7 (7-Day Post Infusion)

YES SADR? NO

STOP & Evaluate Proceed to Infuse Remaining Subjects in Cohort Follow-Up through Visit #7 (7-Day Post Infusion)

YES SADR? NO

STOP & Evaluate Medical Monitor Evaluation including Cohort Lab Results, Proceed to Next Higher Dose Cohort
Timeline for Cohort 3

COHORT 3

- Plasmapheresis 1
- Plasmapheresis 2
- Plasmapheresis 3
- Plasmapheresis 4
- Plasmapheresis 5

~3 weeks LyP manufacture 4 batches

- 5 weeks
- ~3 h FFP or LyP
- >1 week
- ~8 h

SAFETY ALGORITHM DESIGNED FOR CLINICAL SIGNS OF:

- THROMBOSIS
- HYPERVOLEMIA
- CITRATE TOXICITY

Infusion: 7 mL/min
FDP Processing

Transportation to processing facility. Labeling process for traceability.

Thawed FFP is aseptically transferred to processing system

Plasma is processed for ~3-14 days

FFP (meeting 21CFR640.34 (b))
Time to reconstitution

- Cohort 1
- Cohort 2
- Cohort 3

Donor id #

Time to reconstitution (s)

1st infusions
Safety: 1 liter FFP vs. LyP

![Graph showing systolic and diastolic pressure changes over time for LyP and FFP](image)

- **Pre-infusion** pressures:
  - Systolic Pressure (mmHg): Range 115 to 140
  - Diastolic Pressure (mmHg): Range 70 to 86

- **Post-infusion** pressures:
  - Systolic Pressure (mmHg): Range 115 to 140
  - Diastolic Pressure (mmHg): Range 70 to 86

Key:
- **LyP**
- **FFP**
Safety: 1 liter FFP vs. LyP

Cohort 3 - LyP
Cohort 3 - FFP
# LyP Phase 1: Summary of AEs

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Pre-infusion</th>
<th>Post LyP Infusion</th>
<th>Post FFP Infusion</th>
<th>Total AE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>3</td>
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<tr>
<td>2</td>
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<tr>
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<td>3</td>
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<td>15</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
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<td>22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Adverse Event</th>
<th>Treatment/Timing</th>
<th>Therapy for AE</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>1001</td>
<td>Sinusitis</td>
<td>LyP/Post</td>
<td>None</td>
<td>Unlikely</td>
</tr>
<tr>
<td>1002</td>
<td>Exercise Induced Subclinical Rhabdomyolysis</td>
<td>LyP/Post</td>
<td>Ibuprofen, large amount of fluids, po</td>
<td>Not related</td>
</tr>
<tr>
<td>1003</td>
<td>Wasp Sting</td>
<td>LyP/Post</td>
<td>None</td>
<td>Not related</td>
</tr>
<tr>
<td>1005</td>
<td>Dislocated Shoulder</td>
<td>None/Pre</td>
<td>None</td>
<td>Not related</td>
</tr>
<tr>
<td>1008</td>
<td>Colle’s Fracture</td>
<td>None/Pre</td>
<td>Cast of wrist, acetaminophen, ibuprofen</td>
<td>Not related</td>
</tr>
<tr>
<td>1009</td>
<td>Headache</td>
<td>LyP/Post</td>
<td>Need Rx</td>
<td>Unlikely</td>
</tr>
<tr>
<td>1010</td>
<td>Sinus Congestion</td>
<td>LyP/Post</td>
<td>None</td>
<td>Not related</td>
</tr>
<tr>
<td>1010</td>
<td>Pink eye</td>
<td>None/Pre</td>
<td>Need Rx</td>
<td>Not related</td>
</tr>
<tr>
<td>1010</td>
<td>Infiltration</td>
<td>None/Pre</td>
<td>None</td>
<td>Not related</td>
</tr>
<tr>
<td>1012</td>
<td>Headache</td>
<td>/Post</td>
<td>Need Rx</td>
<td>Not related</td>
</tr>
<tr>
<td>1012</td>
<td>Abnormal TAT</td>
<td>/Post</td>
<td>None</td>
<td>Definitely related</td>
</tr>
<tr>
<td>1013</td>
<td>Back/Shoulder Pain</td>
<td>LyP/Post</td>
<td>Need Rx</td>
<td>Not related</td>
</tr>
<tr>
<td>1016</td>
<td>Sore throat/nasal congestion</td>
<td>/Post</td>
<td>Need Rx</td>
<td>Not related</td>
</tr>
<tr>
<td>1016</td>
<td>Headache</td>
<td>LyP/Post</td>
<td>Need Rx</td>
<td>Not related</td>
</tr>
<tr>
<td>1016</td>
<td>Headache</td>
<td>LyP/Post</td>
<td>Need Rx</td>
<td>Not related</td>
</tr>
<tr>
<td>1016</td>
<td>Headache</td>
<td>LyP/Post</td>
<td>None</td>
<td>Not related</td>
</tr>
<tr>
<td>1016</td>
<td>Muscle aches</td>
<td>/Post</td>
<td>Need Rx</td>
<td>Not related</td>
</tr>
<tr>
<td>1016</td>
<td>Fatigue</td>
<td>/Post</td>
<td>Need Rx</td>
<td>Not related</td>
</tr>
<tr>
<td>1017</td>
<td>Decreased</td>
<td>/Post</td>
<td>Fe Therapy</td>
<td>Not related</td>
</tr>
<tr>
<td>1018</td>
<td>Headache</td>
<td>LyP/Post</td>
<td>Ibuprofen</td>
<td>Not related</td>
</tr>
<tr>
<td>1018</td>
<td>Headache</td>
<td>/Post</td>
<td>Ibuprofen</td>
<td>Not related</td>
</tr>
</tbody>
</table>
RePlas FDP
Safety Analysis

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Primary Objective

- Assess the safety of single infusions with autologous FDP product at increasing fixed doses in healthy subjects.

- Eligible to donate per FDA/AABB criteria (less travel-related deferrals), Duke Activity Status Index ≥ 35, and D-Dimer < 0.5 FEU/m, provide informed consent.

- Subject, medical/nurse staff blinded. Transfusion service unblinded.

Cohort 1
- 270 mL FDP

Cohort 2
- 540 mL FDP

Cohort 3 (Double blinded*)
- 810 mL FDP
- Randomized
- 810 mL FFP
- 810 mL FDP
- 810 mL FFP

IND# 17154; NCT02930226; IRB of University of Cincinnati and DoD HRPO
Endpoints for Assessing Safety

• **TEAEs** - AEs that emerged during or following infusion having been absent pre-infusion.

• **Serious Adverse Events (SAEs)**;

• **Suspected, unexpected, serious adverse reactions (SUSARs)**

• **Deaths**

*An independent Data & Safety Monitoring Board reviewed all AE, physical examinations and laboratory values after each cohort.*
Cohorts 1 and 2: Subject Disposition

29 subjects screened

20 subjects enrolled/treatment assigned

FDP-CPD (270 mL) N=4
FDP-ACD (270 mL) N=4
FDP-CPD (540 mL) N=4
FDP-ACD (540 mL) N=4

Cohort 1
Arm 1: ALL completed
Arm 2: ALL completed

Cohort 2
Arm 3: ALL completed
Arm 4: ALL completed

9 screen failures

20 subjects screened

4 discontinued subjects

Physician Decision (1)
Blood Collection problems (3)
Cohort 3: Subject Disposition

11 subjects Screened
- 1 screen failure
- 10 subjects Enrolled
  - FDP-ACD (810mL) N=4
  - FFP-ACD (810mL) N=4

2 discontinued subjects
- Withdraw by subject (1)
- Blood Collection problems (1)

1 discontinued subject
- Bag breakage at infusion time

COHORT 3 ARM 5
- 3 SUBJECTS COMPLETED

COHORT 3 ARM 6
- ALL COMPLETED
Time to reconstitution of RePlas FDP

69 ± 16 s (min: 43, max: 106)
No major effect on BP after 810 mL FDP infusion

Systolic BP (mm Hg)

Diastolic BP (mm Hg)
No major effect on RR after 810 mL FDP infusion
### Summary of AEs

<table>
<thead>
<tr>
<th>Cohort</th>
<th># Subjects w/ AE</th>
<th>Pre-infusion</th>
<th>Post FDP Infusion</th>
<th>Total AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 of 8</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>2 of 8</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>7 of 8</td>
<td>13</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>TOTALS</td>
<td>13 of 24</td>
<td>17</td>
<td>17</td>
<td>34</td>
</tr>
</tbody>
</table>

- No SAEs experienced by any subjects
# Summary of TEAEs

- Require explanation of relationship

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Arm</th>
<th>Subject ID</th>
<th>TEAE</th>
<th>Treatment/Timing</th>
<th>Therapy</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>101107</td>
<td>DAT+ (weak)</td>
<td>FDP (day +29) Unconfirmed</td>
<td>None</td>
<td>Unlikely</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>101110</td>
<td>Transient postprandial Hyperglycemia/Glycosuria</td>
<td>FDP (+4 hours, 30’ post-lunch)</td>
<td>None</td>
<td>Unlikely</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>101111</td>
<td>Transient (1 time point) modest AST/ALT elevation</td>
<td>FDP (day +7)</td>
<td>None</td>
<td>Unlikely</td>
</tr>
<tr>
<td>3</td>
<td>FFP-FDP</td>
<td>101135</td>
<td>Increased both TAT &amp; PF1.2</td>
<td>FFP +30’ – 4 hours</td>
<td>None</td>
<td>Possible FFP?</td>
</tr>
<tr>
<td>3</td>
<td>FFP-FDP</td>
<td>101142</td>
<td>Transient (1 time point) modest AST/ALT elevation</td>
<td>FDP (day +7)</td>
<td>None</td>
<td>Unlikely</td>
</tr>
</tbody>
</table>

* Referred to GP for glucose intolerance
Thrombin activation is not increased in subjects infused with 810 mL FDP.
RePlas FDP: Summary of other laboratory results

• Consistent with absence of clinical evidence of thrombosis, no D-dimer level elevation was observed associated with the infusion of either plasma preparation.

• No significant changes in pro-coagulant and anti-coagulant levels in recipient’s plasma.

• Direct antiglobulin tests (DATs) were always negative before and after infusions in cohort 3.

• Similar, mild Hct reduction after FFP and FDP infusions, probably due to hemodilution.

• No significant changes in other hematological or blood/urine chemistry analysis compared with basal or between FFP and FDP large dose infusions.
RePlas FDP: Conclusions

• No SAEs related to product infusion and no occurrence of predetermined AEs including thromboembolic events, infections, evidence of unusual bleeding/bruising, or relevant changes in coagulation parameters after FDP infusion.

• No relevant clinical difference was observed between large FFP and FDP infusions.

• No signs of either local or systemic allergic reactions were observed after infusions.

• FDP is tolerated well in normal healthy volunteers with no SAEs or safety concerns.
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– Andrew Cap MD, PhD

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  Cellphire Inc.: Grant/Research Support
  USAMMDA/Westat: Grant/Research Support
  NIH: Grant/Research Support
  US DoD/CDMRP: Grant/Research Support
  State of Ohio: Grant/Research Support
– G. Fitzpatrick PhD
  Cellphire Inc.: Full-time/Part-time Employee

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Learning Objectives

• Identify next generation blood products, their indications, clinical safety and efficacy results, and their associated development timelines

• Distinguish advantages of next generation blood products from current traditional blood products, including improved time to initiation of transfusion and inventory management

• Assess the inclusion of next generation blood products into updated first responder protocols