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TITLE: Pathogen-Reduced, Extended Platelet Storage in Platelet Additive Solution (PAS)

PRINCIPAL INVESTIGATOR: Moritz Stolla, MD

CONTRACTING ORGANIZATION: Bloodworks Northwest

Seattle, WA 98104

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
This grant pertains to finding novel approaches for storage of platelets for transfusion. Our project proposes to determine the efficacy of using a pathogen inactivation technique (Mirasol) coupled with a platelet additive solution (PAS) to extend the life of stored platelets. Our project also aims to determine how long acceptable platelet viability can be maintained in platelets stored at 4°C.

15. SUBJECT TERMS
bleeding, extended storage, hemorrhage, hemostasis, InterSol, Mirasol, pathogen inactivation, pathogen reduction technology, platelet additive solution, platelet recovery and survival, platelet storage, platelet storage solution, platelets, thrombocytopenia, transfusion, whole blood

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Pathogen-Reduced, Platelet Additive Solution, Extended Stored Platelets (PREPS)

Grant Number 11105004

Annual Report

15-SEP-2017 to 14-SEP-2018

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Pathogen-Reduced, Platelet Additive Solution, Extended Stored Platelets (PREPS)
(Previously Pathogen-Reduced, Plasmalyte-Extended Stored Platelets)
Grant Number 11105004
Annual Report
15-SEP-2017 to 14-SEP-2018

INTRODUCTION: The purpose of this project is to find better ways to store platelets for patients that need platelet transfusions. A deeper mechanistic understanding of the effects of collection and storage on platelet function could greatly aid in improving the availability and efficacy of platelets both on the battlefield and in the civilian transfusion setting. In this research study, we are interested in evaluating the novel combinations of collection, storage and pathogen reduction approaches on the structural and functional properties of platelets and on platelet viability and function following transfusion.

KEY WORDS: 4°C storage, bleeding, cold storage, extended storage, hemorrhage, hemostasis, Isoplate, InterSol, pathogen inactivation, pathogen reduction, pathogen reduction technology, PRT, platelet additive solution, PAS, platelet recovery and survival, platelet storage, platelet storage solution, platelets, refrigerated storage, thrombocytopenia, transfusion, whole blood.

ACCOMPLISHMENTS: The following major goals are described in the July 2, 2014 revised statement of work, Novel Approaches to Storage of Platelets for Transfusion.

1. Evaluation of structural and functional changes to platelets during enhanced collection, storage and pathogen reduction (enhanced platelets).
2. Evaluation of enhanced platelets in animal models of trauma and hemorrhage.
3. Evaluate enhanced autologous platelets in normal subjects.
4. Evaluation of enhanced platelet recovery and survival, bleeding time and hemostatic activity in thrombocytopenic patients with and without acute hemorrhage.

In the current reporting period we evaluated in vivo platelet recovery and survival of apheresis platelets collected in plasma stored for 5, 10, 15 and 20 days at 4°C in comparison to fresh platelets. We also evaluated 5 day InterSol (PAS) 4°C stored platelets in comparison to fresh platelets. Additionally, we compared in vitro metabolic and functional platelet assays on the day of collection to those at the end of storage.

For this reporting period, we ran four different groups: Day 5, 10, 15 and 20 compared these with fresh platelets. In this study, we target 5 collections with each storage solution and storage time. Some of these collections were completed in the last reporting period. In presenting this data, we are reporting the cummulative completed data sets (n=5 or n=6). We determined that 5 day stored platelets had an average recovery of 34% (n=5), 10 day of 22% (n=6), 15 day of 13% (n=5) and 20 day of 9% (n=5) (46%, 37%, 22%, 13% of their fresh comparator respectively). Platelet survivals averaged 1.6 days for 5 day, 0.9 days for 10 day, 0.7 days for 15 day and 0.7 days for 20 day (20%, 10%, 9% and 9% of their respective fresh comparator).

Over storage time, platelet recoveries (% of fresh) declined significantly from 5 to 10 days (p=0.003), from 10 and 15 days (p=0.009), and 15 to 20 days (p=0.019). Platelet survivals decreased significantly from 5 days to 10 days (p=0.001), but not thereafter (all as % of fresh).

In vitro measurements of integrin activation revealed a marked preactivation over storage comparable to collagen-stimulated fresh platelets. Of note, stored platelets retained their ability to further activate integrins, although this ability was declining over storage. Mitochondrial membrane integrity decreased only non-significantly over storage time. Similarly, apoptosis measured by activation of the effector caspase 3,7 increased non-significantly over storage time. Microparticles increased significantly from 5 to 10 days (p=0.042), P-selectin increased significantly from 10 to 15 days (p=0.049), and phosphatidyl serine exposure (measured by Annexin V binding) increased significantly from 15 to 20 days (p=0.025). We found best correlations between in vivo recovery (% of fresh) and ΔHCO3, Δglucose, Δlactate, Δmicroparticles, Δp-selectin, Δannexin V (all correlation coefficients of at least +/- 0.72, and $p<0.01$).

These data suggest that there is a continuous decline in in vivo viability of plasma stored platelets in the cold. From the current data it is unclear if storing platelets in plasma over 15 days will further decrease recovery and survival. Most in vitro platelet activation parameters increase significantly over the course of storage and noted is continuous metabolic activity from fresh to 20 days of storage. Future studies will need to be performed to look at optimum storage conditions for platelets in the cold.

We also evaluated 5 day InterSol 4°C stored platelets in comparison with fresh platelets. Platelet recoveries averaged 26% (n=5) and 45% of their respective fresh comparator. Platelet survivals averaged 1.8 days and 27% of their respective fresh comparator. These data suggest that storing platelets in InterSol as compared to plasma show a decreased recovery (26% InterSol vs 34% plasma) and slightly increase in survival (1.8 days InterSol vs 1.6 days plasma). Further studies will need to be performed to confirm the trend of the data.

We have nothing to report related to training and professional development. Disseminating results to communities of interest has been performed through both 2018 AABB Abstract/Poster A and 2018 Military Health System Research Symposium Abstract.

IMPACT: A deeper understanding of the effects of cold storage on platelet function could greatly aid in improving the availability of platelets on the battlefield and in the civilian transfusion setting. We have nothing to report related to impact on other disciplines, impact on technology transfer or impact on society beyond science and technology.

CHANGES/PROBLEMS: In January 2018 the University of Washington's IRB approved our modification to allow us store platelets in platelet additive solutions instead of plasma alone, and to conduct in vitro metabolic and functional platelet assays on the day of or day after collection. We increased the total number of study subject we could enroll to 190 for a total of 90 evaluable data sets. Approval was

granted for the change of Principal Investigator from Sherrill Slichter, MD to Mortiz Stolla, MD and the change of location of study procedures to our new Bloodworks Northwest building.

In June 2018 another modification was approved by the IRB to again allow us to store platelets in 35%plasma/65% platelet additive solutions (PAS) (InterSol or Isoplate) instead of plasma alone. Additionally, approval was given for some minor modifications to the protocol to allow some flexibility regarding when the FRESH infusion can take place (7-14 days post 1st infusion) and some flexibility regarding how long the unit can sit at room temperature prior to being refrigerated (24 hours).

Recruiting for our study was on hold from late December 2017 to mid-March 2018 due to a change of PI and a change of location of our laboratory and offices and the resulting revalidation of equipment.

PRODUCTS:

- 2018 AABB Abstract/Poster: "Effects of extended cold-storage on platelet function, apoptosis and in vivo characteristics in healthy human subjects."
- 2018 Military Health System Research Symposium Abstract: "Extended Cold-Storage of Apheresis Platelets in Plasma."

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS: Terumo Corporation is a subcontractor on this grant and will be submitting a separate annual report.

Below is a list of individual who have worked more than one person hour on this grant over this reporting period.

Name:	*Moritz Stolla, MD*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1*
Contribution to Project:	*Design, oversight and conduct of research study.*
Name:	*Shawn Bailey, BS*
Project Role:	*Lab Technologist*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*2*
Contribution to Project:	*Radiolabeling, laboratory testing, unit processing, data capture, data entry.*
Name:	*Lydia Fang, BS, MS*
Project Role:	*Lab Technologist*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*2*
Contribution to Project:	*Laboratory testing, unit processing, data capture, data entry.*

Name:	*Lynda Fitzpatrick, RN*
Project Role:	*Study Coordinator/Research Nurse*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*3*
Contribution to Project:	*Subject enrollment. Apheresis collection. Radiolabeled infusion. Data capture and recording. Regulatory document maintenance. Process development. (Retired September 2018)*
Name:	*Jill S Corson, RN*
Project Role:	*Study Coordinator/Research Nurse*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*0.6*
Contribution to Project:	*Data capture and recording. Regulatory document maintenance.*
Name:	*Barbara Osborne, RN*
Project Role:	*Study Coordinator/Research Nurse*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*0.4*
Contribution to Project:	*Subject enrollment. Apheresis collection. Radiolabeled infusion. Data capture and recording.*

REFERENCES: None

APPENDICES:

- 2018 AABB Abstract/Poster: “Effects of extended cold-storage on platelet function, apoptosis and in vivo characteristics in healthy human subjects.”
- 2018 Military Health System Research Symposium Abstract: “Extended Cold-Storage of Apheresis Platelets in Plasma.”
- Statement of Work - Novel approaches to storage of platelets for transfusion
- Protocol - Cold Apheresis Platelets in Plasma (CAPP)

Effects of extended cold-storage on platelet function, apoptosis and in vivo characteristics in healthy human subjects

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Abstract

Background: Cold-stored platelets are currently under investigation for transfusion in actively bleeding patients. The three day storage limit of the FDA variance severely limits cold-stored platelet availability for clinical applications, and far forward military scenarios. The effects of cold-storage on platelet apoptosis have not been investigated thus far. We sought to test which in vitro parameters correlate best with in vivo recovery.

Study Design/Method: Twenty two healthy human subjects underwent apheresis platelet collections. The cold-stored platelet (CSP) "test" unit was stored for either 5, 10, 15 or 20 days in plasma at 4ºC. Platelet samples for in vitro platelet tests were extracted on the day of donation and after the designated storage period. After storage, all units were radiolabeled and transfused into their respective donors. All donors came back after a week to provide a fresh sample for radiolabeling and reinfusion.

Results/Findings: Over storage time, platelet recoveries (% of fresh) declined significantly from 5 to 10 days (p=0.003), from 10 and 15 days (p=0.009), and 15 to 20 days (p=0.019). Platelet survivals decreased significantly from 5 days to 10 days (p=0.001), but not thereafter (all as % of fresh). Measurements of integrin activation revealed a marked preactivation over storage comparable to collagen-stimulated fresh platelets. Of note, stored platelets retained their ability to further activate integrins, although this ability was declining over storage. Mitochondrial membrane integrity decreased only non-significantly over storage time. Similarly, apoptosis measured by activation of the effector caspase 3,7 increased non-significantly over storage time. Microparticles increased significantly from 5 to 10 days (p=0.042), P-selectin increased significantly from 10 to 15 days (p=0.049), and phosphatidyl serine exposure (measured by Annexin V binding) increased significantly from 15 to 20 days (p=0.025). We found best correlations between in vivo recovery (% of fresh) and ΔHCO3, Δglucose, Δlactate, Δmicroparticles, Δp-selectin, Δannexin V (all correlation coefficients of at least +/- 0.72, and p<0.01).

Conclusion: We performed the first studies with extended storage cold (4ºC)-plasma-stored apheresis platelets up to 20 days and a fresh comparator. We show that there is continuous loss of recovery up to 20 days of storage. Overall, continuous platelet activation is a hallmark of cold-stored platelets, while markers for caspase activation and mitochondrial membrane integrity show a delay of apoptosis during cold-storage. Platelet function tests show preserved integrin activation comparable to fresh up to 20 days of storage. Integrin activation and apoptosis markers did not correlate with recovery, but metabolic parameters and in vitro pre-activation parameters did. Taken together, storage up to 15 days in the cold appears to yield acceptable in vitro and in vivo results.

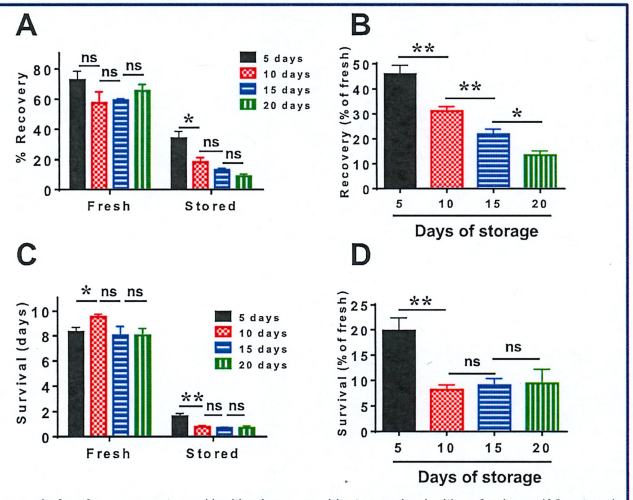

Figure 1: In vivo parameters: Healthy human subjects received either fresh or 4°C- stored autologous platelet transfusions. (**A**) Recovery of transfused fresh or stored platelets at 2 hour time point. (**B**) Recovery of stored platelets as percentage of fresh sample. (**C**) Survival of fresh or stored platelets in days (**D**) Survival of transfused platelets as percentage of fresh. *p<0.05, **p<0.01, ***p<0.001, ns= not significant.

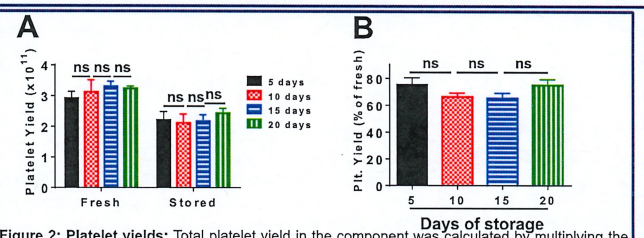

Figure 2: Platelet yields: Total platelet yield in the component was calculated by multiplying the platelet count of the sample times the volume of the component (platelet count x component volume = actual platelet yield) (**A**) Platelets were sampled at baseline (fresh), or after designated storage period. (**B**) Results shown as percentage of fresh samples. ns= not significant

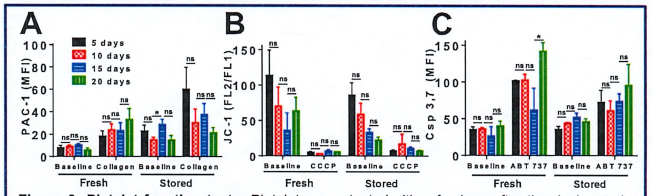

Figure 3: Platelet function tests: Platelets were tested either fresh or after the designated storage time point (**A**) Platelets were tested at baseline or after addition of 20µg/ml collagen. Integrin activation was tested by adding the activation-specific PAC-1 antibody. (**B**) Platelets were tested at baseline or after the addition of the mitochondrial membrane uncoupler CCCP (positive control) and read by flow cytometry after adding the JC-1 dye. (**C**) Platelets were tested either at baseline or after adding the BH3 mimetic ABT737 (positive control). A fluorescently labeled probe to caspase 3,7 was added. p<0.05, ns= not significant.

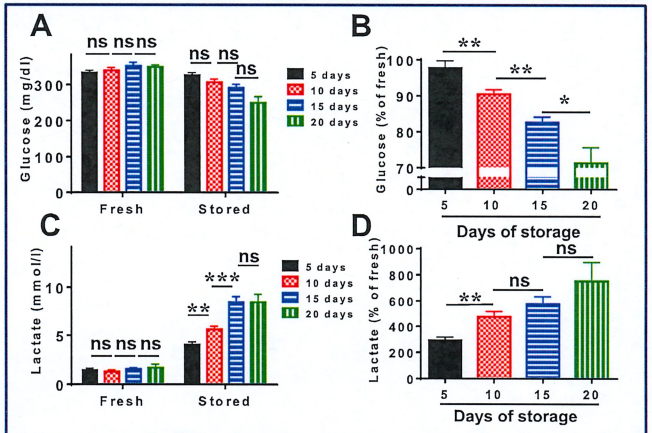

Figure 4: In vitro metabolic parameters: Units were sampled at baseline (fresh) or after designated storage period and tested for glucose or lactate by a commercially available blood gas instrument. (**A**) Absolute glucose values at baseline (fresh) and after designated storage period. (**B**) Glucose values after storage as percentage of fresh samples. (**C**) Absolute lactate values at baseline (fresh) and after designated storage period. (**D**) Lactate values after storage as percentage of fresh samples. . *p<0.05, **p<0.01, ***p<0.001, ns= not significant.

The authors have no conflict of interest to disclose

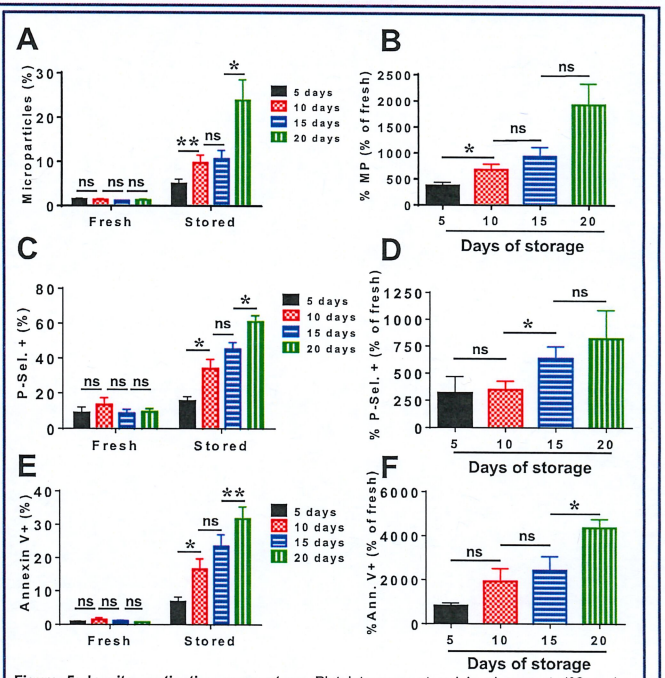

Figure 5: In vitro activation parameters: Platelets were stored in plasma at 4ºC and sampled at baseline (fresh), or after designated storage period. (**A**), CD61-positive microparticles measured by flow cytometry. (**B**), Microparticles as percentage of fresh samples. (**C**), P-selectin (CD62P)-positive events measured by flow cytometry. (**D**), P-selectin events as percentage of fresh samples. (**E**), Annexin V positive events measured by flow cytometry. (**F**), Annexin V as percentage of fresh samples. *p<0.05, **p<0.01, ***p<0.0001, ns= not significant.

Conclusions:

- Recovery continues to decline for up to 20 days of storage (maximum tested), while platelet survivals reach a low plateau after 10 days.
- All in vitro platelet activation parameters increase significantly over the course of storage. Microparticles show an especially large increase from 15 to 20 days of storage.
- The metabolic parameters glucose and lactate indicate that there is continuous metabolic activity over 20 days in the cold: Glucose continues to decline from fresh to 20 days, and lactate increases from fresh to 20 days of storage.
- In vivo recovery correlated best with metabolic parameters and pre-activation parameters, not with apoptosis and functional marker (integrin activation).
- Platelet storage for 15 days at 4ºC in plasma could be used to expand the available supply of platelets to treat bleeding patients.

Extended Cold-Storage of Apheresis Platelets in Plasma 08-2018

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Background: The 5-day storage limit of room temperature (22°C)-stored platelets severely constrains platelet availability. Cold (4ºC)-stored platelets are currently being evaluated by the military for actively bleeding patients and represent an intriguing storage option for far forward military scenarios and remote hospital locations. We do not know how well platelets circulate and function after extended storage at refrigerated temperatures. In this study, we investigate how cold-storage in plasma for up to 15 days affects platelet viability and function compared to fresh platelets. Methods: Seventeen healthy human subjects underwent an apheresis platelet collection. The CSP "test" unit was stored for either 5, 10, or 15 days in plasma at 4ºC. Platelet samples for in vitro platelet function testing were extracted on the day of donation and after the designated storage period. After storage, all units were radiolabeled and transfused into their respective donors. All donors came back after a week to provide a fresh sample for radiolabeling and reinfusion. Results: Over storage time, platelet recoveries declined significantly from 63±10% (fresh), to 34±10% for 5 days (p=0.005), to 18±7% for 10 days (p<0.0001), to 13±3% for 15 days (p<0.0001). There was a significant reduction in recovery from 5 to 10 days (p=0.02), but not between 10 and 15 days (p=0.15). As expected, 5 day platelet survival was low compared with fresh (8.6±1.2 days versus 1.6±0.5days respectively, p<0.0001) and dropped further from 5 to 10 days (1.6±0.5days versus 0.8±0.2 days, p=0.02), but not between 10 and 15 days. We did not observe a significant decrease in platelet survival after prolonged storage to 15 days. The platelet yield dropped significantly from fresh to 5,10,and 15 days ($3.11\pm0.71x10^{11}$, versus $2.21\pm0.61x10^{11}$,p=0.01, $2.11\pm0.74x10^{11}$,p=0.006 and $2.17\pm0.47x10^{11}$, p=0.01 respectively). However, no significant differences were observed between 5,10, and 15 days, suggestive of early microaggregate formation without significant increase over prolonged storage. Glucose concentrations decreased significantly from fresh to 10 and 15 day stored (342±20mg/dl versus 307±23mg/dl, p<0.001, 291±22mg/dl, p<0.0001 respectively) but no significant changes were seen between 5, 10, and 15 days. Lactate concentrations increased significantly when compared to fresh (1.4±0.4mmol/l versus 4±0.6 mmol/l, p=0.0001, 5.6±0.8mmol/l, p=0.0001, and 8.4±1.3mmol/l, p=0.0001, respectively) and between 5, 10, and 15 days of storage. All markers of platelet activation increased over time at storage in the cold when compared to fresh. However, while microparticles and P-selectin increased significantly from 5 to 15 days (5.06±2.18% versus 10.62±4.38%,p=0.04 and 15.65±5.5% versus 45.1±8.5%, p=0.002 respectively), phosphatidyl serine exposure (measured by Annexin V binding) increased from 5 to 10 days (6.8±3.1% versus 16.5±8.4%, p=0.03) and from 5 to 15 days (6.8±3.1% versus 23.4±8%, p=0.002). Conclusions: We performed the first reported studies with extended storage cold (4ºC)-plasma-stored apheresis platelets up to 15 days with FDA-required tests and a fresh comparator. We show that there is continuous loss of recovery up to 15 days of storage, while platelet survivals appear to reach a low plateau after 10 days. Platelet yields drop early and do not further decrease over storage time. We found that cold-stored platelets show signs of ongoing metabolism even at refrigerated storage conditions. *In vitro* markers of pre-activation, including P-selectin expression, microparticle release, and phophatidyl serine exposure increased significantly. Taken together, storage up to 15 days in the cold appears to yield acceptable in vitro and in vivo results.

APPENDIX A
REVISED STATEMENT OF WORK

Title: Novel approaches to storage of platelets for transfusion.

Background: Platelets are transfused to prevent bleeding and induce hemostasis, and can thus be critical in saving lives following trauma. Currently, platelets isolated from volunteers are stored at room temperature with gentle agitation for up to 5 days before transfusion. This short shelf-life severely compromises platelet inventories and creates chronic shortages for two important reasons: (1) platelets age during this period, and are functionally not as desirable as fresh platelets; and (2) storage at room temperature increases the risk of bacterial contamination. There is an urgent need to develop novel methods of storing platelets to minimize or even eliminate these issues. This need is particularly acute in the deployed military setting where platelet products are in especially short supply and are essentially unavailable far-forward, near the point of injury where they might be of greatest utility.

It is possible that manipulation of several collection and storage parameters, such as choice of apheresis systems, storage medium and temperature, and implementation of pathogen reduction technologies may improve platelet shelf life, safety and effectiveness. It is well known that platelets are sensitive to physical stimuli such as shear and contact with artificial surfaces, which may be activating and cause premature release of hemostatic and inflammatory mediators prior to or during storage. Selection of collection systems that minimize physical damage to platelets, in conjunction with storage optimization, could significantly enhance platelet product quality. Similarly, the current common practice of storing platelets in citrated plasma at room temperature may lead to significant product degradation due to the activity of endogenous proteases or other mechanisms. Use of platelet additive solutions (PAS), might reduce platelet stress. Alternatively, storage of platelets within whole blood under refrigeration may provide other factors that maintain important aspects of platelet function that need to be evaluated, as a potentially preferred product for battlefield polytrauma. This was once standard-of-care in transfusion medicine, but was abandoned once it was shown that refrigeration led to accelerated *in vivo* platelet clearance over about 48 hours rather than over one week. While not conducive to maintaining circulating platelet counts in thrombocytopenic cancer patients, this strategy might provide adequate platelet hemostatic capacity to bleeding trauma patients and improve platelet availability for such patients. This possibility has been inadequately evaluated, particularly in clinical studies. Finally, addition of a pathogen reduction technology to some combination of the preceding approaches may yield further benefit by impeding bacterial growth, which is the principal lethal transfusion risk associated with platelet transfusion. Pathogen reduction would have the greatest impact in resource-constrained settings such as the deployed military environment or the

developing world, where full transfusion transmitted disease testing is unavailable.

A deeper mechanistic understanding of the effects of collection and storage on platelet function could greatly aid in improving the availability and efficacy of platelets both on the battlefield and in the civilian transfusion setting. In this research proposal we are interested in evaluating the effect of novel combinations of collection, storage and pathogen reduction approaches on the structural and functional properties of platelets and on the functional consequences during transfusion.

We hypothesize that the *"collection of platelets in a manner that minimizes physical damage combined with an alternative storage medium and/or temperature and pathogen reduction technology will improve platelet shelf life, safety and function*." We propose the following Specific Aims to test this hypothesis.

Aim 1. Evaluation of structural and functional changes to platelets during enhanced collection, storage and pathogen reduction (enhanced platelets)

We will evaluate changes in the structural and functional properties of platelets including metabolism, protein and microRNA expression, shape changes, cytoskeletal rearrangement, membrane fluidity, receptor expression and distribution, microparticle formation, aggregation in response to agonists, whole blood clotting function, adhesion and aggregation under high shear conditions.

Aim 2. Evaluation of enhanced platelets in animal models of trauma and hemorrhage

We will evaluate the hemostatic efficacy and inflammatory characteristics of enhanced platelets and these observations will be correlated with *in vitro* findings from Aim 1. Enhanced platelets will be optimized for collection methods, storage mediums (plasma, PAS etc.), Temperature (refrigerated, room temperature, temperature cycling etc.)and exposure to pathogen reduction technology.

Aim 3. Evaluate enhanced autologous platelets in normal subjects

As the first step in the in vivo evaluation of human platelets, autologous platelets will be obtained from whole blood or apheresis procedures. The whole blood or apheresis platelets will have been subjected to various storage conditions with or without pathogen reduction. The efficacy of the platelets obtained from these products will be assessed by determining the recovery and survival of the subjects' autologous radiolabeled platelets following re-infusion. The results of these studies will be correlated with the studies performed in Aims 1 and 2.

Aim 4. Evaluation of enhanced platelet recovery and survival, bleeding time and hemostatic activity in thrombocytopenic patients with and without acute hemorrhage.

We will evaluate the shelf life, safety, and efficacy of enhanced platelets in patients and correlate these findings with observations from Aims 1, 2, and 3 in order to optimize platelet product collection and storage conditions.

We expect our results to generate important information on how changes in platelet collection, storage medium and temperature, and exposure to pathogen reduction technologies affect stored platelet structure and function, as well as shelf life and *in vivo* efficacy.

Our collaborators at the Puget Sound Blood Center, led by Dr. Sherrill J. Slichter, have extensive experience in studying platelet biology and transfusion medicine. Dr. Slichter's laboratory and clinical study group has made a number of the seminal observations on the effectiveness of platelet transfusion strategies.

A CRADA will cover collaborative research between the Puget Sound Blood Center and the USAISR Coagulation and Blood Research Task Area. This collaborative effort is envisioned to lead to development of new platelet storage techniques. Joint authorship in publications and inventors rights will be shared by both parties.

Collaboration:

USAISR agrees to:

1. evaluate changes in the structural and functional properties of platelets following collection, storage and pathogen reduction approaches that incorporate a number of combinations of currently available technologies, or technologies in advanced development (enhanced platelets).

2. evaluate the hemostatic efficacy and inflammatory characteristics of enhanced platelets in animal models of trauma and hemorrhage, and these observations will be correlated with *in vitro* findings.

3. evaluate the shelf life, safety, and efficacy of enhanced platelets in thrombocytopenic patients with acute hemorrhage and correlate these findings with observations from Aims 1 and 2 in order to optimize platelet product collection and storage conditions.

4. engage in analysis of data and validation of findings related to changes in platelet structure, function, and viability following enhanced collection, storage and pathogen reduction.

5. write manuscripts and scientific reports, submit invention disclosures and patents.

Puget Sound Blood Center agrees to:

1. identify candidate platelet collection, storage and pathogen reduction approaches to test in model *in vitro* and *in vivo* systems with the goal of improving platelet storage life, safety and efficacy. As needed, transfer candidate technologies to USAISR for *in vitro* and *in vivo* testing as described above.

2. conduct *in vitro* and *in vivo* platelet product testing as described above.

3. conduct clinical studies of enhanced platelets such as recovery and survival experiments in normal volunteers and thrombocytopenic patients. In addition, PSBC will perform bleeding time assays and trials of hemostatic efficacy studies in thrombocytopenic patients with and without acute hemorrhage. The first collection/storage conditions to be evaluated will be platelets stored within whole blood units under refrigeration.

4. engage in analysis of data and validation of findings related to changes in platelet structure, function, and viability following enhanced collection, storage and pathogen reduction procedures.

5. write manuscripts and scientific reports, submit invention disclosures and patents.

From time to time, USAISR personnel may work in the Puget Sound Blood Center's laboratories and Puget Sound Blood Center's personnel may work in USAISR's laboratory as necessary to accomplish the goals of this collaboration.

Cold Apheresis Platelets in PAS (CAPP)
Platelet Additive Solution (PAS)

I. PROTOCOL INFORMATION
Title: Cold Apheresis Platelets in PAS (CAPP)
Phase of Study: Phase I/II. Proof of Principle.
Version/Date of Protocol: Version 4/May 17, 2018

II. SPONSOR INFORMATION
The study is being sponsored by the Department of Defense (DOD) Congressionally Directed Medical Research Program (CDMRP).

III. PRINCIPAL INVESTIGATOR'S INFORMATION
PI Name: Moritz Stolla, MD
Title: Director Platelet Transfusion Research
Name & Address of Research Institution: BloodworksNW (formerly Puget Sound Blood Center)
Phone #: 206-689-6541
FAX #: 866-791-4098
Email: mstolla@BloodWorksNW.org

IV. ROLES AND RESPONSIBILITIES
Principal Investigator (PI): The PI will have overall responsibility for the study. He will ensure compliance with the protocol, institutional policies, and all applicable regulations. The PI will supervise the use of the test articles and review study data at regular intervals. The PI will permit and comply with audits and monitoring requirements. The PI will report all unanticipated problems involving risk to subjects or others to the Research Monitor, appropriate regulatory bodies, including the University of Washington Human Subjects Division and the USAMRMC, ORP, HRPO.

Study Coordinator (SC): The SC will assist in the preparation of the protocol, Institutional Review Board (IRB) applications and amendments, required quarterly reports, and other regulatory documents as needed. The SC will manage implementation of the research protocol under the supervision of the Principal Investigator. She will identify and recruit eligible subjects, review information on source documents to ensure data are complete and correct, and assist in rectifying discrepancies. She will maintain study records and logs and assist in evaluating study results. In addition the SC may perform all tasks ascribed to the Clinical Research Staff (below).

Clinical Research Staff (CRS) will perform research-related interventions under the direction of the PI and/or the SC. CRS will ensure that subjects have read and understand the informed consent document and have all questions appropriately answered and that informed consent documents are properly signed and dated. CRS will schedule study subject visits; explain study procedures; assess and document study subject's clinical status as required by research protocol; collect apheresis units; obtain subject blood samples; administer radiolabeled platelets as required by the protocol (only trained Registered Nurse CRS will perform this task); monitor study subject's progress and report adverse effects to the PI.

Laboratory Research Staff will perform research-related laboratory testing and platelet radiolabeling in accordance with the study protocol. Laboratory Research Staff will perform data entry into the study data base. Upon occasion Laboratory Research Staff may also collect blood samples from subjects. BloodworksNW Staff (either research or non-research) will collect follow-up blood samples and hold them for pick up and processing by Laboratory Research Staff.

Research Monitor: The Research Monitor will act as the safety advocate for study subjects. The Research Monitor will review all unanticipated problems involving risk to subjects or others and will provide an unbiased written report of the event to appropriate regulatory bodies, including the University of Washington Human Subjects Division and the USAMRMC, ORP, HRPO.

V. SITE INFORMATION

All study activities with the exception of the laboratory tests noted below will occur at BloodworksNW (formerly Puget Sound Blood Center) under the direction of Dr. Moritz Stolla. Bacterial testing and Gram Staining will be conducted by the University of Washington (UW) Microbiology Laboratory in Seattle, or by LabCorp in Seattle. All samples sent to outside microbiology laboratories will be stripped of all personal identifiers and labeled with a study ID number only.

VI. STUDY INFORMATION

Type of Research: Biomedical

VII. STUDY DESIGN

Background

Platelets are transfused to prevent bleeding and induce hemostasis, and can thus be critical in saving lives following trauma and in supporting thrombocytopenic cancer patients. Currently, platelets collected from volunteers are stored at room temperature. Room temperature storage has been demonstrated to maximize platelet recovery and survival in transfused patients; however it also increases the opportunity for bacterial growth in the platelet unit. The FDA limits the shelf life of platelets to 5 days or less to minimize this bacterial risk. Recently, the FDA has allowed 7 day storage with additional point-of-release bacterial testing. Nonetheless, transfusion associated sepsis remains the principal lethal risk associated with platelet transfusion.

Cold storage (4°C) is known to reduce post transfusion platelet recoveries but the effect is no more than 10% to 20% after 3 days of platelet concentrate storage. However, survivals are reduced to 1 to 2 days compared to an average survival of 4 to 5 days at 22°C storage[1-3]. In addition, there is controversy regarding the ability of 4°C stored platelets to correct bleeding times in thrombocytopenic patients compared to 22°C stored platelets[1]. However, we have demonstrated, in preliminary studies that platelets stored within whole blood for 15 days have radiolabeled autologous recoveries of 27±11% (49% of the same donor's fresh autologous recoveries) and survivals averaging 1.2±0.4 days (16% of the same donor's fresh autologous survivals)[4]. These data suggest that 4°C storage of apheresis platelets, as proposed in this study, may clearly show similar or even better platelet viability as platelet storage within whole blood.

Platelet Additive Solution (PAS)

Platelet Additive Solutions are isotonic solutions used to replace a portion of the plasma to store leukocyte reduced apheresis platelets. Several of these solutions are FDA approved for storage in a 65% PAS/35% plasma mixture for up to 5 days at 20-24°C with continuous agitation. Some FDA licensures associate the particular PAS with a specific collection devices or storage bag. None are approved for use

with platelets stored in the cold. We will be using these storage solutions in an off-label manner as regards to the collection device and storage conditions. However, all of the collection devices and storage solutions are FDA approved for platelet collection and storage.

Cold Stored Platelets

While not conducive to maintaining circulating platelet counts in thrombocytopenic cancer patients, transfusion of refrigerated platelets for deployed military medical units might provide adequate platelet hemostatic capacity to bleeding trauma patients and improve platelet availability for such patients. Based on recent in vitro studies of 4°C versus 22°C stored platelets, clot strength, platelet aggregation and sheer induced platelet aggregation are all better maintained at 4°C[6-8].

A deeper understanding of the effects of cold storage on platelet function could greatly aid in improving the availability of platelets on the battlefield and in the civilian transfusion setting. In this research proposal, we are interested in evaluating metabolic, functional and viability changes to apheresis platelets preserved in an additive solution and stored at 4°C. We will also determine the recovery and survival of these platelets by radiolabeling an aliquot of the apheresis platelets and re-infusing it into the donor/subject.

Current Research Approach

Cold stored platelets suspended in a mixture of PAS and plasma as well as cold stored platelets suspended in plasma without PAS will be evaluated.

For PAS suspended platelets. a single hyperconcentrated apheresis platelet unit (target platelet yield 3.0 X 10^{11}/unit and concentration of ~4000 x 10^3 platelets/µL) will be collected from a healthy adult volunteer subject using the Trima Accel® Automated Blood Collection System. Concurrent plasma (150 mL) will also be collected. After collection the unit will be re-suspended in a platelet additive solution (either InterSol or Isoplate) and plasma. The ratio of PAS to plasma will be 65% PAS: 35% plasma.

For plasma suspended platelets, a standard single apheresis platelet unit (target platelet yield 3.0 X 10^{11}/unit and concentration of ~1500 x 10^3 platelets/µL) will be collected as described above. Fifty milliliters of concurrent plasma will also be collected.

Regardless of the storage medium, each unit will achieve a final platelet concentration of 700 – 2100 x 10^6 platelets/µL, as per allowable bag parameters. The unit will be stored for a predetermined period of up to 20 days at 4°C. Various in vitro assays (see "In Vitro Tests Performed on Test Units") will be performed on the day of or day after collection.

At the end of the storage period, the subject will return to receive an ^{111}Indium Oxine (Indium 111, In-111) radiolabeled aliquot of their 4°C stored platelets. Follow-up samples from the subject will be collected approximately 2 hours post-infusion and on Days 1 (2X), 2 (2X), and 3 to calculate recovery and survival of the subject's 4°C stored platelets. The Day 1 and Day 2 the sample draws will be 2 - 10 hours apart.

In addition to radiolabeled platelet recovery and survival measurements, the same in vitro assays that were performed on the unit on the day of or day after collection will also be performed on the unit at the end of 4°C storage.

Seven to 14 days after the infusion of the radiolabeled aliquot, the subject will return to receive a second radiolabeled aliquot of fresh platelets. To facilitate this, on the morning of the second infusion, the subject will return for collection of a 43 mL blood sample. The blood will be processed to obtain a fresh sample of the subject's platelets to serve as a control comparator. The platelets will be radiolabeled with In-111. The subject will return later in the day for infusion of the radiolabeled fresh 'control' comparator aliquot. Follow-up blood samples will be drawn at ~2 hours after the infusion on Day 0 and then on Days 1, 2, 3, 4 or 5, and 6 or 7 day post infusion to calculate recovery and survival of the subject's fresh vs. stored platelets.

VIII. INCLUSION / EXCLUSION CRITERIA

Inclusion Criteria

The subject is in good health, is taking no excluded medications and meets platelet donor suitability requirements aimed at assuring donor safety. Recipient safety restrictions (e.g. travel and sexual contact) do not apply for this study. No infectious disease testing will be performed.

Specific inclusion criteria are:

- Weight: ≥110 pounds
- Hematocrit: ≥38% for females, ≥39% for males, but not >55% *
- Platelet count ≥150X10^3/mm^3 *
- Temperature: ≤99.5°F
- Resting blood pressure: systolic ≤ 180 mmHg; diastolic ≤100 mmHg
- Resting heart rate: 40 to 100 beats per minute
- Subjects must be ≥ 18 years old, of either sex
- Subjects must be able to read, understand and sign the informed consent document and commit to the study follow-up schedule. The ability to read and speak English is required for participation.
- Subjects must have good veins for apheresis platelet collection and drawing blood samples.
- Subjects of child-bearing potential (either male or female) must agree to use an effective method of contraception during the course of the study. The following methods of contraception will be considered 'effective' when self-reported by subject; abstinence, intrauterine contraception devices, hormonal methods, barrier methods or history of sterilization.

* The CBC will be run in duplicate from a single sample and the results averaged. Results > 10% difference from each other will be repeated.

Exclusion Criteria

Healthy subjects will be excluded from the study for any of the following reasons:

- Unable to achieve target platelet yield of 3.0 X 10^{11}/unit per Trima Accel (apheresis machine) configuration parameters.
- Ever received radiation therapy.
- Already participated in 4 research studies involving radioisotopes within the current calendar-year.
- Taken aspirin, non-steroidal anti-inflammatory, or other platelet affecting drugs within 72 hours of collection or infusion. Subjects who have ever been prescribed anti-platelet medications (e.g. clopidogrel) will be excluded from study participation regardless of the interval to their last dose.

- Currently pregnant or nursing as assessed during interview. A urine pregnancy test prior to radioisotope infusion is required for women of childbearing potential.
- Unable to comply with the protocol in the opinion of the investigator.
- Donated granulocytes within the last 2 days.
- Donated whole blood within the last 7 days.
- Donated platelets or plasma within the last 28 days.

IX. SUBJECT RECRUITMENT & SCREENING

The study will advertise for healthy adult volunteers on websites, newspapers and/or bulletin boards. Prospective subjects will be asked to contact the Study Coordinator by email or phone. Email inquiries will be answered, by the Study Coordinator, with a summary email along with attachments of study documents (study consent, HIPAA policy, directions to BloodworksNW and a schedule of study visits). The subject will be encouraged to call the Coordinator to discuss the study by phone before making a screening appointment. The Study Coordinator may reference *Talking Points for Volunteer Inquiries* during the phone conversation.

Prospective subjects responding by phone will speak with the Study Coordinator, as described above, and will be offered an email with attached study documents.

Individuals who wish to make an in person appointment for consent and screening will make those arrangements by phone or email with the Study Coordinator. An email confirmation and reminder will be sent by the Study Coordinator. Contact information from people who do not make appointments will not be retained.

A total of 90 subjects with evaluable complete data sets may be enrolled. Allowing for screenfails and withdrawals this may require consenting of as many as 190 subjects.

X. INFORMED CONSENT PROCESS

At the time of the recruitment visit, Clinical Research Staff, usually the Study Coordinator will review the consent with the study subject in a private space at the BloodworksNW. The purpose of the study, the study procedures, the risks and options to not participate or to withdraw will be discussed. The number of venipunctures, the radioisotope exposure and the time demands of multiple blood draws will be emphasized. Throughout the process the subject will be encouraged to ask questions or make comments.

Subjects will sign the consent form in the presence of the staff administering the consent and that person will also sign the consent. The subject will be given a copy of the consent and HIPAA document.

After the subject has given informed consent eligibility screening will be performed. See Study Procedures section below. Screening questions are related to establishing that the subject is in good health. See Section 8, Inclusion/Exclusion Criteria.

All Clinical Research Staff have been trained and are certified in the Protection of Human Research Subjects.

XI. STUDY PROCEDURES

Screening

An abbreviated version of blood donor screening will be performed including completion of a study specific health history questionnaire, check of vital signs and a blood draw to obtain a 2 mL sample for a complete blood count (CBC) to obtain the hematocrit and platelet count. Only criteria aimed at assuring donor safety will apply. Recipient safety restrictions (e.g. travel and sexual contact) do not apply for this study. No infectious disease testing will be performed. If the subject meets eligibility criteria an appointment for apheresis platelet collection, within the next 35 days, will be made.

Apheresis Platelet Collection

Prior to apheresis, the pre-apheresis health history questionnaire and check of vital signs will be completed. The subject's platelets will be collected using the Trima Accel Automated Blood Collection System which is licensed by the FDA for this purpose. A venipuncture site will be selected and cleaned using standard BloodworksNW procedures. A needle will be placed in one of the subject's arms at the antecubital area. A CBC sample is obtained using an inline diversion pouch. Whole blood is drawn into the apheresis machine and the blood components are separated by centrifugation. Platelets and plasma are collected into the Terumo ELP storage bags and the red blood cells are returned to the subject. Along with the return of the subject's red blood cells the subject receives approximately 350 mL of ACD (citrate) anticoagulant during the collection process. The platelet apheresis collection lasts ~2 hours. Subjects are observed throughout the collection by a nurse or technician specifically trained in apheresis.

Suspension in PAS and Cold Storage

Immediately after apheresis collection, using sterile technique, research laboratory staff may suspend the platelets in a mixture of PAS/plasma at a 65%/35% ratio or leave the platelets in the attendant plasma, depending upon the predetermined storage medium being tested. After suspension units will rest for 1-24 hours at room temperature prior to sampling for in vitro assays. Units are weighed to calculate platelet yield. The units are placed in a locked cage in a refrigerator at 4±2°C and are not agitated during storage.

Temperature monitors will record temperatures and trigger alarms for out of range conditions. End of storage will be defined as the date and time when the aliquot for radiolabeling and infusion is removed from the stored unit.

Autologous infusion of radiolabeled platelets

Radiolabeling will be done according to a modified Biomedical Excellence for Safer Transfusion (BEST) method. In the BEST method, a concurrent, dual, fresh and stored label using two different isotopes is used to achieve an evaluable survival and recovery calculation. This approach is not practical as the error corrections inherent in the BEST method arrive at numerous irrational data outputs when comparing products of very different signal strengths. Therefore, Indium-111, will be used for both test and control platelets. In-111 infusions will be separated by at least one week. The In-111 administered on Day 0 will be undetectable by Day 7 and therefore re-use of the same isotope to measure both cold stored (test) and fresh (control) platelets is valid. To confirm this, we will collect a pre-infusion radioactivity sample to account for any residual In-111, and adjust our calculations accordingly.

Prior to infusion the subject's health will be reassessed via interview. If the subject feels unwell, has flu-like symptoms, or has any significant negative change to his or her health status, then he/she will be considered ineligible for the radiolabeled infusion and will exit the study. Pre-menopausal female

subjects who do not report sterilization will have a urine pregnancy test to confirm that they are not pregnant prior to the infusion. Any subject with a positive pregnancy test will be ineligible to continue with the infusion and will exit the study. Vital signs (temperature, pulse and blood pressure), height and weight will be assessed and recorded. Prior to infusion, microbiological tests (bacterial testing and Gram stain) of the platelet unit will be verified as negative.

Height and weight will be measured at the time of infusion. After venous access has been established, a blood sample (20 mL) will be obtained to determine baseline radioactivity. Approximately 10 mL (2-10 mL) of autologous cold-stored Indium-111 labeled platelets will be infused into the subject. During each platelet infusion, the subject will be carefully monitored for adverse reactions; i.e., fever, chills, dyspnea, urticaria or pain (infusion site, chest pain or other). Any adverse reactions will be recorded and reported to the study investigator.

After infusion, the line will be flushed with saline and removed. The subject will remain at, or return to, BloodworksNW for the Day 0 post-infusion blood sample, which will be collected ~2 hours after the infusion.

Seven to 14 days after the 1st radiolabeled aliquot is infused, the subject will return to have a 43 mL sample of whole blood collected to obtain a 'fresh' sample of the subject's platelets. The whole blood sample will be processed using a soft centrifugation to obtain platelet-rich-plasma (PRP). The PRP will be hard spun to produce a fresh sample of concentrated platelets. The fresh platelets will be radiolabeled with ^{111}Indium Oxine and infused into the subject as described in the above paragraphs.

Follow-up

The subject will return to BloodworksNW for sample collection (10 mL of blood) for measurement of radioactivity to calculate platelet-survival curves; Day 0, Day 1 (twice, 2-10 hours apart), Day 2 (twice, 2-10 hours apart), and Day 3 post infusion #1 for a total of 6 sample draws. After the second infusion the subject will return for the same 10 mL blood sample collections on Day 0, Day 1, Day 2, Day 3, Day 4 or 5 and Day 6 or 7 post infusion #2 for a total of 6 sample draws. (See Schedule of Events below). These samples will be used to determine platelet recovery and survival using computerized modeling of a multiple hit decay function.

Schedule of Events

Study Day	Study Procedures
1 – 35 days before apheresis	Informed consent process
	Screening (including collection of a 2 mL blood sample for hematocrit and platelet count) and enrollment
Apheresis platelet collection day	Pre-apheresis vital signs and health assessment
	Apheresis platelet collection
	In vitro testing on platelet unit (some testing may occur day after collection)
	Platelet unit reconstituted with 65% PAS/35% plasma or left on attendant plasma with no PAS , placed into storage at 4±2°C (may occur 1 -24 hours after collection)
1 day after apheresis	Bacterial culture sample collected from platelet unit and sent to UW microbiology laboratory
Day 0 Infusion day (3-20 days after apheresis platelet collection day)	Platelet storage ends
	Aliquot removed from stored platelets and processed for ^{111}Indium radiolabel (test)
	In vitro testing on stored unit
	Bacterial cultures evaluated
	Gram stain on stored unit sent to UW microbiology laboratory and evaluated
	Pre-infusion ID check, vital signs and health assessment. Urine pregnancy test if woman subject of childbearing potential.
	20 mL blood sample from subject for baseline radioactivity
	Infusion of 4°C stored radiolabeled platelet aliquot
	Post infusion recovery and survival (R&S) sample from subject (≥2 hours post infusion)
Post Infusion Day 1	Post infusion R&S sample from subject (twice, 2 - 10 hours apart)
Day 2	Post infusion R&S sample from subject (twice, 2 - 10 hours apart)
Day 3	Post infusion R&S sample from subject
Day 7 (visit can occur 7 – 14 days after the Day 0 infusion. All subsequent visits, below, will be pushed out accordingly)	43 mL fresh blood sample collected from subject and processed for ^{111}Indium radiolabel (control)
	Pre-infusion ID check, vital signs and health assessment. Urine pregnancy test if woman subject of childbearing potential.
	20 mL blood sample from subject for baseline radioactivity
	Infusion of fresh radiolabeled platelet aliquot
	Post infusion recovery and survival (R&S) sample from subject (≥2 hours post infusion)
Day 8	Post infusion R&S sample from subject
Day 9	Post infusion R&S sample from subject
Day 10	Post infusion R&S sample from subject
Day 11 or 12	Post infusion R&S sample from subject
Day 13 or 14	Post infusion R&S sample from subject
	Subject exits study

Total Volume of Blood Collected

The total amount of blood loss during the course of the study is approximately 290 mL. This includes CBC (2 mL), diversion pouch sample (~25 mL), apheresis platelets (~60 mL residual in disposable kit), immediate pre-infusion for baseline radioactivity (20 mL, twice),fresh whole blood sample on morning of infusion for fresh platelet control comparator (43 mL), and post infusion blood samples (10 mL each X 12) to determine circulating radioactivity.

In addition to the above volumes, 75 mL – 200 mL apheresis platelets in plasma and 50 - 150 mL of concurrent plasma will be collected.

In Vitro Testing Schedule

In addition to the in vivo platelet viability measurements after re-infusion, a number of in vitro laboratory measurements will be performed. Samples for these experiments will be obtained from the apheresis unit on the day of or day after collection and at the end of storage. These tests will be performed using standardized methods.

A sample from the platelet product will be sent for bacterial culture to an outside microbiology laboratory one day after the platelet collection. At the end of the storage period, a sample from the stored platelet unit will be sent to the University of Washington Microbiology Lab for a Gram stain. If either test is positive, the subject's stored platelets will not be reinfused and the subject will be withdrawn from the study.

The following table provides a list of the tests that will be performed on the apheresis platelet unit at the end of storage. These are the standard in vitro assays that the FDA requires for platelet licensing.

In Vitro Tests Performed on Stored Apheresis Unit at the End of Storage

Test Type	Day of or day after collection testing	End of storage testing
Platelet Concentration	✓	✓
Volume	✓	✓
Platelet yield	✓	✓
Blood Gases (pH and pCO2, PO2, HC03)	✓	✓
Glucose and Lactate	✓	✓
P-selectin	✓	✓
Morphology	✓	✓
Annexin V binding	✓	✓
Extent of Shape Change	✓	✓
Hypotonic Shock Response	✓	✓
Platelet Microparticles	✓	✓
Swirling	✓	✓
Mean Platelet Volume (MPV)	✓	✓
Mitochondria assay: JC-1 (±)	✓	✓
Apoptosis assay: Caspase 3/7 activation (±)	✓	✓
Integrin PAC-1 activation (±)	✓	✓
Flow chamber adhesion and aggregation assay: collagen and vWF (±)	✓	✓
Bacterial Culture*	✓	✓
Gram stain		✓

All samples will be discarded once testing is complete and no residual radiation is detectable.

*Bacterial Culture sample removed from unit 1 day after collection and evaluated at end of storage.

(±) May or may not be performed depending on staffing and reagent availability.

Adverse Event (AE) Assessments

During apheresis collection and infusion of platelets, the subject will be carefully monitored for adverse reactions; e.g., fever, chills, dyspnea, urticaria, or pain (infusion site, chest pain or other). Adverse reactions will be recorded in the study file and reported to the study investigator. Subjects will be instructed to report changes in health condition over the course of the study to the study coordinators. Minor AEs that are associated with venipuncture and blood collection, such as minor bruising at the needle site, will not be recorded as AEs, unless they worsen over time (e.g., become infected, etc.).

XII. DATA and ANALYSIS

Laboratory and other evaluable results will be transcribed from source documents (e.g. lab result print-outs) into an electronic database.

Summary statistics (means, medians, standard deviations, interquartile range) will be calculated for all in-vitro assays.

Tables of recovery and survival summary statistics will display values by group from fresh and stored platelets. Recovery and survival of stored platelets as percentage of corresponding fresh platelets will be plotted against days stored. Regression methods will be used to determine if there is evidence of any trend in the mean storage or recovery of 4°C stored platelets with respect to storage time as a percentage of each subject's fresh platelet results. Histograms of recovery and survival as percentage of 4°C stored platelet measurements will be plotted, and corresponding confidence intervals will be calculated.

XII. LABELING & STORAGE OF DATA & SPECIMENS

Study records, samples, and test results will be identified with a unique identifier and access will be limited to sponsor authorized personnel, the investigator, site research staff, and authorized regulatory authorities, including representatives of the FDA.

An alpha-numeric code that is unique to this study will be used as study identifiers. The study ID number will be associated with the subject's name on a study ID log. That log and the study database will be kept in separate folders on an electronic network at BloodworksNW. BloodworksNW uses Active Directory NT Authentication along with Access Control Lists (ACL's) for all network folders. File and folder access is logged on network shares. Security is enforced by the Information Technology Department. A network firewall is used to prevent unauthorized access to the network from outside entities.

Source paper documents will be kept in the Study Coordinator's office at BloodworksNW which is a security-card-restricted-access-building. The door to the coordinator's office is kept locked. Any documents not needed for source documentation will be shredded using a secure records-destruction service.

The link between the subject's identify and their study data will be destroyed/deleted when the research ends and any required monitoring of the study is finished, which will be no later than December 31, 2025. Consents will be destroyed six years after the conclusion of data analysis.

BloodworksNW utilizes an independent waste management contractor to dispose of research samples. The waste management contractor is contractually obligated to be in compliance with all applicable regulations regarding the pick-up, transport and treatment of regulated medical waste.

Subject samples that are radioactive at the time of collection are stored on a secure-access floor until such time as they have no detectable residual radiation. This is generally about 2 weeks. At that point they are disposed of as described above.

XIII. RISK AND INJURY

Apheresis-Related Risks and Precautions

Risks associated with standard platelet-product apheresis procedures are listed below. A single apheresis procedure typically lasts about 2 hours.

- Venipuncture-related risks: Venipuncture may lead to apprehension, discomfort, pain, bruising or infiltration at the venipuncture site. A vasovagal response, such as lightheadedness or fainting, nausea, or vomiting may occur. There is a very small risk of infection at the venipuncture site.
- Citrate infusion related risks (hypocalcaemia): Citrate (Acid-Citrate-Dextrose) is added to the apheresis circuit as an anticoagulant. This may result in perioral tingling or paresthesias. Non-specific mild symptoms of hypocalcaemia include headaches, nervousness, irritability, lightheadedness, flushing, shivering, nausea, vomiting, chest discomfort and abdominal cramping. Slowing the collection rate, pausing the collection and/or administering oral calcium (TUMS) will effectively address these symptoms. Rarely, intravenous calcium is administered when symptoms do not resolve. If allowed to progress citrate toxicity could potentially manifest as muscle cramps, tremors, tetany, laryngospasm, seizures and life threatening cardiac arrhythmias.
- Blood Loss: In rare and unusual circumstances, blood loss has occurred due to inability to complete the procedure.

The following precautions will be taken: The subject's pre-apheresis vital signs (blood pressure, heart rate, temperature) and pre-apheresis hematocrit will be determined. Subjects will be visually monitored for signs of distress during all procedures by trained and experienced staff. Citrate reactions will be treated according to the standard treatments at the site, which includes oral or, rarely, intravenous calcium supplementation, and/or slowing, pausing or stopping the procedure.

Radioisotope Infusion-Related Risks and Precautions

The radiation dose in this study is less than annual background radiation (3 mSv) and is not known to be associated with any health hazard. The amount of the isotope that will be infused is ≤30 μCi of Indium-111. The total radiation dose is approximately ≤30 μCi for a splenic dose of 8 mGy and a total body effective dose equivalent of 0.8 mSv. The risks of radiation exposure to a fetus are unknown. Therefore, women of childbearing potential will have a pregnancy test performed prior to the radiolabeled platelet infusion.

BloodworksNW's Platelet Transfusion Research Department will maintain a record of each subject's participation and will limit the number of studies any one individual can participate in to four studies in a calendar year. Patients who have received radiation therapy will be excluded from the study.

Platelet Transfusion-Related Risks and Precautions

Risks associated with receiving any blood product include chills, fever, hives, itching, immune response against blood cells, and/or blood infection from bacterial contamination. There is a rare risk of receiving the wrong subject's cells upon infusion, which could cause symptoms similar to those listed above.

The following precautions will be taken: In this study, subjects will be infused with their own cells; confirmation of identification will be done by two person verification of the infusion material. To prevent bacterial contamination, the product will be bacterially screened before infusion and sterile technique will be used for all manipulations of the study platelets.

Venipuncture-Related Risks and Precautions

Risks associated with venipuncture for blood sampling are apprehension, pain, discomfort, venospasm, fainting, bleeding, or bruising or infiltration at the venipuncture site.

The following precautions will be taken: Trained and experienced phlebotomists will perform the venipuncture procedures so that discomfort of the subject should be minimal.

XIV. BENEFIT(S)

There is no direct benefit to the study subject. Real benefits are altruistic in nature: subjects participating in this study will assist the scientific and medical communities in gathering important information to improving the availability of platelet transfusions.

XV. COMPENSATION

Subjects will receive $900.00 at the conclusion of the study for their time involved in study participation. If the subject is unable to complete the entire study or has to be withdrawn from the study, they will receive partial payment for their time involved in the study. The partial payment scale is the following (number in parentheses equals the number of times each procedure occurs during the course of the study):

Initial screening (Day -35 to Day -1, one visit during this time period)		$30
Apheresis collection (Day 0)		$200
Infusion of radiolabeled platelets, including pre-infusion sample draw (Day 0 and Day 7 - 14. Two separate infusions)	$100 (x2) =	$200
Collection of 43 mL of whole blood on morning of 2nd infusion day		$35
Follow-up blood sample, platelet recovery and survival calculation	$35 (X12) =	$420
End of study exit		$15
Total for completing all study procedures		$900

XVI. CONFIDENTIALITY

BloodworksNW considers all data and information collected during this study confidential. All data used in the analysis and summary of this study will be anonymous, and without reference to specific subject names. Study records, samples, and test results will be identified with a unique identifier and access will be limited to sponsor authorized personnel, the investigator, site research staff, and authorized regulatory authorities, including representatives of the FDA.

XVII. USAMRMC REPORTING REQUIREMENTS FOR SAE

All unanticipated problems involving risk to subjects or others will be promptly reported by telephone (301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301-619-7803) to the Human Research Protection Office (HRPO). A complete written report will follow the

initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

XX. LITERATURE REVIEW

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Pathogen-Reduced, Extended Platelet Storage in Platelet Additive Solution (PAS)
EDMS 5570/11105004 W81XWH-12-1-0441

PI: Moritz Stolla MD **Org:** Bloodworks Northwest

Award Amount: $4,100,464 which includes funds contracted to Terumo of $1,402,000

Study/Product Aim(s)

- Research related to cold storage of platelets derived from whole blood and apheresis
- 4° C storage of platelets in Platelet Additive Solution (PAS)
- 4° C extended storage of platelets in plasma

Approach

This is a non-clinical, exploratory study of apheresis platelets stored in the cold (4° C). We are testing radiolabeled recovery and survival in health volunteers utilizing two sequential Indium-111 labels. The first radiolabeled infusion is performed using an aliquot from the extended stored apheresis platelet unit. The test unit is stored from 3-20 days. The second infusion is a fresh comparator obtained from and administered to the subject one week later. Various invitro tests are also performed. Study title Cold Apheresis Platelets in Plasma (CAPP).

Refrigerated platelets in plasma- stored for up to 20 days

Accomplishment: Enrollment ongoing to evaluate apheresis platelets at 4° C

Timeline and Cost

Activities CY	15	16	17	18
Study evaluating platelets in WB at 4° C				
Development and regulatory approval of apheresis platelets in PAS at 4° C study				
Apheresis platelets in PAS at 4° C study enrollment, data collection and analysis				
Apheresis platelets in plasma at 4° C study enrollment, data collection and analysis				
Estimated Budget ($K)	**$1.124**	**$1.592**	**$1.745**	**$2.698**

Updated: 22OCT18

Goals/Milestones

CY 18 Goal – Continued enrollment, data collection and analysis

☑ Complete analysis of apheresis platelets stored in **plasma** for 3-20 days at 4° C to same subject's fresh platelets

☑ Complete approvals, conduct enrollment, data collection and analysis of apheresis platelets stored in **Isoplate/plasma or InterSol/plasma** for 3-20 days at 4° C to same subject's **fresh** platelets

☑ Complete analysis of apheresis platelets stored in **plasma** for 20 days at 4° C to same subject's fresh platelets

CAPP is an exploratory study only. For confirmation of results the FDA requires a full set of in vivo platelet recovery/survival data and complimentary in vitro platelet quality data for 22-24 subjects for the selected cold storage period.

Budget Expenditure to Date

Projected Expenditure: $4,100,464 ($2,698,464 to Bloodworks + $1,402,000 to Terumo)

Actual Expenditure: $2,684,658 ($13,806) remaining for Bloodworks)