CHIlled Platelet Study (CHIPS)

Protocol Version 6.0
Version Date: 04 January 2024

PROTOCOL TITLE:

CHIlled **P**latelet **S**tudy

Short Title: CHIPS

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Protocol Version: 6.0
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I confirm that I have read this protocol, I understand it, and I will conduct the study according to the protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and will adhere to the Ethical and Regulatory Considerations as stated. I confirm that if I or any of my staff are members of the Institutional Review Board/Ethics Committee (IRB/EC), we will abstain from voting on this protocol, its future renewals, and its future amendments.

Principal Investigator Name: _____

Principal Investigator Signature: _____

Date: _____

PROTOCOL AMENDMENTS

Table 1: Protocol Amendment Summary

Section	Text	Amendments	Rationale for Change
Amendment #6 (dated 04 January 2024)			
Synopsis, 7.4.4, 11.1	Revised	Increased the maximum number of study platelet units or doses that can be given to 8 units or 8 doses.	Recommendation by the DSMB
Synopsis, 4.3.1, 7.13	Revised	Inclusion of neonate population in study.	Recommendation by the DSMB
2	Included	Referenced FDA Guidance for Industry issued in June 2023	New information available
7.4.2	Revised	Updated short-hand nomenclature for Intersol	Consistency and clarity of terminology
Throughout Protocol	Clarified	Corrected grammatical and typographical errors, updated minor items that do not change protocol but clarifies intent.	Consistency and clarity of protocol
Amendment #5 (dated 30 May 2023)			
Title Page, 13.2	Revised	Changed Washington University to the University of Pittsburgh	Employer change of Dr. Spinella (study PI)
Synopsis, 4.4, 9.5, 13.4	Revised	Updated number of anticipated study sites and removed planned sites in Canada and the UK	Operational change based on enrollment
Synopsis	Revised	Revised the planned study period	Based on first patient enrolled and enrollment rate
Synopsis, 3.3	Revised	Included the relative change in the peri-operative bleeding score as an exploratory outcome.	Consistency and clarity of protocol
Synopsis, 7.2, Appendix A	Removed	Removed duration of screening period to allow assessing eligibility and obtaining consent more than 30 days prior to scheduled surgery	Operational flexibility at sites
Synopsis, 4.3.1	Clarified	Clarified study eligibility to include only subjects for whom there is an expectation of bleeding that will require platelet transfusion during the intervention period	Consistency and clarity of protocol
Synopsis, 6.5, 8.3, Appendix A & B	Revised	Changed use of Pettersson Cleveland score to Surgical Complexity score to assess cardiac surgery severity score in adult patients	More practical to collect data for this score
Synopsis, 6.5	Clarified	Updated age range in the Mortality Group to 12 to 64	Clarification range
3.1	Revised	Corrected the Perioperative Bleeding Score table (used to calculate hemostatic efficacy)	Made definitions clearer
7.4.1	Clarified	Clarified process informing blood banks of changes to the maximum cold-stored platelet storage duration	Operational consistency for study team and at sites
7.4.4	Removed	Removed minimum number of study platelets anticipated to be available at the site prior to randomization	Detailed instructions included in manual of operations

Section	Text	Amendments	Rationale for Change
8.1	Clarified	Clarified subjects to be entered into EDC (who are not subsequently randomized)	Operational consistency at sites
10.1	Revised	Included processes to cover study being conducted in Australia	Update with new study information
11.2.1, Appendix D	Clarified	Added notes outlining adverse event definitions for this study.	Operational consistency at sites
11.2.2, Appendix D	Clarified	Added AE classification definitions	Operational consistency at sites
11.2.5, 11.2.6, 11.2.7	Corrected	Updated to reflect addition of Australian clinical site and the alignment of team responsibilities	Operational consistency for study team
Appendix C	Added	Added protamine as anticoagulant to be recorded	Commonly used for heparin reversal
Throughout Protocol	Clarified	Corrected grammatical and typographical errors, updated minor items that do not change protocol but clarifies intent	Consistency and clarity of protocol
Amendment #4 (dated 12 July 2021)			
10.4, 11.2, 11.2.3, Appendix D	Revised	Changed the reporting timeframe of SAEs from 14 to 28 days following first study platelet transfusion	FDA request
10.4, 11.2.3, Appendix D	Revised	Changed collection timeframe of thrombotic/embolic and bleeding events (AEs of special interest) to be recorded for a maximum of 28 (rather than 7) days regardless of seriousness	FDA request
Synopsis, 3.4, 10.4, Appendix D	Revised	Corrected SAE reporting timeframe to indicate recording will start at time of 1st study platelet transfusion	FDA request
7.4.2	Revised	Updated Zika testing requirements	Updated FDA guidance available
Throughout Protocol	Clarified	Corrected grammatical and typographical errors, updated minor items that do not change protocol but clarifies intent	Consistency and clarity of protocol
Amendment #3 (dated 07 May 2021)			
Throughout protocol	Revised	Changes study from double blind to partial blind	Inability to completely blind temperature of study platelets to person performing transfusion
Synopsis, 3.3	Added	Added amount of blood products and hemostatic adjuncts at 24 hours and 72 hours after first study platelet transfusion as exploratory outcomes	Identified during preparation of Statistical Analysis Plan (SAP)
Synopsis, 4.3.2, 8.2, 8.2.1, Appendix A	Clarified	Clarified that labs to confirm treatment eligibility will be performed within 72 hours of the date of surgery	Operational clarity at sites
Synopsis, 6.5	Added	Added ABO compatibility between donor and recipient and pre-operative antiplatelet agent use status as subgroups of interest to primary and secondary endpoints	Subgroups identified during preparation of SAP

Section	Text	Amendments	Rationale for Change
4.1	Revised	Corrected definitions of enrolled subjects and mITT population	Incorrect as previously written
4.1	Clarified	Slightly modified Figure 1 to clarify subjects are enrolled when platelets are ordered	Clarity and readability of protocol
7.4.2	Removed	Removed cold-stored platelet information needed only by blood suppliers	Detailed instructions included in manual of operations and procedures implemented by suppliers
7.4.4, Appendix C	Removed	Removed data collection of other fluids (including colloids, crystalloids, hetastarch)	Deemed unnecessary for safety or efficacy assessments
7.4.4, 8.5, Appendix A	Clarified	Changed renal replacement therapy to support	Clarity and readability of protocol
7.4.4, 11.1	Revised	Changed notification to DSMB of >6 doses or units of all study platelets within the intervention period to only notification if >6 doses or units of cold-stored platelets are administered	Administration of >6 doses or units of room temperature plates is not considered a potential safety concern
8.3	Removed	Deleted collection of CPB circuit features, priming & blood components and cooling duration	Deemed unnecessary for safety or efficacy assessments
8.4	Removed	Removed collection of CMV status	Deemed unnecessary information
8.5	Added	Added protocol deviations to be summarized	Operational clarity identified during preparation of SAP
10.3	Revised	Updated definition of waivers to be requested for screening information obtained prior to consent	Incorrect as previously written
10.4, 11.2.3, Appendix D	Revised	Changed the reporting timeframe of SAEs to 14 days following first study platelet transfusion	SAEs occurring after 14 days are not anticipated to be due to study platelet administration
10.4, 11.2.3	Clarified	Clarified that patients who are discharged from the hospital prior to Day 28 will receive a telephone call on Day 28 to assess mortality	Confirmation of safety outcome
Appendix C	Removed	Removed data collection of products used to prime the CPB pump	Deemed unnecessary for safety or efficacy assessments
Appendix D	Clarified	Clarified that transfusion-related AEs that occur, by definition, less than 7 days after transfusion of platelets will be recorded according to the defined event definition	Operational clarity at sites
Throughout Protocol	Clarified	Corrected grammatical and typographical errors, updated minor items that do not change protocol but clarifies intent	Consistency and clarity of protocol
Amendment #2 (dated 29 March 2021)			
Prior to Section 1	Added	Added protocol amendment summary table, synopsis and list of abbreviations	Clarity and readability of protocol

Section	Text	Amendments	Rationale for Change
1, 4.4, 7.4, 7.4.2, 9.5, 10.1, 10.2, 11.2.7, 13.1, 13.2, 13.4, 13.5, 13.6	Revised	Changed anticipated number of sites and updated protocol language to allow inclusion of non-U.S. sites	Flexibility to include enrollment at non-U.S. sites
3.2, 3.3, 3.4, 8.5, 10.4	Clarified	Clarified measurement and timing of outcomes, including if study platelets are first administered in the ICU; need for & duration of mechanical ventilation; unplanned extracorporeal support post-op	Clarity of outcome measurement & increased consistency
3.4, 10.4	Revised	Changed 'severe sepsis' to 'septic shock' as a safety outcome measure	New definitions of shock only include sepsis and septic shock
4	Revised	Defined eligibility methodology terms to clarify study and treatment eligibility	Clarity of protocol & enrollment of suitable subjects
5.2.1	Clarified	Clarified the DSMB will review the interim analysis to determine the maximum storage duration of cold-stored platelets for the next cohort	Clarity of protocol
6.5	Revised	Changed 'gender' to 'sex'	Clarity of data to be collected
6.5	Clarified	Clarified that TEG samples from 'at least' 50 subjects from at least 4 sites will be collected	Flexibility to obtain additional samples at more sites
7.1	Added	Added options to use phone or electronic consent	Operational flexibility at sites
7.2	Revised	Extended randomization window from 21 to 30 days prior to scheduled surgery	Operational flexibility at sites
7.2, 7.3, 9.4.4	Clarified	Clarified unblinded and blinded roles	Clarity and readability of protocol
7.4.2	Added	Added platelet transport details including cold-stored platelet transfer between clinical sites	Informational
7.4.2	Added	Specified that all platelets for the study will be collected by a U.S. FDA registered or licensed blood collection facility	FDA request
7.4.3	Added	Specified that 'paid donor' or 'volunteer donor' will be specified on the platelet unit label	FDA request
7.4.3	Removed	Removed details of blinding procedures	Specific blinding details will be provided in the manual of operations
7.4.4	Clarified	Clarified the intervention period for receipt of study platelets	Clarity and readability of protocol
7.4.4, 8.1	Revised	Revised the minimum number of study platelets to be available to randomize study subjects & updated dosing to only specify weight (rather than pediatric and adult)	To minimize waste based on patient weight
7.5, 7.6	Clarified	Clarified procedures to subject discontinuation (study or intervention)	Operational clarity

Section	Text	Amendments	Rationale for Change
8.2	Revised	Clarified which baseline labs must be performed for research and extended window from 48 to 72 hours prior to cardiac surgery	Operational clarity & increased flexibility at sites
8.4, 8.5, Appendix C	Added	Added complete list of concomitant medications to be collected & associated reporting timeframe	Recommendation of independent DSMB
8.5	Added	Added ±2 hour window to 6 and 24 hour lab assessments collected after first study platelet transfusion	Operational flexibility at sites
9.1	Clarified	Clarified site study files may be paper or electronic	Operational clarity
9.3, 11.2.6	Revised	Changed EDC system from OpenClinica to REDCap	Operational change
9.4.2	Revised	Removed budget as factor for monitoring visit frequency	Operational change
10.4, 11.2, 11.2.2, 11.2.3, 11.2.4, Appendix D	Added	Added list of adverse events to be collected and associated reporting timeframe	Recommendation of independent DSMB
11.2, 11.2.3	Clarified	Clarified vital signs will be recorded at Day 28 or hospital discharge, whichever comes first	Prevent subject from returning for vital signs (only) at Day 28 if discharged earlier
13.2	Revised	Changed entity to issue data use agreement for subject data protection	Operational change
14	Added	Added citation to newly published data on a pilot trial of cold-stored platelets	Informational
Appendix A	Added	Added Schedule of Events	Operational clarity
Appendix B	Added	Added list of pre-operative comorbidities to be collected and associated reporting timeframe	Recommendation of independent DSMB
Throughout	Revised	Changed ‘patient’ to ‘subject’	Consistency of protocol
Throughout	Clarified	Edited text to consistency use ‘cold-stored’ and ‘room temperature’ platelet and ‘study platelet’ to refer to blinded platelets administered during the intervention period	Consistency and clarity of protocol
Throughout	Clarified	Edited text to define abbreviations (first use)	Consistency and clarity of protocol
Throughout	Clarified	Corrected grammatical and typographical errors, updated minor items that do not change protocol but clarifies intent	Consistency and clarity of protocol
Amendment #1 (dated 16 October 2020)			
7.2, 8.1	Revised	Changed randomization responsibility to site clinical coordinator	Blood bank staff will not be responsible for randomization
~~7.4.2~~	~~Revised~~	~~Changed text to say “All platelets for the study may be collected by a U.S. licensed blood collection facility” versus “will”.~~	~~Increased flexibility~~ (Not changed per FDA request)

Section	Text	Amendments	Rationale for Change
7.4.2	Added	Added Secure Transfusion Services to the list of cold platelet providers	Added another establishment to the list of platelet providers
8.5	Revised	Changed lab assessments from 0-30m and 0-60m prior to first transfusion to 30 minutes prior to first transfusion	Consistency of lab assessments
8.2, 8.5	Revised	Changed lab measurement times from 24 and 72 hours after the first transfusion to 48 hours after the first transfusion	Consolidated time point to reduce blood sample collection
10.1	Revised	Changed Utah to Washington University as sIRB	Administrative change
11.2.6	Added	Added research monitor (medical monitor)	IRB request
3.1	Revised	Changed text to say “Patients weighing greater than 50 kg or patients weighing 50 kg or less” versus specifying pediatric/adult patients	Increased flexibility since we may have adults weighing less than 50 kg
6.3	Revised	Changed text to indicate patient analysis being done by treatment received versus study arm	Safety analysis are based off of what patients receive
7.4.4	Revised	Changed text from infusions to transfusions	Text entry error
7.4.4	Removed	Removed the following sentence “The maximum amount of cold stored platelets that can be administered to subjects in the cold platelet arm will be 6 units or pediatrics doses (10-15 mL/kg) during the 24 hours post- operative period”	Duplicative text
7.5	Revised	Changed text to state that discontinued study intervention patients will remain in the safety analysis	Added for clarification of intended practices
Original Protocol (dated 07 June 2020)			

SYNOPSIS

Name of Sponsor/Company: Philip C. Spinella / University of Pittsburgh

Name of Finished Product: Cold-Stored Platelets

Title of Study: CHIlled Platelet Study (CHIPS)

Number of Planned Centers: Approximately 30 centers in the United States and Australia

Planned Study Period: ~3.5 years
First Patient In: 4Q 2021
Last Patient Last Visit: 1Q 2025
Phase of Development: Phase 3

Study Type: Randomized, Partial Blinded, Adaptive, Non-inferiority

Objectives:
This study aims to demonstrate that platelets stored at 4°C are non-inferior in hemostatic efficacy to standard, room temperature platelets stored at 22°C when transfused to adult and pediatric patients requiring cardiac surgery and are actively bleeding. The study also aims to determine the maximum storage duration of platelets stored at 4°C, and to demonstrate comparable safety of administration of platelets stored at 4°C versus platelets stored at 22°C to adult and pediatric patients requiring cardiac surgery who are actively bleeding. These aims will be evaluated through:

Primary:

- Test the hypothesis that platelets stored at 4°C are non-inferior (or superior) in hemostatic efficacy to standard, room temperature platelets stored at 22°C when transfused to adult and pediatric patients requiring complex cardiac surgery who are actively bleeding.

***Secondary*:**

- To determine the maximum duration (up to 21 days) of storage at 4°C that maintains non-inferiority.

Exploratory:

- To demonstrate comparable safety of administration of platelets stored at 4°C versus platelets stored at 22°C to adult and pediatric patients requiring cardiac surgery who are actively bleeding.

Study Hypothesis: The hypothesis of CHIPS is that platelets stored at 4°C are non-inferior (or superior) in hemostatic efficacy to standard, room temperature platelets stored at 22°C when transfused to adult and pediatric patients requiring cardiac surgery who are actively bleeding.

Number of Patients Planned: 1,000 patients enrolled

Study Design:
This is a phase 3, multicenter, international, randomized, partial blinded, adaptive, non-inferiority, storage duration ranging trial in adult and pediatric subjects undergoing complex cardiac surgery using cardiopulmonary bypass (CBP) that will compare the transfusion of cold-stored platelets at multiple storage durations to standard room temperature stored platelets. Study platelets may be transfused to eligible subjects for active bleeding either during surgery or in the first 24 hours following ICU admission post complex cardiac surgery. With the exception of clinical providers directly transfusing study platelets at the bedside (e.g., anesthesiologist in the Operating Room or ICU nurse), all other clinical providers will be blinded to the intervention.

The study consists of a screening period, intervention period (24 hours) and follow-up period (28 days). Subjects (viable neonates ≥ 3 kg at the time of enrollment or patients > 28 days – less than 85 years at time of consent) will be allocated randomly in a 2:1 ratio to cold-stored or room temperature platelets, with the maximum allowable duration of cold storage changing adaptively. The maximum allowable duration of cold storage begins at 7 days. After each successive cohort of 200 patients, an interim analysis will be conducted that allows the maximum duration to change depending on the accumulating trials results. The DSMB will review each interim analysis and determine the maximum cold stored platelet storage duration for the next cohort. If a duration appears inferior to room temperature platelets, the trial will reduce the maximum storage duration to both fully understand the maximal non-inferior duration and protect patients from potential harm. The maximum storage duration will remain blinded during the study, with only **designated unblinded** site or study team members being aware of the actual maximum storage duration prior to database lock.

At the end of the trial, the primary analysis tests whether any duration of cold storage greater than or equal to 7 days is non-inferior to room temperature platelets. If non-inferiority is achieved in the primary analysis, the trial will report not only non-inferiority of cold-stored platelets, but an appropriate duration of storage.

The primary outcome measure is hemostatic efficacy, assessed by adapting the five-level scoring system developed by Dyke. The maximum score recorded from the start of the first platelet transfusion through 24 hours after will be compared between the two study groups, where a higher numerical signifies poorer hemostatic control (i.e., larger bleeding volume/faster bleeding rate). The score will be determined by post-operative blood drainage, blood products transfused, hemostatic agents given, and sternal closure as described by Dyke, except for minor modifications to allow for the specific context of this clinical trial. The primary outcome measure will be derived by the Data Coordinating Center after relevant information has been entered into the database. Clinical staff that are directly involved with transfusing the study platelets will not assess any outcome measures for the trial.

The secondary outcome measure is 24-hour total chest tube output. Exploratory and safety outcome measures are described in the Endpoints section of this synopsis, below.

Diagnosis and Eligibility Criteria

***Study* Eligibility:**
Inclusion Criteria
(1) Viable neonates ≥ 3 kg at time of enrollment or age greater than 28 days and less than 85 years of age at time of consent; and
(2) Planned complex cardiac surgery with planned use of cardiopulmonary bypass, with an expectation of bleeding requiring platelet transfusion.

Exclusion Criteria
(1) Expected order for washed or volume-reduced platelets;
(2) Patient with known anti-platelet antibodies (e.g., anti-HPA-1a, ITP);
(3) Platelet transfusion refractoriness due to anti-HLA antibodies;
(4) Known or suspected pregnancy;
(5) Previously randomized in this study;

(6) Conscious objection or unwillingness to receive blood products;
(7) Known IgA deficiency;
(8) Known congenital platelet disorder;
(9) Known congenital bleeding disorder (e.g., Hemophilia A, von Willebrand disease, Ehlers-Danlos syndrome);
(10) Planned post-operative extracorporeal membrane oxygenation (ECMO), ventricular assist device (VAD), and/or continuous renal replacement therapy (CRRT)/hemodialysis;
(11) Patients intended to receive whole blood either intra-operative or post-operative for bleeding.

Once a subject is determined to be eligible to participate in the study, the subject must then meet the *treatment* eligibility criteria prior to receiving a transfusion of study platelets.

***Treatment* Eligibility:**

Inclusion Criteria

(1) Must have pre-operative labs (CBC) completed within 72 hours prior to the date of surgery (inclusive of the day of surgery).

Exclusion Criteria

(1) Platelet transfusion of any type within 24 hours prior to the date of surgery;
(2) Preoperative thrombocytopenia, defined as platelet count $<75x10^9$/L, based on the most recent labs completed within 72 hours prior to the date of surgery (inclusive of the day of surgery).

Test Product, Dose, Mode of Administration:
Up to eight units or eight pediatric doses of cold-stored (4°C) platelets transfused within a 24-hour period.

Duration of Treatment: 24 hours

Reference Therapy, Dose and Mode of Administration:
Up to eight units or eight pediatric doses of room temperature (22°C) platelets transfused within a 24-hour period.

Criteria for Evaluation (Endpoints):

Primary

- The primary evaluation is an adapted Perioperative Bleeding Score measured from the start of the first platelet transfusion through 24 hours.

Secondary

- The secondary evaluation is the measure of chest tube output from the time of ICU admission, if the first platelet transfusion occurs in the OR, or from the start of the first platelet transfusion, if the first transfusion occurs after ICU admission, through 24 hours.

Exploratory

- Total and individual blood product administration (donor exposures and mL/kg/component) for RBCs, plasma, platelets, cryoprecipitate, and whole blood. *Note that blood used for CPB priming and RBC transfusion for preoperative anemia will not be included.*
  - From initiation of surgery to 24 hours from the start time of the first platelet transfusion.
  - From initiation of surgery to 72 hours from the start time of the first platelet transfusion.
  - From the start time of the first platelet transfusion to 24 hours after the start time of the first platelet transfusion.
  - From the start time of the first platelet transfusion to 72 hours after the start time of the first platelet transfusion.
- Total dose of individual hemostatic adjuncts (antifibrinolytics and coagulation factor concentrates)
  - From initiation of surgery to 24 hours from the start time of the first platelet transfusion.
  - From initiation of surgery to 72 hours from the start time of the first platelet transfusion.
  - From the start time of the first platelet transfusion to 24 hours after the start time of the first platelet transfusion.
  - From the start time of the first platelet transfusion to 72 hours after the start time of the first platelet transfusion.
- Need for and duration of mechanical ventilation for up to 28 days after first study platelet transfusion.
- ICU and hospital length of stay.
- The relative change in the peri-operative bleeding score from pre-intervention (i.e., before the first study platelet transfusion) to post-intervention
- Relative change in hemostatic parameters: CBC (including platelet count and hemoglobin), INR, PTT, and fibrinogen from before first platelet transfusion to 6 and 24 hours after the first platelet transfusion. In a subset of patients, thromboelastogram (TEG) with platelet mapping will also be compared between study groups at the above timepoints.

Safety

The safety and tolerability of cold-stored platelets will be measured by:

- Unplanned sternal closure delay.
- Re-exploration for bleeding within 24 hours of ICU admission.
- Unplanned extracorporeal support post operatively within 48 hours of first study platelet transfusion.
- Morbidities within 7 days of first study platelet transfusion:
  - Acute respiratory distress syndrome.
  - Need for and duration of renal support.
  - Renal failure.
  - Septic shock.
- Measures of end organ injury to include BUN, creatinine, lactate, troponin, alanine aminotransferase (ALT) within 48 hours of the first platelet transfusion, if drawn clinically.
- Transfusion associated adverse events classified as per the Center for Disease Control and Prevention (CDC) National Healthcare Safety Network Biovigilance Component Hemovigilance Module Surveillance Protocol (www.cdc.gov/nhsn) within 7 days of first study platelet transfusion as assessed by a blinded investigator (or designee) at the site.
- Arterial thrombotic events (stroke, MI) within 7 days of first study platelet transfusion.

- Venous thrombotic events within 7 days of first study platelet transfusion (deep vein thrombosis or pulmonary embolism confirmed by Doppler ultrasound, venography, perfusion scan, spiral CT, MRI or pulmonary angiogram).
- 28-day all-cause mortality.

Statistical Methods:

The following populations will be defined during statistical analyses:

Screened population: All patients screened (i.e., who signed the informed consent).

Safety population: The safety dataset will use all consented subjects who were transfused at least one platelet product. Subjects will be analyzed by treatment received, and those who are transfused both room temperature and cold platelets will be analyzed as an independent group. Subjects who are randomized and then not transfused platelets will not be analyzed.

Modified ITT analysis: All subjects will be analyzed as they are randomized into either study group with the exception of subjects who are not transfused platelets after randomization. Unless otherwise specified, only subjects who were randomized, underwent cardiac surgery and received at least one platelet transfusion during their surgery or in the 24 hours following ICU admission post-cardiac surgery will be analyzed.

The primary and secondary analyses will be compared in the following subgroups of interest:

- Subjects ABO group
- Sex
- Race/ethnicity
- Cardiac surgery severity score, defined as Surgical Complexity Score (adult) or O'Brien STAT Mortality Category (pediatric)
- Age group (<12, 12 to 64, ≥65 years of age)
- Volume of platelets transfused (< versus ≥ 15 mL/kg for under 12 years of age, < versus ≥ 10 mL/kg for 12 years or older)
- ABO compatibility of platelets between donor and recipient (compatible, major and minor incompatibility)
- Preoperative antiplatelet agent use status

Regressions will be performed using an interaction between randomization arm and subgroup of interest to determine if effects differ by subgroup levels.

The primary analysis is tested using a Bayesian model, described in Section 5.3, that relates the duration of cold storage to the hemostatic efficacy score.

Among the secondary, exploratory, and safety endpoints, continuous outcomes will be compared between treatment groups using a Van Elteren test stratified by site. Dichotomous outcomes will be compared between treatment groups using a Cochran-Mantel-Haenszel test stratified by site. In cases of small counts, Fisher's Exact test may be used. For sensitivity analyses, appropriate regressions will also be performed using treatment group as a predictor and will control for relevant confounders.

Sample Size:

The study intends to enroll 1000 subjects.

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ADP	Adenosine Diphosphate
ALT	Alanine Transaminase
Anti-HLA	Anti-Human Leukocyte Antigen
Anti-HPA	Anti-Human Platelet Antigen
ASD	Atrial Septal Defect
AV	Atrioventricular
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CCC	Clinical Coordinating Center
CDC	Centers for Disease Control
CHIPS	Chilled Platelet Study
CPB	Cardiopulmonary Bypass
CRRT	Continuous Renal Replacement Therapy
CT	Computerized Tomography
DCC	Data Coordinating Center
DIC	Disseminated intravascular coagulation
DSMB	Data Safety and Monitoring Board
EC	Ethics Committee
ECMO	Extracorporeal Membrane Oxygenation
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GI	Gastrointestinal
ICCBBA	International Council for Commonality in Blood Banking Automation
ICU	Intensive Care Unit
IRB/EC	Institutional Review Board/Independent Ethics Committee
ISBT	International Standard for Blood and Transplant
ITP	Immune Thrombocytopenia
ITT	Intent to Treat
LVAD	Left Ventricular Assist Device
MA	Maximum Amplitude
MI	Myocardial Infarction
mITT	Modified Intent to Treat
MRI	Magnetic Resonance Imaging
NYHA	New York Heart Association
OR	Operating Room PACU Post Anesthesia Care Unit
PAS	Platelet Additive Solution
PFO	Patent Foramen Ovale
PI	Principal Investigator
PPA	Per Protocol Analysis
PTT	Partial Thromboplastin Time
INR	International Normalized Ratio
PTP	Post Transfusion Purpura

RCT	Randomized Controlled Trial
sIRB	Single Institutional Review Board
STAT	Society of Thoracic Surgeons-European Association for Cardio-Thoracic Surgery
TACO	Transfusion Associated Circulatory Overload
TAD	Transfusion Associated Dyspnea
TA-GVHD	Transfusion Associated Graft Versus Host Disease
TEG	Thromboelastography
TGA	Therapeutic Goods Authority
TMF	Trial Master File
TRALI	Transfusion Related Acute Lung Injury
UDPB	Universal Definition of Perioperative Bleeding
U.S.	United States
VAD	Ventricular Assist Device
VSD	Ventricular Septal Defect

1 STUDY SUMMARY

The desired effect of transfusing platelets is to control sites of active bleeding without contributing to thromboembolic complications. During intra-operative and post-operative bleeding following cardiopulmonary bypass (CPB), effective hemostasis without thromboembolic complications is critical. Whether cold-stored platelets will accomplish hemostatic control as well as or better than standard, room temperature stored platelets in the bleeding CPB subject has not been determined in a large multicenter clinical trial.

CHIPS (CHIlled Platelet Study) is a phase 3, multicenter, international, randomized, partial-blinded, adaptive, non-inferiority, storage duration ranging trial in adult and pediatric subjects undergoing complex cardiac surgery that will compare the transfusion of cold-stored (1 to 6°C, henceforth referred to as 4°C or cold-stored) platelets at multiple storage durations to standard room temperature stored (20 to 24°C, henceforth referred to as 22°C or room temperature) platelets. The goal of the trial is to determine whether platelets stored at 4°C are non-inferior (or superior) in terms of hemostatic efficacy relative to platelets stored at 22°C and, if so, to determine the maximum duration of storage at 4°C that maintains non- inferiority. The data generated in this trial with cardiac surgery patients may be generalizable to other patient populations with life-threatening hemorrhage to include traumatic injury, and gastrointestinal and obstetric bleeding. If trial results support the use of cold-stored platelets, the data will be used to request FDA approval of a longer storage duration of cold-stored platelets.

1.1 Hypothesis

The hypothesis of this study is that platelets stored at 4°C are non-inferior (or superior) in hemostatic efficacy to standard, room temperature platelets stored at 22°C when transfused to adult and pediatric patients requiring cardiac surgery who are actively bleeding.

1.2 Specific Aims

This project has the following Specific Aims:

Specific Aim 1. Test the hypothesis that platelets stored at 4°C are non-inferior (or superior) in hemostatic efficacy to standard, room temperature platelets stored at 22°C when transfused to adult and pediatric patients requiring complex cardiac surgery who are actively bleeding.

Specific Aim 2. If the hypothesis in Specific Aim 1 is supported, determine the maximum duration (up to 21 days) of storage at 4°C that maintains non-inferiority.

Specific Aim 3. Demonstrate comparable safety of administration of platelets stored at 4°C versus platelets stored at 22°C to adult and pediatric patients requiring cardiac surgery who are actively bleeding.

2 RATIONALE AND BACKGROUND

The rationale to perform a trial comparing 4°C platelets (maximum storage up to 21 days) to 22°C platelets (maximum storage duration 7 days) is based on the hypothesis that platelets stored at 4°C have increased hemostatic efficacy, improved safety, and are logistically superior due to the extended shelf life. Platelet components in the U.S. are currently FDA-approved for room temperature storage for up to 5 to 7 days. In June 2015, the FDA approved apheresis-collected platelets to be stored at 1 to 6°C for up to 3 days for the resuscitation of bleeding patients (21 CFR 610.53; 2016 and 21 CFR 606.65(e); 2016), and in June 2023, the FDA issued a Guidance for Industry for "Alternative Procedures for the Manufacture of Cold-Stored Platelets Intended for the Treatment of Active Bleeding when Conventional Platelets Are Not Available or Their Use is Not Practical".

Platelet components in Australia are currently TGA-approved for room temperature storage for up to 7 days.

2.1 Potential Increased Hemostatic Efficacy

Increased efficacy of platelets at 4°C is based on *in vitro* data indicating superior aggregation, viscoelastic properties, clot retraction, and occlusion time in microfluidic models when compared to platelets at 22°C. The improved function is maintained for up to 21 days with 4°C platelets when compared to 22°C platelets.[1–8]

Clinical data from a randomized controlled trial (RCT)[7] indicated 4°C platelets improved bleeding time significantly compared to 22°C platelets in thrombocytopenic patients; this has also been demonstrated in patients on aspirin.[9] Another RCT in children where platelets were stored at 4°C in whole blood reported reduced blood loss and improved platelet aggregation compared to platelets stored at 22°C. Recently, a pilot RCT in Norway indicated less blood loss and blood utilization when platelets at 4°C were transfused compared to platelets stored at 22°C when the storage duration was 7 days in each group (PI: Geir Strandenes, "Transfusion of Cold-stored Platelet Concentrates", NCT02495506.) This trial was extended to include platelets out to 14 days of storage. There was no statistical difference in chest tube output for patients transfused room temperature platelets up to 7 days of storage compared to cold-stored platelets out to 14 days of storage.[10]

Cold-stored platelets have been shown to retain improved hemostatic function and produce stronger clot formation for up to at least 21 days of storage.[1–3, 5, 7, 11]

2.2 Reduced Bacterial Contamination

Improved safety with platelets stored at 4°C compared to 22°C platelets is based upon reduced bacterial contamination risk.

2.3 Logistical Advantages

Logistical benefits of 4°C platelets compared to 22°C platelets are based upon the potential increased storage duration with 4°C and the resultant reduced waste. Increased storage duration to 21 days for 4°C platelets would also permit for them to be stored in more rural hospitals and therefore provide availability of platelets in hospitals that could not previously maintain an inventory. This logistic benefit may lead to improved outcomes by facilitating the use of platelets

for patients with severe bleeding at hospitals that would not otherwise have platelets available (if the storage duration was only 5 to 7 days with 22°C storage).

3 STUDY OUTCOMES

3.1 Primary Outcome: Hemostatic Efficacy

The primary outcome measure of this clinical trial is hemostatic efficacy, assessed by adapting the objective five-level scoring system developed by Dyke[12] (Table 2). The maximum score recorded from the start of the first platelet transfusion through 24 hours after will be compared between the two study groups. A higher numerical score signifies poorer hemostatic control (i.e., larger bleeding volume/faster bleeding rate). The Perioperative Bleeding Score has been adapted by the addition of weight-adjusted drainage and transfusion volume criteria for subjects weighing 50 kg or less (Table 2b).

Table 2: Universal Definition of Perioperative Bleeding Score

UDPB SCORE (Bleeding Definition)	**Sternal closure delayed**	**Post-op chest tube blood loss within 12 h (mL)**	**RBC[a] (units)**	**Plasma (units)**	**PLT[b]**	**Cryo**	**PCCs**	**FVIIa**	**Re-exploration/ tamponade**
1 Insignificant	No	< 600	0	0	0	No	No	No	No
2 Mild	No	600–800[c]	1	1[c]	0	No	No	No	No
3 Moderate	No	801–1000	2–4	2–4	Yes	Yes	Yes	No	No
4 Severe	Yes	1001–2000	5–10	5–10	N/A	N/A	N/A	No	Yes
5 Massive	N/A	> 2000	> 10	> 10	N/A	N/A	N/A	Yes	N/A

(a) Patients weighing greater than 50 kg.

UDPB SCORE (Bleeding Definition)	**Sternal closure delayed**	**Post-op chest tube blood loss within 12 h (mL/kg)**	**RBC[a] (mL/kg)**	**Plasma (mL/kg)**	**PLT[b]**	**Cryo**	**PCCs**	**FVIIa**	**Re-exploration/ tamponade**
1 Insignificant	No	< 8	0	0	0	No	No	No	No
2 Mild	No	8 – <12	>0 – <8	>0 - <8	0	No	No	No	No
3 Moderate	No	12 – <14	8 – <18	8 – <18	Yes	Yes	Yes	No	No
4 Severe	Yes	14 – 29	18 – 40	18 – 40	N/A	N/A	N/A	No	Yes
5 Massive	N/A	>29	> 40	> 40	N/A	N/A	N/A	Yes	N/A

(b) Patients weighing 50 kg or less.

a Red blood cell (RBC) units should only be counted when accompanied by bleeding. RBCs administered for dilutional anemia are not counted.
b The first platelet transfusion, whether it occurs in the Operating Room (OR) or post-operatively in the intensive care unit (ICU) will not count for purposes of determining the Universal Definition of Perioperative Bleeding (UDPB) Score.
c Modified from the original score developed by Dyke et al.

The score will be derived by the Data Coordinating Center (DCC) after all relevant information, including post-operative blood drainage, blood products transfused, hemostatic agents given, and

sternal closure as described by Dyke, except for the following minor modifications to allow for the specific context of the proposed clinical trial, has been entered into the database.

The first platelet transfusion that occurs during the study period of the trial (intra- operative time or 24 hours post-operative in the ICU) will not be included in the calculation of the Perioperative Bleeding Score. This is to allow for the entire 5-point system of the score to be used and increase the discriminatory power of the outcome. If there is a second platelet transfusion, it will be included in the score as an appropriate component of the subject's clinical outcome.

The Perioperative Bleeding Score has also been adapted to include both intra-operative and post-operative time periods to account for intra-operative bleeding, chest tube output, and transfusion. Both intra- and post-operative assessments are essential in this trial to assess the hemostatic effect of platelet storage temperature. Most patients receive their first platelet transfusion intra-operatively. In one recent pilot RCT, 87% of patients transfused platelets received the first unit in the OR. Intra-operative assessment may be highly illustrative of an intervention's hemostatic effect but not correlate with subsequent post-operative assessments. A significant amount of bleeding may occur intra-operatively when drainage tubes are not yet present.

3.2 Secondary Outcome Measure

The secondary outcome measure of this clinical trial is 24-hour total chest tube output as measured from time of ICU admission if study platelets were administered during surgery or from the start of the first study platelet transfusion if study platelets were administered in the ICU. The use of chest tube output as the primary outcome would not capture all of the blood loss that occurs intra-operatively because drains are not typically placed until intra-operative bleeding is primarily controlled before chest closure. Chest tube output measurement is also confounded if there is re-exploration for bleeding, and late drainage usually becomes serous.

3.3 Exploratory Outcome Measures

1. Total and individual blood product administration (donor exposures and mL/kg/component) for RBCs, plasma, platelets, cryoprecipitate, and whole blood. Note that blood used for CPB priming and RBC transfusion for preoperative anemia will not be included.
    (a) From initiation of surgery to 24 hours from the start time of the first study platelet transfusion.
    (b) From initiation of surgery to 72 hours from the start time of the first study platelet transfusion.
    (c) From the start time of the first platelet transfusion to 24 hours after the start time of the first study platelet transfusion.
    (d) From the start time of the first platelet transfusion to 72 hours after the start time of the first study platelet transfusion.
2. Total dose of individual hemostatic adjuncts (antifibrinolytics and coagulation factor concentrates):
    (a) From initiation of surgery to 24 hours from the start time of the first study platelet transfusion.

(b) From initiation of surgery to 72 hours from the start time of the first study platelet transfusion.
(c) From the start time of the first platelet transfusion to 24 hours after the start time of the first study platelet transfusion.
(d) From the start time of the first platelet transfusion to 72 hours after the start time of the first study platelet transfusion.

3. Need for and duration of mechanical ventilation for up to 28 days after first study platelet transfusion.

4. ICU and hospital length of stay.

5. The relative change in the peri-operative bleeding score from pre-intervention (i.e., before the first study platelet transfusion) to post-intervention.

6. Relative change in hemostatic parameters: CBC (including platelet count and hemoglobin), INR, PTT, and fibrinogen from before first platelet transfusion to 6 and 24 hours after the first platelet transfusion. In a subset of patients, thromboelastogram (TEG) with platelet mapping will also be compared between study groups at the above timepoints.

3.4 Safety Outcome Measures

1. Unplanned sternal closure delay.

2. Re-exploration for bleeding within 24 hours of first study platelet transfusion.

3. Unplanned extracorporeal support post operatively within 48 hours of first study platelet transfusion.

4. Morbidities within 7 days of first study platelet transfusion:

(a) Acute respiratory distress syndrome
(b) Need for and duration of renal support
(c) Renal failure
(d) Septic shock

5. Measures of end organ injury to include BUN, creatinine, lactate, troponin, alanine aminotransferase (ALT) within 48 hours from the start of the first platelet transfusion if drawn clinically.

6. Transfusion associated adverse events classified as per the Center for Disease Control and Prevention (CDC) National Healthcare Safety Network Biovigilance Component Hemovigilance Module Surveillance Protocol (www.cdc.gov/nhsn) within 7 days of first study platelet transfusion as assessed by a blinded investigator (or designee) at the site.

7. Arterial thrombotic events (stroke, MI) within 7 days of first study platelet transfusion.

8. Venous thrombotic events within 7 days of first study platelet transfusion (deep vein thrombosis or pulmonary embolism confirmed by Doppler ultrasound, venography, perfusion scan, spiral CT, MRI or pulmonary angiogram).

9. 28-day all-cause mortality.

4 SITE AND SUBJECT ELIGIBILITY AND ACCRUAL

4.1 Eligibility Methodology Terms

Eligible subject. A subject who meets all preoperative *study* eligibility criteria but who may not yet meet *treatment* eligibility criteria.

Consented subject. An eligible subject who has been consented and has assented when appropriate to participate in CHIPS.

Randomized subject. An eligible subject who has been consented and been assigned a randomized treatment group.

Enrolled subject. A randomized subject who has met both study eligibility criteria and treatment eligibility criteria *and for whom platelets have been ordered to be transfused* for active bleeding during the CHIPS study intervention period (intra-operative or within 24 hours of admission into the ICU). Platelets being sent to the operating room on hold for transfusion does not meet the definition of an order to be transfused. An enrolled subject is the ITT population as described below.

ITT population. Any subject who meets the definition of enrolled whether or not platelets are actually administered.

mITT population. A subset of the ITT population. An enrolled subject who receives platelets will be included in the modified intent to treat population (mITT).

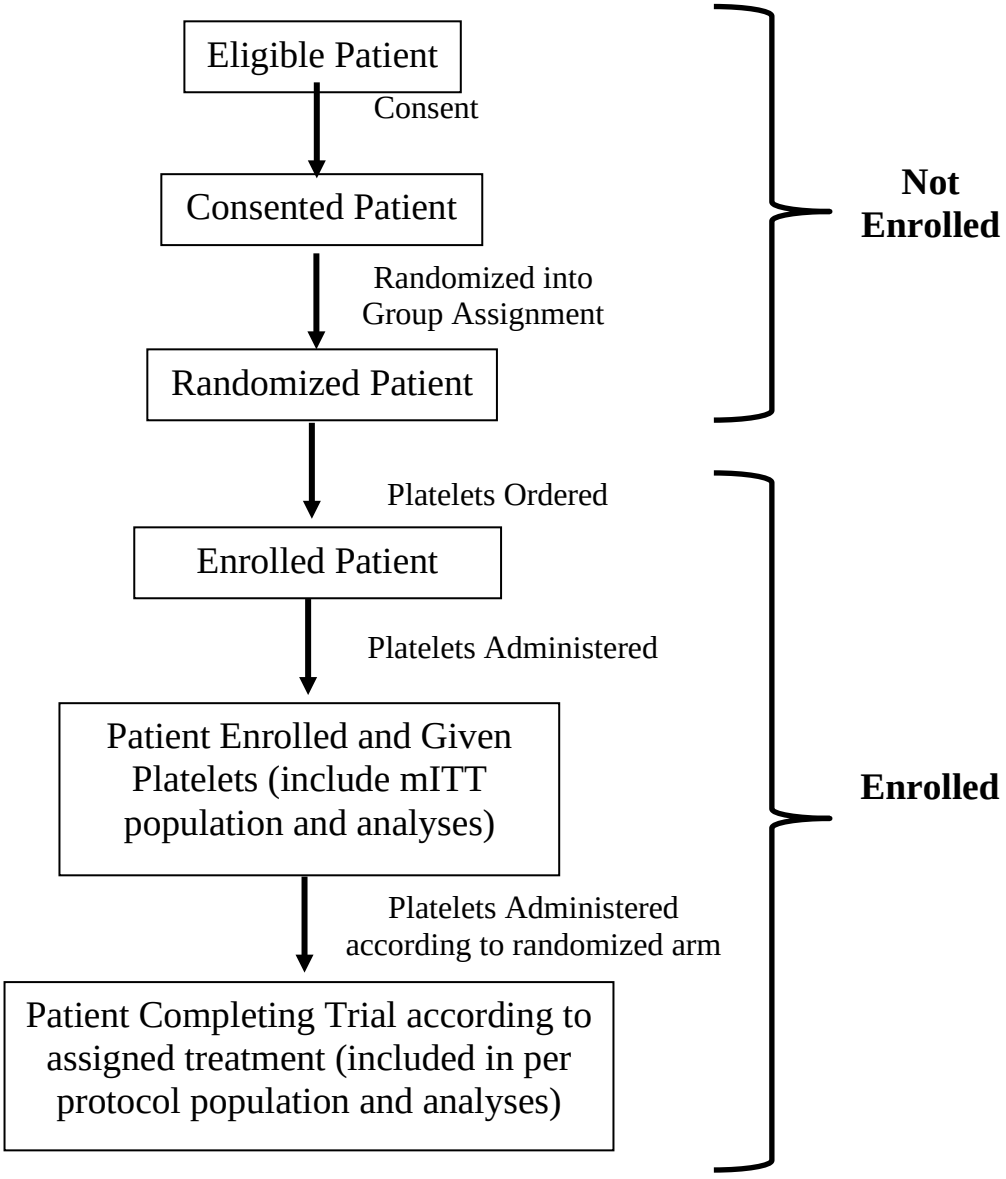


Figure 1: **Eligibility flow chart.**

4.2 Site eligibility criteria

Eligible sites *must* routinely use apheresis platelets for bleeding from cardiac surgery. Sites that routinely use whole blood derived platelets or cold-stored whole blood for bleeding from cardiac surgery are *not* eligible to participate in this trial.

4.3 Subject eligibility criteria

Eligible participants will be identified by on-site study staff. There will be two sets of eligibility criteria assessed at different time points prior to enrolling the subject: Study Criteria and Treatment Criteria.

4.3.1 Study eligibility criteria

Study eligibility criteria may be assessed at any time prior to the subject signing consent.

Study inclusion criteria are:

1. Viable neonates ≥ 3 kg at time of enrollment (as defined in Section 4.1) OR age greater than 28 days and less than 85 years of age at time of consent; AND
2. Planned complex cardiac surgery with planned use of cardiopulmonary bypass, with an expectation of bleeding requiring platelet transfusion.

A “neonate” subject is a newborn from date of birth through day 28 after birth; furthermore, "viable neonate” means being able, after delivery, to survive (given the benefit of available medical therapy) to the point of independently maintaining heartbeat and respiration as outlined as in 45 CFR 46.202(h).

Complex adult cardiac surgery includes but is not limited to: repeat sternotomy, planned combined procedures as described by Pettersson[13] which consists of single procedures plus at least one additional surgical component, or cardiac surgery with an expectation of bleeding requiring platelet transfusion. Single procedures include coronary artery bypass graft, aortic or mitral valve surgery, or surgery on the aorta (aortic root, ascending aorta, or arch replacement).

Complex surgical procedures (adult or pediatric) also would include, at the discretion of the site investigator, outflow tract repairs or revisions, ventricular restoration or reconstruction procedures, tumor resection, cardiac transplantation, or any procedure that according to the investigator is at high risk of bleeding and requiring a platelet transfusion.

Complex pediatric cardiac surgery includes repeat sternotomy, or at least one of the following procedures:

- Absent pulmonary valve repair
- Anomalous left coronary artery reimplantation
- Aortic stenosis repair, valvular or supravalvular or bicuspid
- Complex coarctation of the aorta repair
- Damus-Kaye-Stansel procedure
- Double Aortic Arch repair
- Double inlet left ventricle repair
- Double outlet right ventricle repair
- Ebsteins Anomaly repair
- Eisenmenger Complex repair
- Endocardial cushion defect repair (AV canal repair)
- Hypoplastic left heart syndrome repair (Glenn, Fontan)
- Interrupted aortic arch repair
- Partial anomalous pulmonary venous repair
- Pulmonary atresia repair
- Ross procedure
- Other single ventricle physiology repair
- Tetralogy of Fallot repair
- Total anomalous pulmonary venous repair
- Transposition of the great arteries
- Truncus arteriosus repair

Isolated simple PFO, ASD, or VSD initial repairs are not considered complex surgical procedures and those children should not be enrolled.

Study exclusion criteria are:

1. Expected order for washed or volume reduced platelets;
2. Patient with known anti-platelet antibodies (e.g., anti-HPA-1a, ITP);
3. Platelet transfusion refractoriness due to anti-HLA antibodies;
4. Known or suspected pregnancy;
5. Previously randomized in this study;
6. Conscious objection or unwillingness to receive blood products;
7. Known IgA deficiency;
8. Known congenital platelet disorder;
9. Known congenital bleeding disorder (e.g., Hemophilia A, von Willebrand disease, Ehlers-Danlos syndrome);
10. Planned post-operative extracorporeal membrane oxygenation (ECMO), ventricular assist device (VAD), and/or continuous renal replacement therapy (CRRT)/hemodialysis;
11. Patients intended to receive whole blood either intra-operative or post-operative for bleeding.

4.3.2 Treatment eligibility criteria

Treatment eligibility criteria must be confirmed within 24 hours prior to the date of surgery. Labs to confirm treatment eligibility must be completed within 72 hours prior to the date of surgery (inclusive of the day of surgery). See Appendix A, Schedule of Events, for more detail regarding timing of assessment of treatment eligibility criteria.

Treatment exclusion criteria are:

1. Platelet transfusion of any type within 24 hours prior to the date of surgery.
2. Preoperative thrombocytopenia, defined as platelet count $<75x10^9$/L, based on the most recent labs completed within 72 hours prior to the date of surgery.

4.4 Subject Accrual and Study Duration

Accrual of 1,000 subjects enrolled is anticipated to require up to 3.5 years of enrollment at approximately 30 clinical sites in the United States and Australia.

5 CHIPS ADAPTIVE TRIAL DESIGN

The CHIPS adaptive trial design was prepared by Berry Consultants.

5.1 Introduction

The CHIPS trial investigates cold-stored platelets compared to room temperature platelets in cardiac surgery patients. The primary endpoint is the adapted 5-point Hemostatic Efficacy scale developed by Dyke, with a primary non-inferiority analysis using a non-inferiority margin of 1 point on the 5-point scale (lower scores are desirable on the Dyke scale). Recognizing that longer durations of cold storage may result in changing (likely decreasing) efficacy, the trial adaptively explores durations of cold storage, up to 21 days. The trial begins with shorter durations of cold storage and increases durations of cold storage if non-inferiority is demonstrated for shorter durations, up to the maximum duration. If a duration appears inferior to room temperature platelets, the trial will reduce the maximum storage duration to both fully understand the maximal non-inferior duration and protect patients from potential harm. At the end of the trial, the primary analysis tests whether any duration of cold storage greater than or equal to 7 days is non-inferior to room temperature platelets. If non-inferiority is achieved in the primary analysis, the trial will report not only non-inferiority of cold platelets, but an appropriate duration of storage.

Define $\eta(x)$ as the true mean hemostatic efficacy score for cold platelets stored x days and define μ_{warm} as the mean hemostatic efficacy score for room temperature platelets. The primary analysis is a test of the hypotheses:

H_0**:** No $\eta(x) < \mu_{warm} + 1$ for $x \geq 7$

H_A**:** At least one $\eta(x) < \mu_{warm} + 1$ for $x \geq 7$.

The primary analysis is tested using a Bayesian model, described below, that relates the duration of cold storage to the hemostatic efficacy score. Using this model, we obtain a posterior distribution for all $\eta(x)$ and for μ_{warm} based on the accumulating trial data. From this posterior distribution, we can compute the posterior probability of non-inferiority:

$$\Pr(NI)_x = \Pr(\eta(x) < \mu_{warm} + 1)$$

for each value of x. The primary analysis rejects H_0 and declares non-inferiority of cold platelets if any $\Pr(NI)_x > 0.975$ for $x \geq 7$. This analysis controls Type I Error at 0.025, demonstrated by simulation below. No alpha penalty is taken here because the model itself is assumed to be monotonically increasing (e.g., higher durations of storage perform worse than shorter durations) and is sufficiently conservative to control type I Error even accounting for the adaptive trial and multiple durations.

If non-inferiority is declared for any $x \geq 7$, a gated 2.5% one-sided Type I Error controlled test of superiority of any duration of cold-stored platelets over room temperature platelets will be conducted, also described below.

5.2 Trial Design Summary

5.2.1 Adaptive Duration Selection and Futility Rule

Patients will be allocated randomly in a 2:1 ratio to cold-stored and room temperature platelets, with the maximum allowable duration of cold storage changing adaptively. The maximum allowable duration of cold storage begins at 7 days. After each successive cohort of 200 patients, an interim analysis will be conducted that allows the maximum duration to change depending on the accumulating trial results. The DSMB will review each interim analysis and determine the maximum CSP storage duration for the next cohort. Additional details are provided in the statistical analysis plan and DSMB charter. Let CUR be the current maximal allowable duration going into an interim analysis.

Using the Bayesian model for $\eta(x)$ (described in the next section), we compute the posterior probability of non-inferiority of cold platelets at each integer duration x. We then identify the largest x for which $\Pr(NI)_x > 0.33$. Define this as the candidate duration CAN.

If the candidate duration is at least 7 days, then the new maximal allowable duration after the interim analysis is the minimum of CAN, CUR+5, and 21. This construction will often use the candidate, but we limit increases to at most 5 days at any interim analysis, and of course the maximum possible duration of cold storage is 21 days.

If the candidate duration is less than 7 days, a futility rule will be applied. The trial will stop enrollment for futility if $\Pr(NI)_7 < 0.1$. If the futility rule is not met, the maximum allowable duration will be set to 7 days after the interim analysis.

There is no provision for early stopping for success.

5.2.2 Gated Superiority Analysis

If the primary analysis results in a claim of non-inferiority, then a gated secondary superiority analysis (for any cold storage duration) will be conducted on the hypotheses:

H_0**:** No $\eta(x) < \mu_{warm}$

H_A**:** At least one $\eta(x) < \mu_{warm}$

This analysis is prone to multiplicities. To test these hypotheses, we compute $\Pr(Sup)_x = \Pr(\eta(x) < \mu_{warm} - \delta_x)$ where δ_x is given in the table below.

x	1	2	3	4	5	6	7	8	9	10+
δ_x	.21	.19	.17	.14	.12	.09	.05	.03	.01	0

The secondary analysis will claim superiority if any $\Pr(Sup)_x$ exceeds 0.983. The δ_x and 0.983 thresholds are a necessary penalty to obtain 2.5% Type I Error for this analysis. Details of Type I Error appear in the simulations section.

5.3 Statistical Model

At each interim analysis, and after full enrollment, the trial decisions outlined above are made based on the estimated efficacy of cold platelets stored at each duration. These efficacy estimates are made by a statistical model that estimates the relationship between cold storage duration and hemostatic efficacy. The hemostatic efficacy estimates at each whole number cold-storage duration (1, 2, 3, . . . , 19, 20, 21 days) are compared to the estimated mean hemostatic efficacy of platelets stored at room temperature to determine at which durations cold-stored platelets are at least non-inferior to room temperature platelets.

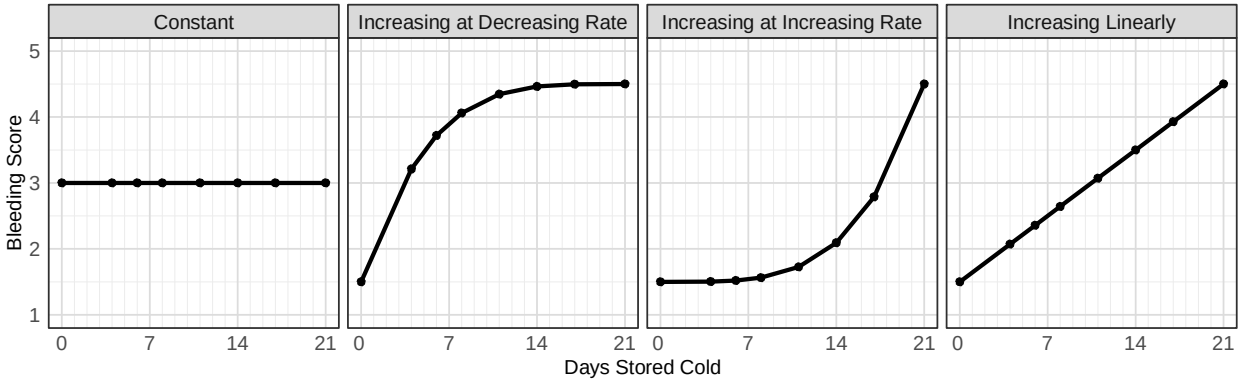


Figure 2: Examples of fit that could be obtained by a monotone piecewise linear model. Points in the plots represent the "pivot" points of the model where the slope changes

To model the relationship between cold storage time and the bleeding score we utilize a monotonic piece-wise linear regression model. This model assumes that the duration response curve $\eta(x)$ is continuous and non-decreasing over the entire 21-day duration range. The estimated model has no points of discontinuity, but at 4, 7, 8, 11, 14, and 17 days the slope of the duration response model is allowed to change. Figure 2 shows different possible curves that can be fit by this model.

Formally, let:

$$\eta(x) = \beta_0 + \sum_{i=1}^{7} \beta_i (R_{i+1} - R_i)\mathbb{I}(x > R_{i+1}) + \sum_{i=7}^{7} \beta_i (x - R_i)\mathbb{I}(R_i < x \leq R_{i+1})$$

where R_i is i_{th} element of the vector $\{0, 4, 7, 8, 11, 14, 17, \infty\}$ representing the pieces of the piece-wise regression model duration axis, and $\mathbb{I}$ is the indicator function. The first summation contributes the expectations for the duration regions smaller than x, and the second summation contributes to the expectation for the duration region that x falls within.

The β_0 parameter estimates the cold platelet efficacy at 0 days storage time. β_1 estimates the slope for the decay in efficacy for the region of 0 to 4 days stored cold, β_2 for the second region (4–7), and so on up to β_7 representing the slope for days over 17 days. To enforce monotonicity of the decay, the slopes $\beta_1, \beta_2, \ldots, \beta_7$ are bound to be greater than or equal to 0.

The observed data are assumed to be normally distributed with mean bleeding score $\eta(x)$ and variance σ_2.

5.3.1 Prior Specification

The prior for the intercept parameter β_0 is uniform from 1 to 5, which is the possible range of bleeding scores. β_1 has a truncated (restricted to non-negative values) Laplace prior with a location parameter of 0 and a scale parameter of 0.075. β_2 and β_3, representing slopes from 4 to 7 and 7 to 8, respectively, have truncated Laplace priors with their locations at the previous region's slope and scale parameters of 0.075 (e.g., $\beta_3 \sim \text{Laplace}^+(\beta_2, 0.075)$). The parameters β_4 through β_7 have truncated Laplace distributions with location at the previous β and scale parameters of 0.03. The combination $\beta_0 + \sum_{i=1}^{7} \beta_i(R_{i+1} - R_i)$ (where R is the vector {0, 4, 7, 8, 11, 14, 17, 21}) is constrained to be between 1 and 5, similar to the constraint on β_0. Restricting this value prevents model estimates across all durations from being outside the range of possible bleeding score values. Finally, the data variance parameter σ^2 is given an inverse gamma prior with shape parameter 1 and scale parameter 1.

The mean of room temperature platelets μ_{warm} is given a non-informative $N(2, 10_2)$ prior. This prior is non-informative because the large variance relative to the 1–5 scale makes the prior essentially uniform across the possible values of μ_{warm}.

5.3.2 Model Fit Example

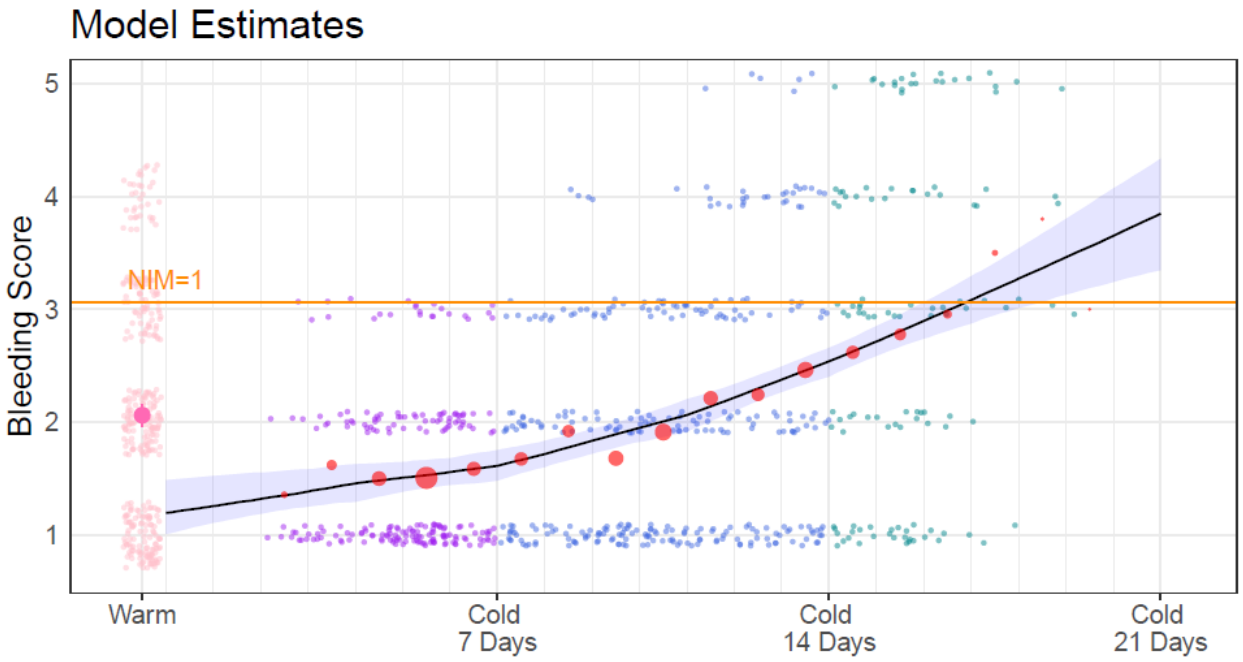


Figure 3: Model fit on a simulated dataset

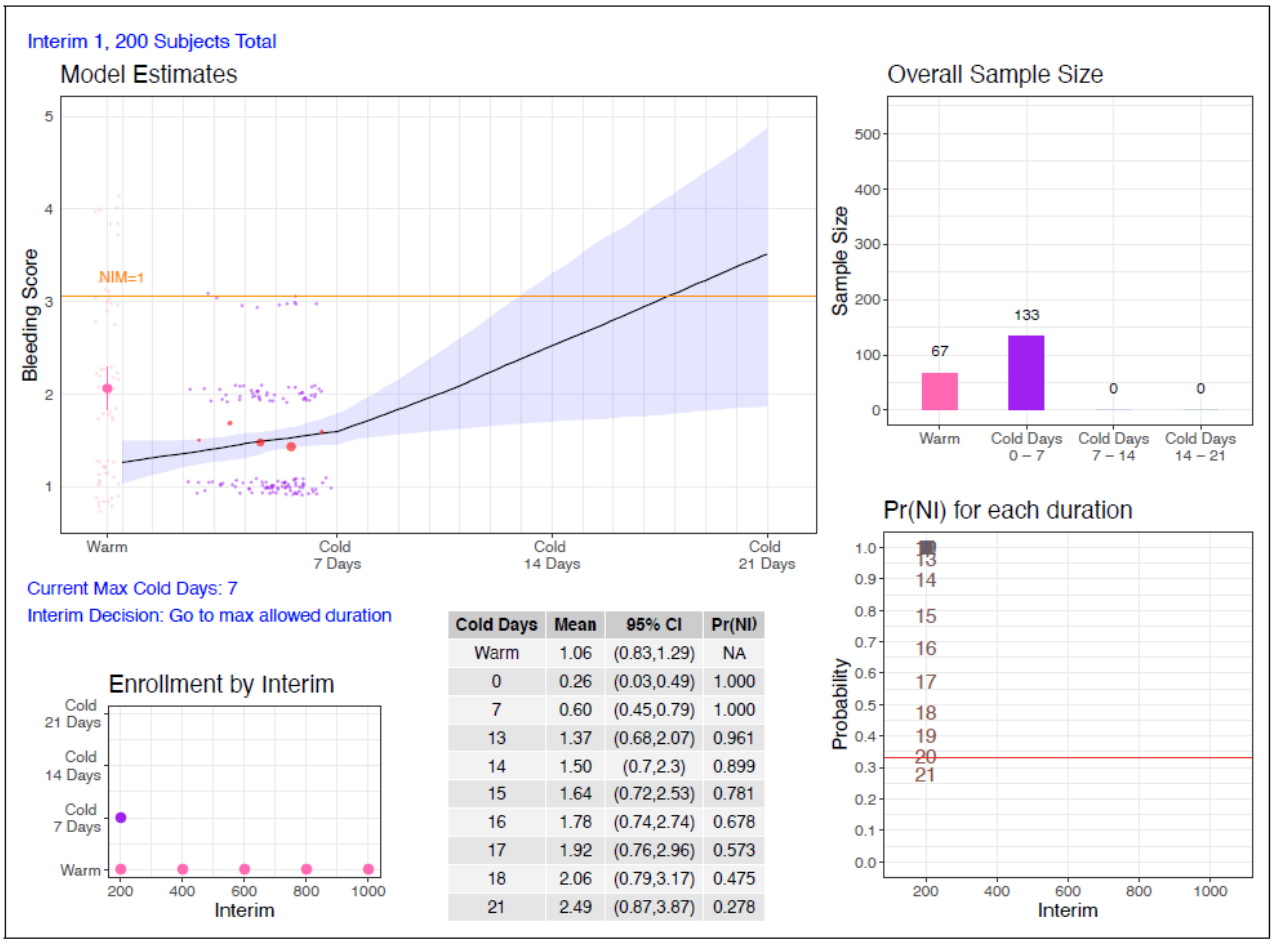
Figure 3 shows an example of the above-described model fit to simulated data. The x-axis shows the storage duration (room temperature platelets are shown separately on the left) and the y-axis shows the hemostatic efficacy score, with "jitter" added to make the separate points visible. Each small blue dot is an individual patient, showing their actual observed duration of cold storage and

bleeding score. In the simulated dataset the majority of the cold platelets were administered between the 3-day to 16-day durations. Up to 14 days the model median, shown as the black line, is mostly linear. The binned means of the data, shown as red dots, have a generally linear pattern, so the model does not try to bend. After 14 days the mean efficacy begins to deteriorate more rapidly than at the lower durations, and the model responds by increasing its slope at the 14-day pivot point. When it can, the model tries to fit the points linearly, but when the data shows a non-linear trend, the model adjusts accordingly. Due to the smaller amount of data below 3 days and above 16 days the variability of the model is higher in that region, shown by the increase in the width of the blue 95% credible interval.

5.4 Example Trial

This section shows an example of how a potential trial would progress for simulated data. The escalation and de-escalation rules are demonstrated, as well as the final decision-making criteria at final enrollment.

Interim Analysis 1



Cold Days	Mean	95% CI	Pr(NI)
Warm	1.06	(0.83,1.29)	NA
0	0.26	(0.03,0.49)	1.000
7	0.60	(0.45,0.79)	1.000
13	1.37	(0.68,2.07)	0.961
14	1.50	(0.7,2.3)	0.899
15	1.64	(0.72,2.53)	0.781
16	1.78	(0.74,2.74)	0.678
17	1.92	(0.76,2.96)	0.573
18	2.06	(0.79,3.17)	0.475
21	2.49	(0.87,3.87)	0.278

Figure 4: Simulated trial results after 200 subjects enrolled (Interim Analysis 1)

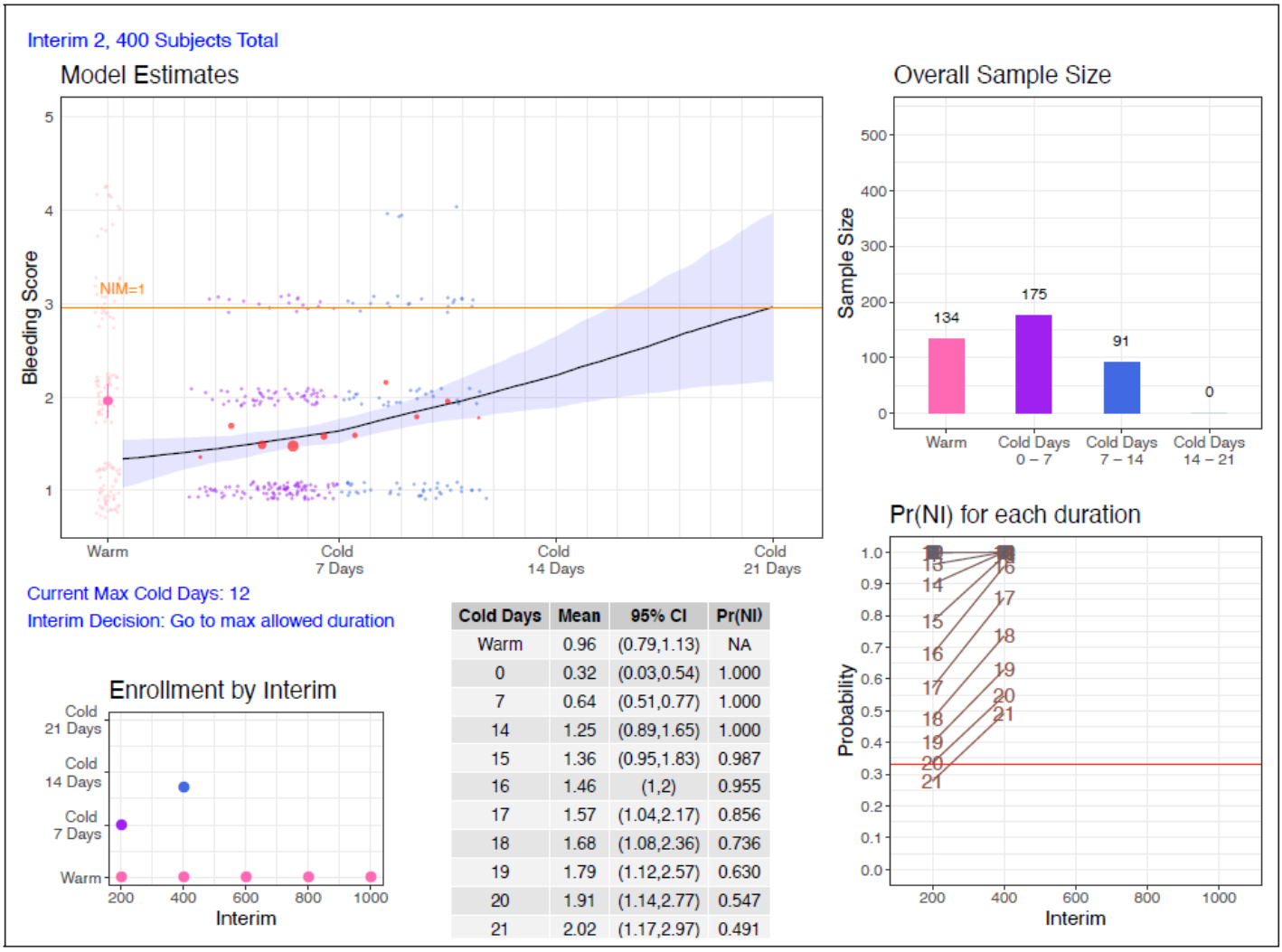
At the first interim analysis 200 patients have been enrolled. The top right pane of Figure 4 shows that 67 patients were randomized to room temperature platelets and 133 were randomized to cold platelets of various durations. Up to the first interim analysis the maximum cold duration is 7 days, so all cold platelets were stored between 0 and 7 days. This can be seen via the small purple points' locations on the X-axis of the top left plot. The pink points on the far left of the X-axis are labeled warm and represent the control group. The pink dot with the vertical line through it shows the estimate and variability of the mean efficacy on the room temperature control arm. The horizontal yellow line shows the mean control efficacy+1. The red points show binned averages of the individual data points to facilitate visualization. The black line shows the median estimated model fit across each duration, and the blue colored ribbon shows the 95% credible intervals for each $\eta(x)$. It's clear that as we extrapolate beyond where data has been collected the model variability increases.

The collected data is well below the NI cutoff, so the model is optimistic about the effectiveness of the cold-stored platelets. The table in the bottom middle pane shows the Pr(NI) at various cold storage durations. Up to 20 days the probability of non-inferiority is greater than 0.33, and at 21 days and beyond the Pr(NI) is lower than 0.33. Since it is clear to the model that cold platelets are non-inferior at 7 days there is no futility stop at this interim.

This holds for the remainder of this example trial, so futility will not be triggered in this example.

The model would propose escalation to 20 days max cold platelet storage, but the rule preventing escalation of more than 5 days limits the escalation up to 12. The trial continues with a 12-day maximum cold storage duration.

Interim Analysis 2

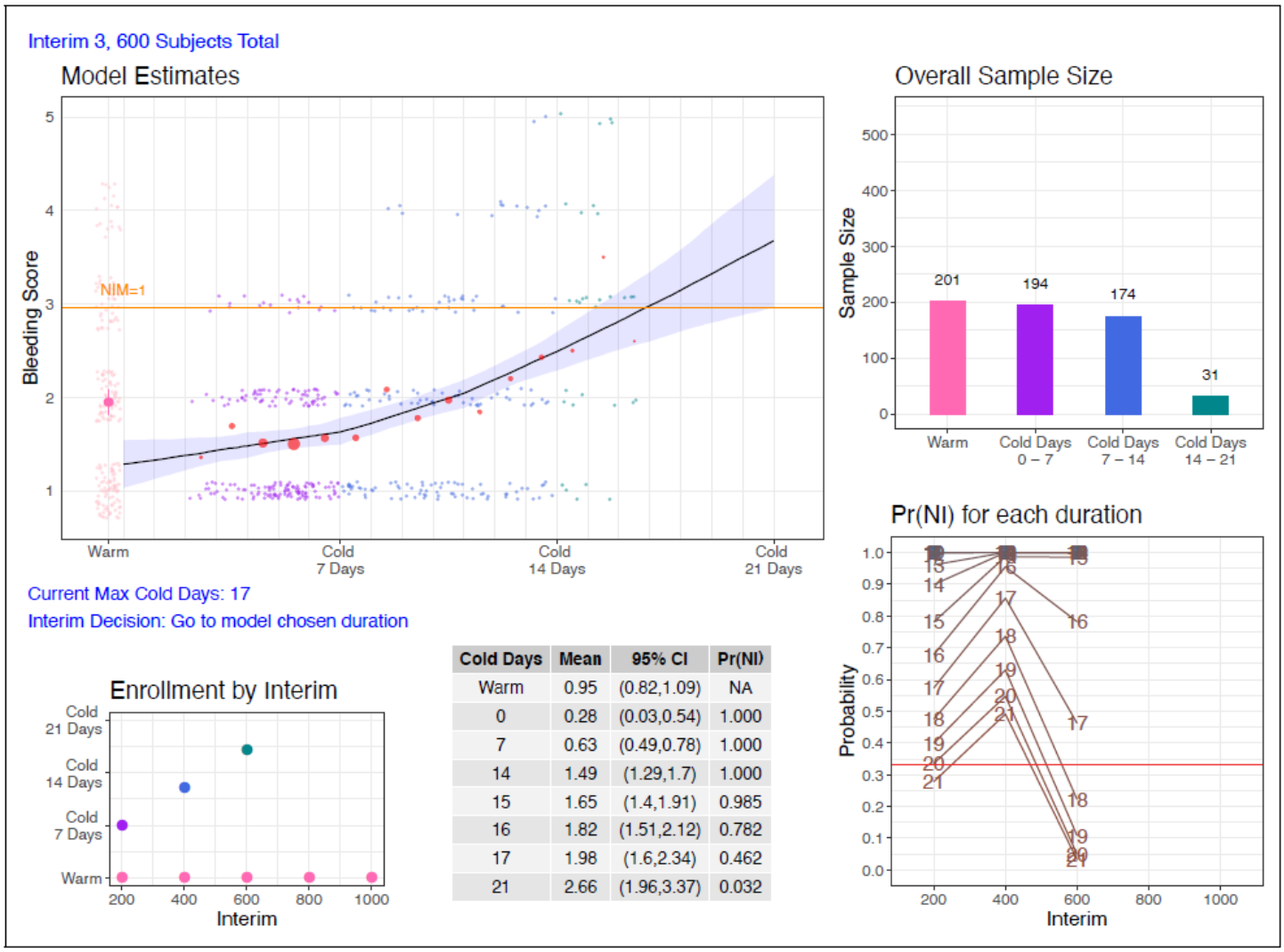


Cold Days	Mean	95% CI	Pr(NI)
Warm	0.96	(0.79,1.13)	NA
0	0.32	(0.03,0.54)	1.000
7	0.64	(0.51,0.77)	1.000
14	1.25	(0.89,1.65)	1.000
15	1.36	(0.95,1.83)	0.987
16	1.46	(1,2)	0.955
17	1.57	(1.04,2.17)	0.856
18	1.68	(1.08,2.36)	0.736
19	1.79	(1.12,2.57)	0.630
20	1.91	(1.14,2.77)	0.547
21	2.02	(1.17,2.97)	0.491

Figure 5: Simulated trial results after 400 subjects enrolled (Interim Analysis 2)

At the second interim analysis 400 patients have been enrolled in total. Again 1/3 of the patients have been randomized to the room temperature cohort and 2/3 are randomized to cold platelets. The maximum cold storage time for cold randomized patients is now 12 days, but patients are still administered platelets stored cold between 0 and 7 days. The top right pane of Figure 5 shows the total allocations by cold storage duration. Since the last interim, 42 new patients were randomized between 0 and 7 days, and the remaining 91 cold randomized patients were given platelets stored between 7 and 12 days. Again, we look at the Pr(NI) for each duration. Based on the positive data collected up to this interim analysis, the model thinks that up to 21 days the Pr(NI) is sufficient for escalation, but we are again limited by the 5 day escalation rule. The maximum allowable cold storage duration is increased from 12 days to 17 days.

Interim Analysis 3

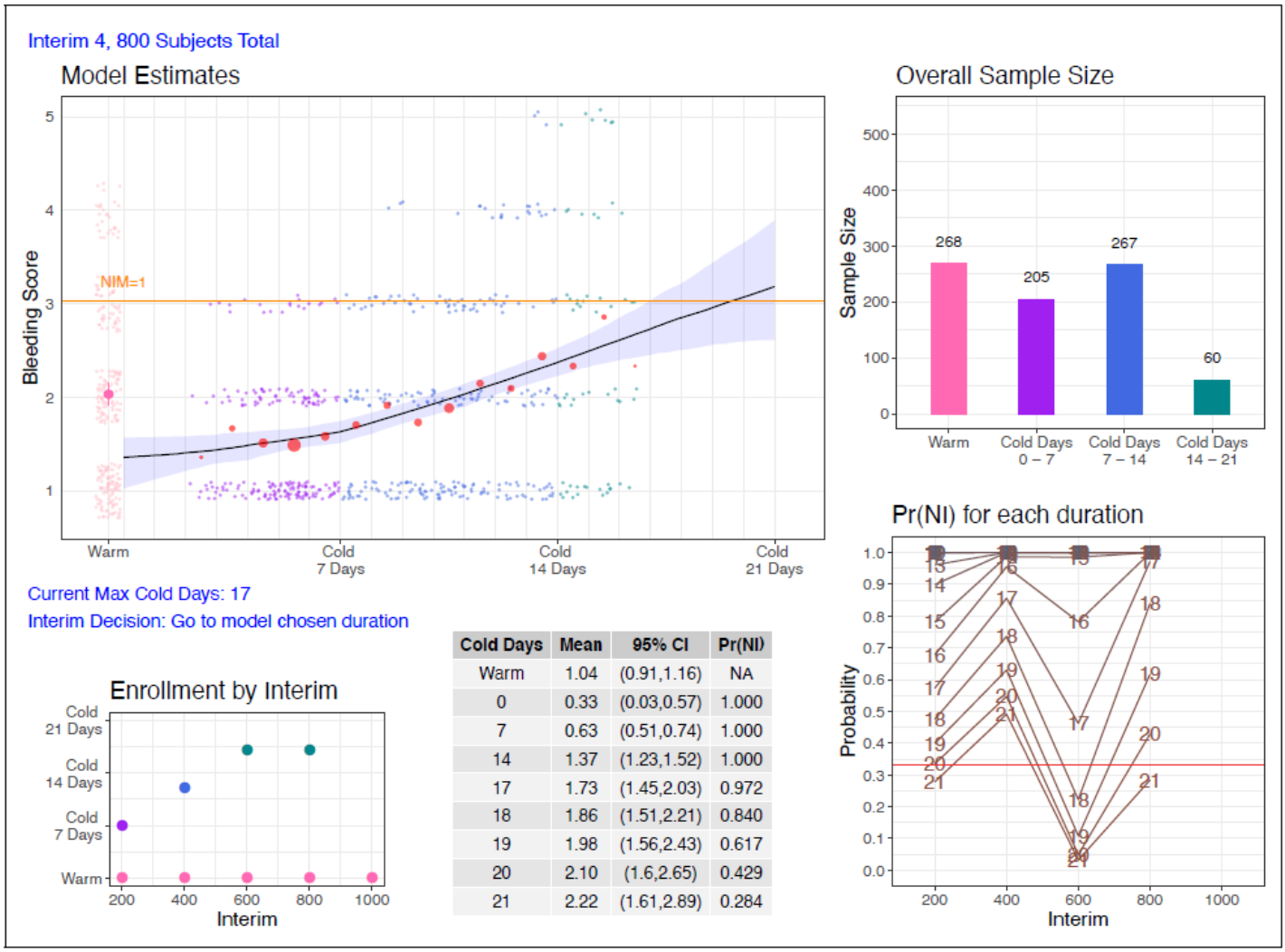


Cold Days	Mean	95% CI	Pr(NI)
Warm	0.95	(0.82,1.09)	NA
0	0.28	(0.03,0.54)	1.000
7	0.63	(0.49,0.78)	1.000
14	1.49	(1.29,1.7)	1.000
15	1.65	(1.4,1.91)	0.985
16	1.82	(1.51,2.12)	0.782
17	1.98	(1.6,2.34)	0.462
21	2.66	(1.96,3.37)	0.032

Figure 6: Simulated trial results after 600 subjects enrolled (Interim Analysis 3)

After fitting a roughly linear decay for cold storage times up to 7 days, the model begins to curve as the collected data shows a non-linear worsening trend for durations between 7 and about 14 days (Figure 6). After 14 days the model returns to roughly linear estimation, but with a steeper decay slope than the linear piece below 7 days. This decay profile leads the model to cross the estimated non-inferiority threshold at around 17 days cold storage time. While $\Pr(NI)_{17}$ is greater than 33%, $\Pr(NI)_{18}$ is less than 33% (this can be seen in the plot in the bottom right pane of the interim dashboard). The third interim analysis decision is to keep the maximum cold storage time at 17 days.

Interim Analysis 4

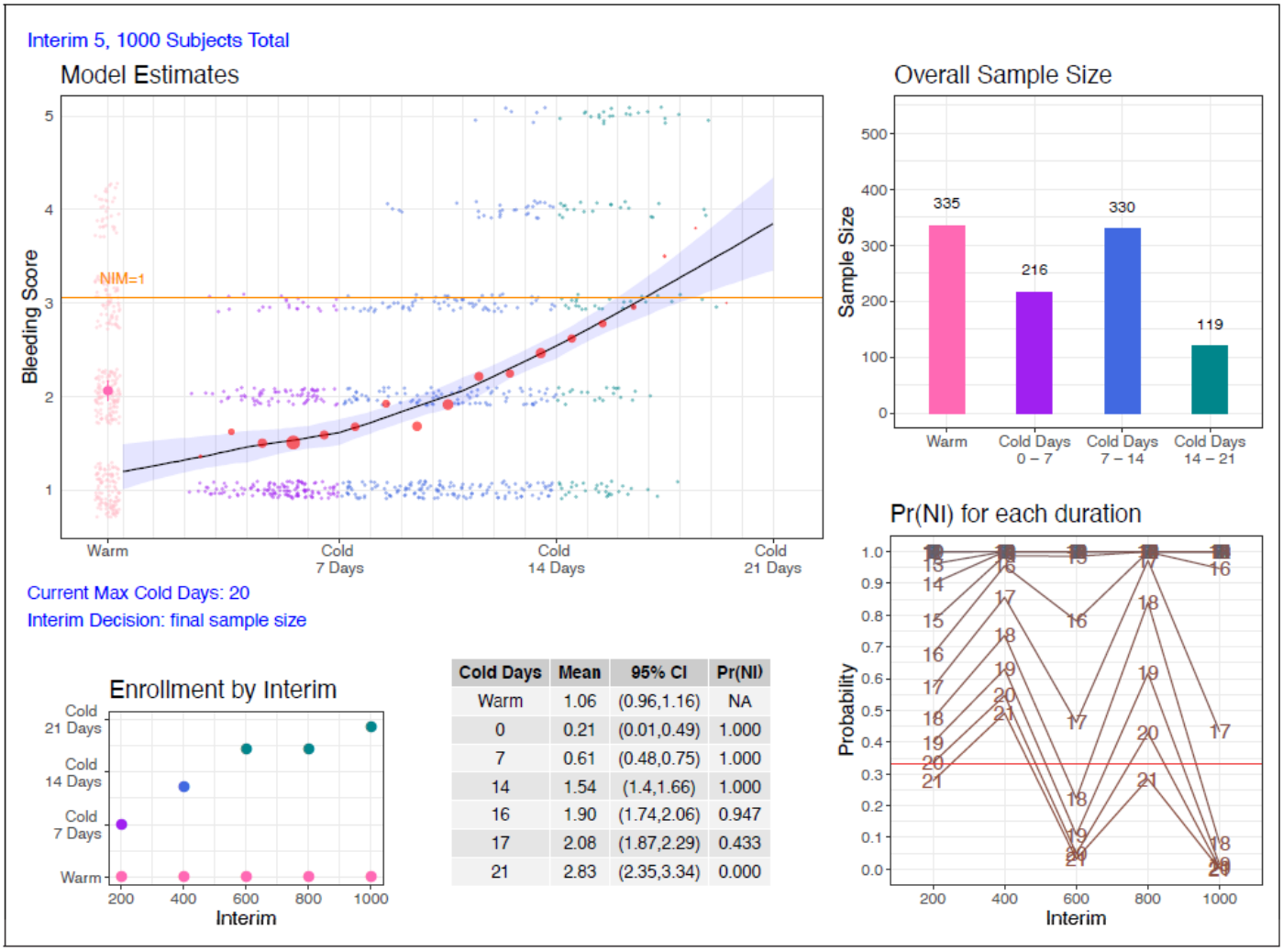


Cold Days	Mean	95% CI	Pr(NI)
Warm	1.04	(0.91,1.16)	NA
0	0.33	(0.03,0.57)	1.000
7	0.63	(0.51,0.74)	1.000
14	1.37	(1.23,1.52)	1.000
17	1.73	(1.45,2.03)	0.972
18	1.86	(1.51,2.21)	0.840
19	1.98	(1.56,2.43)	0.617
20	2.10	(1.6,2.65)	0.429
21	2.22	(1.61,2.89)	0.284

Figure 7: Simulated trial results after 800 subjects enrolled (Interim Analysis 4)

At the fourth interim analysis, with 800 patients enrolled, the Pr(NI)$_x$ is below 0.33 for all x except 21 days (Figure 7). The trial continues to the final analysis with a maximum cold storage duration of 20 days.

Final Analysis



Cold Days	Mean	95% CI	Pr(NI)
Warm	1.06	(0.96,1.16)	NA
0	0.21	(0.01,0.49)	1.000
7	0.61	(0.48,0.75)	1.000
14	1.54	(1.4,1.66)	1.000
16	1.90	(1.74,2.06)	0.947
17	2.08	(1.87,2.29)	0.433
21	2.83	(2.35,3.34)	0.000

Figure 8: Simulated trial results after 1000 subjects enrolled (Final Analysis)

At the final analysis we have full enrollment and full information for each patient. The top right plot in Figure 8 shows the allocations across different cold durations and to the control. Most of the cold randomized patients were given platelets between 0 and 14 days, with fewer patients (18%) enrolled between 14 and 21.

The final analysis, like the escalation rules, is based on $\Pr(NI)_x$ across all durations x. The largest NI duration is the maximum duration with a $\Pr(NI)_x$ larger than 0.975. In this example trial, $\Pr(NI)_{16} = 0.947$ and $\Pr(NI)_{15} > 0.999$, so 15 days is the reported maximum non-inferior duration.

Since $\Pr(NI)_7 > 0.975$, the gated superiority analysis is performed to estimate the largest duration at which cold platelets are superior to room temperature platelets. Figure 8 shows the estimated mean bleeding score of room temperature platelets as the pink dot with the small vertical line representing the variability of the estimate. The room temperature platelet mean bleeding score is estimated to be just above 2, and the model fit crosses from better than 2 to worse than 2 on the bleeding score between about 9 and 12 days.

x	1	2	3	4	5	6	7	8	9	10	11	12	13+
$\Pr(Sup)_x$	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.00	0.92	0.49	0.02	0.00

Table 3: Probabilities of superiority by duration of storage

Table 3 shows the probabilities of superiority $\Pr(Sup)_x$ estimated by the model at each duration x from 1 to 21 days. $\Pr(Sup)_9 > 0.983$ and $\Pr(Sup)_{10} < 0.983$, so 9 days is the maximum cold storage duration that is superior to room temperature platelets.

5.5 Simulations

The goals of this trial are to determine if any duration of cold-stored platelets is non-inferior to room temperature stored platelets, and to identify the maximal non-inferior duration. Thus, it is important to determine how well the specified design achieves those goals. We would like to achieve high power, meaning that if the truth is that some cold storage duration is non-inferior, we would like a high probability of concluding so. Additionally, when we conclude that cold-stored platelets are non-inferior, we would like to accurately estimate the highest non-inferior duration. In simpler statistical designs the performance of the design can be calculated through mathematical formulas. In an adaptive trial there are no such formulas. Nevertheless, we can determine the performance of the trial through trial simulation. Given an underlying true duration response curve, we simulate thousands of trials under that condition, and for each simulation we record the primary analysis conclusion, the identified maximal non-inferior duration, and the identified maximal superior duration. After simulating thousands of trials, we estimate the power of the trial (the probability of declaring some duration non-inferior) by simply looking at the observed proportion of simulated trials which drew this conclusion. Similarly, we can look at the distribution of selected maximal durations and determine how accurately they reflect the true maximal duration.

We perform a set of simulations for a large number of possible underlying truths (scenarios). These scenarios reflect an array of possible realities, any of which could be the actual true description of cold platelets performance. We would like our design to work well under any possible scenario and making sure that the trial performs as expected across our wide range of simulated scenarios helps verify this.

5.5.1 Scenarios

The 13 scenarios we will analyze are broken into 2 groups, the null scenarios and the alternative scenarios. A null scenario is defined as a scenario where no duration 7 days or greater is non-inferior to room temperature stored platelets. Figure 9 shows the set of 7 null scenarios that are used to verify that the design controls Type I Error, and Figure 10 shows the alternative scenarios used to assess power and the duration finding abilities of the trial.

In the two sets of plots the gray line dictates the true efficacy of cold platelets at each duration from 0 to 21 days cold storage. The pink dot above the ‘Warm’ label on the x-axis represents the assumed efficacy of room temperature platelets. The horizontal orange line is exactly 1 point on the bleeding score above the pink dotted line, so it marks the non-inferiority cutoff bleeding score. The point at which the gray and orange lines cross (if they do) is the point at which cold platelets become inferior and is the ideal duration that could be selected by the trial.

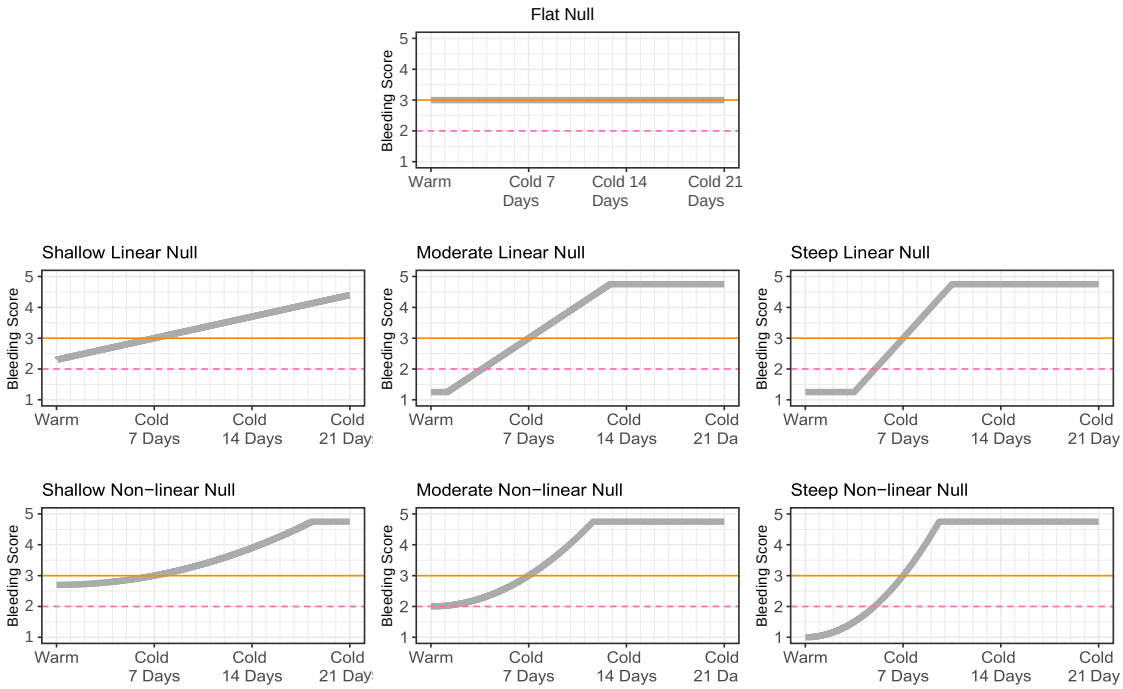


Figure 9: The set of 7 scenarios representing null cases

In each of the 7 null scenarios the 7-day cold-stored platelets have efficacy exactly equal to the non-inferiority cutoff. The top plot shows the null case when every cold duration has efficacy on the NI border. The middle row has null scenarios in which cold platelet efficacy decays linearly, and the bottom row has null scenarios in which the cold platelet efficacy decays non-linearly. In both of the rows with 3 plots the slope of the lines gets steeper from the left to the right. Since in each of these scenarios the cold platelets are not non-inferior at 7 days, the proportion of simulated trials that declare 7 days non-inferior represents the Type I Error of the design. We provide nulls of various shapes and slopes to show that Type I Error is controlled under a variety of possible ineffective duration response curves.

The alternative cases in Figure 10 are intended to span across a set of possible scenarios in which cold platelets are truly non-inferior to room temperature platelets. The No Decay scenario mimics a truth in which cold platelets have the same efficacy as room temperature platelets for all 21 days and never get worse. There are 2 linear decay alternative cases, one with a shallow slope and one with a steeper slope, but both crossing the non-inferiority cutoff at 16 days. There is also a set of 3 cases where the cold platelet bleeding score increases non-linearly with duration. These non-linear scenarios have crossings at 9, 14, and 19 days and slopes that are flatter for larger duration crossings. The slope of the cold platelet effectiveness curve at the place where it crosses the non-inferiority cutoff is a large factor in the difficulty of the scenario. Scenarios with steeper crossings are easier to estimate, and scenarios with narrower crossings are harder to estimate.

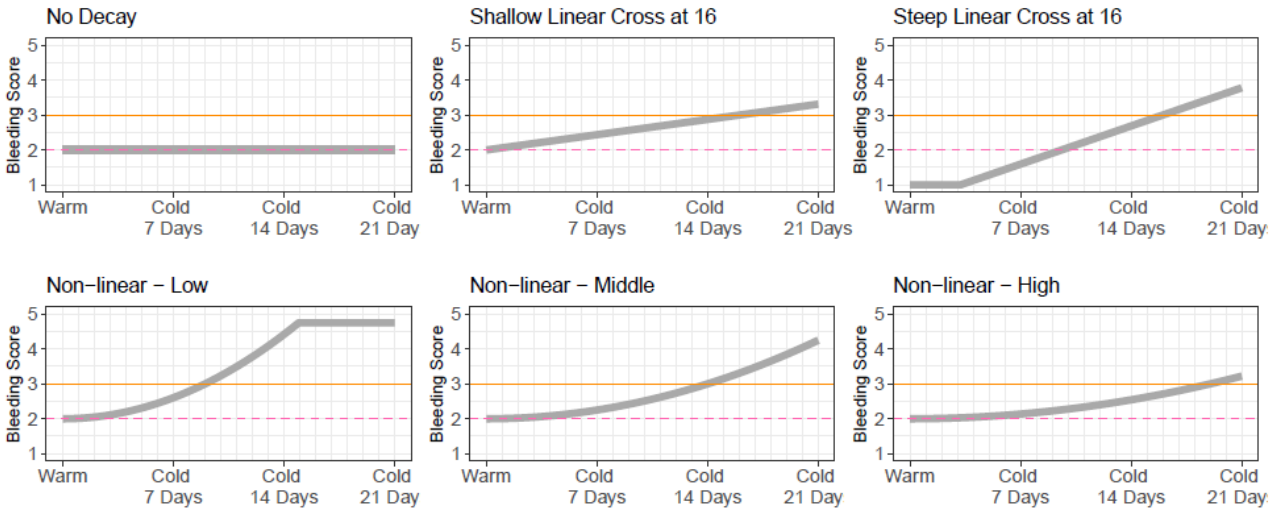


Figure 10: The set of 6 alternative scenarios

Null Scenario	Type I Error
Flat	0.008
Shallow Linear	0.015
Moderate Linear	0.017
Steep Linear	0.018
Shallow Non-linear	0.020
Moderate Non-linear	0.020
Steep Non-linear	0.018

Alternative Scenario	Power
No Decay	1.000
Shallow Linear Cross at 16	1.000
Steep Linear Cross at 16	1.000
Non-linear - Low	0.991
Non-linear - Middle	1.000
Non-linear - High	1.000

Table 4: Type I Error in null scenarios and Power in alternative scenarios

5.5.2 Operating Characteristics

Power & Type I Error. We first show the Type I Error results for the null scenarios, and then the power for the alternatives. A trial in a null scenario is a Type I Error if 7 days cold-stored platelets are called non-inferior, and it is considered to have failed if 7 days is not non-inferior. In these simulations we ran 25,000 simulated trials on each scenario using a seed of 3500.

The Type I Error table shows that the trial limits the false positive rate to less than 0.025 in all null scenarios. In the flat null case, the Type I Error is very small, below 1%.

The power of the trial is nearly 100% for every alternative scenario. The sample size was selected for accurate assessment of the maximal non-inferior duration, not power. Duration selection requires a larger sample size.

Duration Selection. Ideally, we would like each trial to perfectly identify the maximal non-inferior duration of cold storage. Obviously, this is impossible under uncertainty, but in this section, we determine the distribution of the duration selection for each alternative scenario listed above. Note that if the maximal duration is estimated incorrectly, we would prefer an

underestimation rather than an overestimation. With underestimation, all identified non-inferior durations are truly non-inferior.

In the simulation results plots in Figure 11 we have zoomed in on the scenarios shown in Figure 10. The bottom value of the y-axis, labeled "Warm", denotes the room temperature platelet efficacy for that scenario. The highest value of the y-axis of each plot represents the NI cutoff, or warm efficacy + 1. The red line shows the path of the cold platelet efficacy truth as the storage duration goes from 0 to 21 days while it is between the room temperature platelet efficacy and the non-inferiority cutoff. The dashed vertical red line marks the duration at which the red simulation truth line becomes worse than the NI cutoff. The vertical blue bars represent the proportion of the simulations that each duration is selected as the maximum non-inferior duration. Within each pane of Figure 11 the blue bar heights add to 1, and the closer the blue bars are to the red dotted line the better the decisions of NI durations were.

Figure 11 illustrates that the maximal duration is typically underestimated (the desirable direction) but in many scenarios is typically within 2 days of the correct maximal duration. In scenarios with shallower slopes, estimation of the maximal duration is harder.

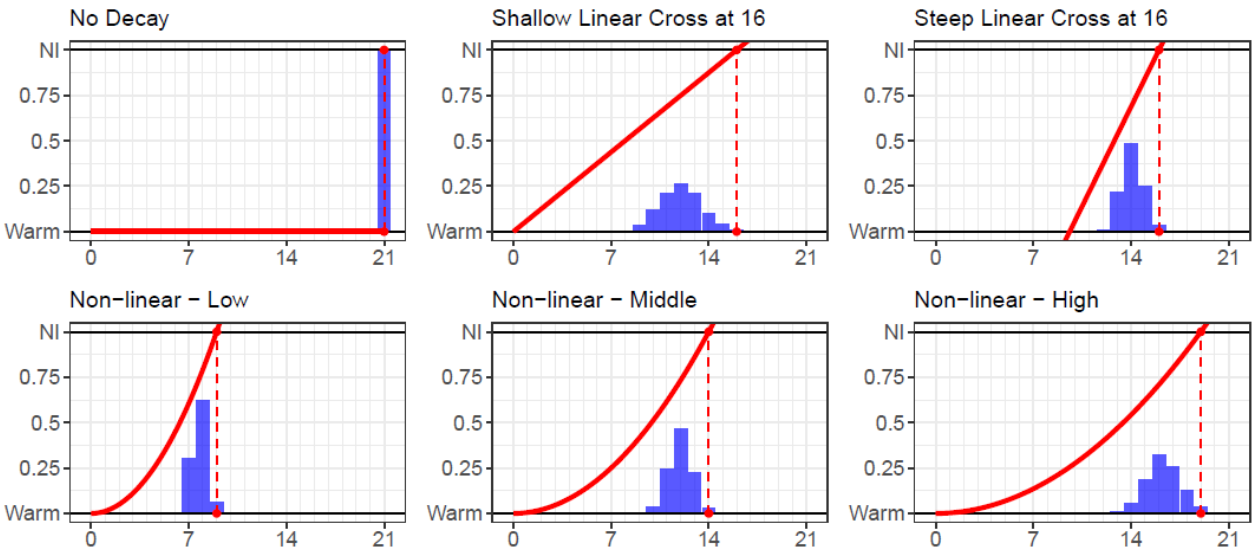


Figure 11: Simulated dose selection for alternative scenarios

In the No Decay case, as expected, every simulated trial resulted in the selection of 21 days as the highest non-inferior duration. In the Shallow Linear and Steep Linear cases with crosses at 16 we can see the effect that the slope of the response curve has on duration selection. In the shallow case the modal duration is about 4 days below the truth and at a duration that is 0.25 points better than the non-inferiority cutoff in that case. In the steep linear case the modal duration is about 2 days below the truth, but again at a duration about 0.25 points better than the non-inferiority cutoff. This pattern of the modal duration appearing when the curve is 0.25 points better than the NI cutoff is repeated in each of the non-linear scenarios as well. This means that again, the steeper sloped scenarios have durations selected closer to their true crossing durations.

Superiority Outcomes. To assess operating characteristics of the superiority analysis we provide six scenarios designed to determine the Type I Error and duration selection results for the gated superiority test after successful determination of non-inferiority at 7 days. A trial results in a Type I Error in the testing for superiority if the duration chosen as the maximum superior duration is larger than (or exactly equal to) the true maximum superior duration of the simulated duration response curve.

We provide a flat duration response curve in which no duration is superior, two scenarios in which cold-stored platelets lose superiority in a region with a non-zero $\delta(x)$ margin, and three non-linearly decaying scenarios that lose superiority at durations at which there is no $\delta(x)$. In the flat "No Decay" scenario no duration is superior, so declaring any duration from 1 to 21 superior is a Type I Error. In the "Shallow Linear" and "Steep Linear" scenarios the cold-stored platelets lose superiority at 8 days, so declaring a duration 8 or larger as superior is considered a Type I Error. In the three non-linear scenarios cold platelets lose superiority at 16 days, so declaring a duration of 16 or larger superior is a Type I Error.

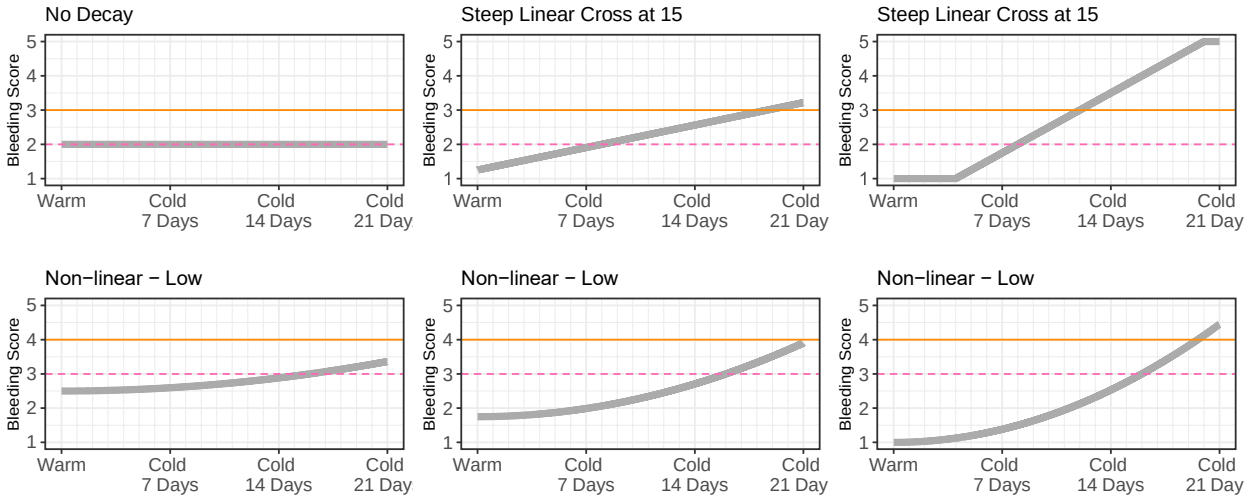


Figure 12: Non-null scenarios for superiority outcomes

For the above 6 scenarios, we provide two different measurements for the superiority simulations. In the second column of Table 5 we provide the proportion of simulated trials in which we declare a duration superior when it is not (Type I Error). Power provides the proportion of simulated trials

that result in any duration 1 day or longer being declared as superior. The fourth column of Table 5 provides the proportion of trials that estimated a maximum superior cold storage duration crossing that is within 3 (including the actual crossing) of the true superiority crossing duration of the scenario. This column does not include trials that selected durations larger than the point of losing superiority. The right-most column provides the proportion of simulated trials that resulted in declaring a duration superior that is greater than the true duration at which superiority is lost.

Superiority Scenario	Type I Error	Power	Proportion of Trials Selecting Durations within 3 days of True Superior Duration	Over Estimated
No Decay	0.023	0.023	-	0.023
Shallow Linear	0.005	0.685	0.345	0.00004
Steep Linear	0.008	1.00	1.00	0
Shallow Non-linear	0.011	0.941	0.216	0.00228
Moderate Non-linear	0.020	1.00	0.956	0.00084
Steep Non-linear	0.024	1.00	1.00	0.00004

Table 5: Operating characteristics of superiority analysis

In each scenario the Type I Error is controlled at the 2.5% level for the superiority analysis. In the duration selection portion of Table 5 the proportion of simulations close to the true superiority crossing increases as the slope of the duration response model increases.

Sample Size. The rule for early stopping for futility allows trials that do not show any evidence of non-inferiority to terminate early. The futility stopping rule for this trial, as presented earlier in the report, is to stop if at any interim analysis the probability of non- inferiority at 7 days is less than 0.10. A productive futility rule stops null trials that are truly not non-inferior at 7 days early but allows the alternative scenario simulations to enroll to the final analysis.

In the alternative scenarios, none of the simulated trials stopped for futility.

The stopping rule is more interesting in the null scenarios, when 7 days falls on the non-inferiority margin. Our set of null scenarios represent some of the most effective scenarios in which we would ideally like to stop for futility. Figure 13 shows, for each of the 7 null scenarios we simulated, the distribution of early stopping timing by interim analysis. In the flat null case over 50% of simulated trials were stopped before 1000 patients was reached, including 30% of trials stopping at 200 patients. In each of the other null scenarios stopping early is fairly unlikely. By making the futility rule more aggressive we could surely save patients on average, but at the risk of terminating trials that could have shown non-inferiority if they were allowed to continue.

The mean sample size for each scenario is provided in Table 7 at the end of the operating characteristics section.

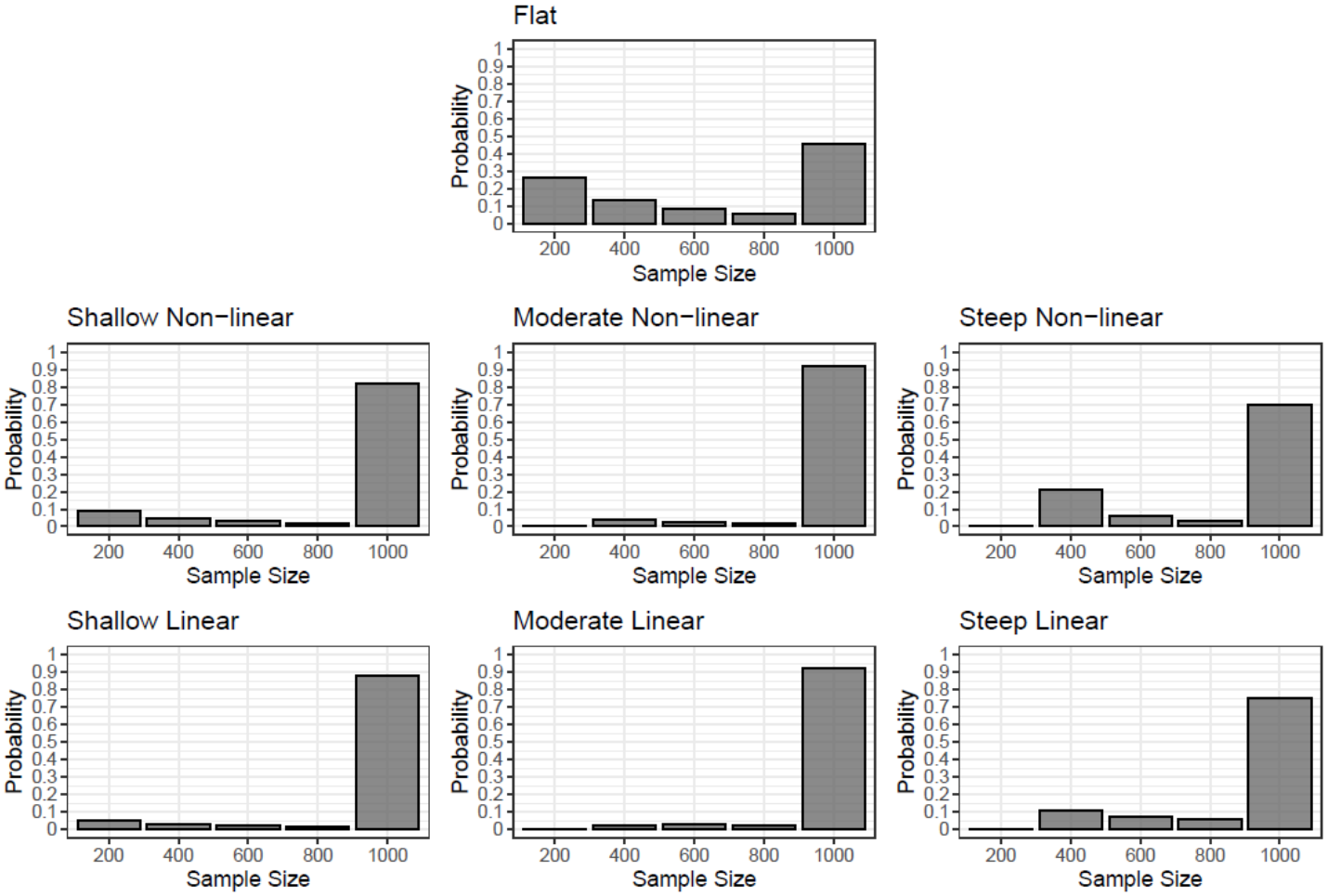


Figure 13: Simulated distribution of stopping times for null scenarios

Escalation Rule Analysis. One of the difficult statistical components of this design is that patients near the durations that are the best candidates for maximum non-inferior cold storage durations are fairly rare. The 33% NI probability threshold is used because it balances enrollment of patients near the maximal non-inferior duration (required for accurate estimation of the maximum) with avoiding large number of patients enrolled at inferior durations. Table 6 demonstrates this trade-off by showing the number of patients enrolled at inferior durations weighed the accuracy of duration selection, as measured by the proportion of trials which select a maximal duration within 3 days of the true maximal duration. This trade-off is shown for each scenario and for thresholds of 10% and 50% in addition to the chosen 33%.

The general pattern across all of the simulations is that as the escalation threshold decreases and the allowed cold storage durations get more aggressive the number of patients randomized to inferior durations increases. This is what we would expect to happen. The increase in patients from 10% to 33% is larger than the increase in patients from 33% to 50%, especially in the Null scenarios.

	Number of Patients On Inferior Durations			Proportion of Trials Selecting Durations within 3 days of True Maximal Non-Inferior Duration		
Scenario	10%	33%	50%	10%	33%	50%
No Decay	0	0	0	1.00	1.00	1.00
Shallow Linear Cross at 16	50	15	4	0.22	0.16	0.11
Steep Linear Cross at 16	26	11	6	0.84	0.77	0.72
Non-linear – Low	94	64	47	0.99	0.99	0.99
Non-linear – Middle	64	33	20	0.76	0.72	0.69
Non-linear – High	18	12	7	0.45	0.41	0.36
Flat	123	25	7			
Shallow Non-linear	142	55	26			
Moderate Non-linear	96	75	58			
Steep Non-linear	90	78	67			
Shallow Linear	157	67	34			
Moderate Linear	100	74	56			
Steep Linear	89	73	60			

Table 6: Effect of NI probability on patient exposure to inferior arm

The opposite side of this trade-off is that as the threshold gets more aggressive the number of trials that choose durations close to the truth increases. This change is roughly equal from 10% to 33% and 33% to 50%. As is usual, the benefits are larger when there is room to benefit, because the proportion of close trials is further away from 1.

Summary Table. Table 7 provides five different metrics for assessing the operating characteristics of our design.

Power. The proportion of simulations that resulted in the selection of a duration of 7 days or longer as the largest NI duration. The power in the null scenarios is the simulated Type I Error.

Inferior Patients. The mean number of patients across all simulations that were given cold platelets stored for durations that were longer than the true maximum non-inferior cold storage duration.

Within 3 Days. The proportion of simulations that resulted in the selection of a duration *less than* 3 days before the true non-inferior duration crossing without going over. So, if the simulated cold platelet non-inferiority crossing is at 14 days, e.g., this is the proportion of trials selecting 14, 13, or 12 days. In the Flat Null and No Decay scenarios the crossing durations are set to be 7 and 21, respectively, since there is not crossing. These are the largest appropriate duration that can be chosen under these scenarios.

Sample Size. The mean sample size for simulations in this scenario.

	Scenario	Power	Inferior Patients	Within 3 Days	Sample Size	Over Crossing
Alternative	No Decay	1.000	0	1.000	1000	0.000
	Shallow Linear Cross at 16	1.000	15	0.156	1000	0.003
	Steep Linear Cross at 16	1.000	11	0.772	1000	0.001
	Non-linear – Low	0.991	64	0.991	1000	0.000
	Non-linear – Middle	1.000	33	0.719	1000	0.001
	Non-linear – High	1.000	12	0.414	1000	0.006
Null	Flat	0.008	25	0.036	663	0.004
	Shallow Non-linear	0.015	55	0.406	886	0.001
	Moderate Non-linear	0.017	75	0.990	964	0.000
	Steep Non-linear	0.018	78	1.000	843	0.000
	Shallow Linear	0.020	67	0.708	931	0.001
	Moderate Linear	0.020	74	0.999	970	0.000
	Steep Linear	0.018	73	1.000	891	0.000

Table 7: Operating characteristics with 33% NI probability

Over Crossing. The proportion of simulations that chose a duration that is *larger than* the true crossing duration. So, for a scenario with a true crossing of 14 days, this is the proportion of trials that select 15 days or longer. For scenarios with no crossing point the same crossing points are enforces as in the Within 3 Days metric.

6 STATISTICAL PROCEDURES AND DATA ANALYSIS

6.1 Sample Size Calculations

The anticipated sample size is 1000 subjects. The sample size may likely be smaller than 1000 if the null scenario occurs (initial cooled durations are at the NIM). Otherwise, full enrollment is anticipated using the adaptations previously described.

6.2 Randomization Methods

The randomization sequence will allocate subjects on a 2:1 ratio to cold versus room temperature platelets. Block sizes of 3, 6, and 9 will be used in the creation of the randomization sequence.

6.3 Population Definitions

Modified ITT analysis. We will analyze all subjects as they are randomized into either study group with the exception of subjects who are not transfused platelets after randomization. Unless otherwise specified, only subjects who were randomized, underwent cardiac surgery and received at least one platelet transfusion during their surgery or in the 24 hours following ICU admission post-cardiac surgery will be analyzed.

Per protocol analysis (PPA). The PPA will only include subjects in the standard stored (22°C) group who received 100 percent standard stored (22°C) platelets, and subjects in the cold-stored (4°C) group who received 100 percent cold-stored (4°C) platelets during the 24-hour study intervention period.

Safety data set. The safety dataset will use all consented subjects who were transfused at least one platelet product. Subjects will be analyzed by treatment received, and those who are transfused both room temperature and cold platelets will be analyzed as an independent group. Subjects who are randomized and then not transfused platelets will not be analyzed for safety.

6.4 Primary Endpoint Analyses

Primary hemostatic efficacy analyses have been described in detail in Section 5.2. Sensitivity analyses of the primary outcome will be performed in the per-protocol population. A different sensitivity analysis of the primary outcome will control for the volume of platelets transfused.

6.5 Secondary, Exploratory, and Safety Endpoints Analyses

Among the secondary, exploratory, and safety endpoints, continuous outcomes will be compared between treatment groups using a Van Elteren test stratified by site. Dichotomous outcomes will be compared between treatment groups using a Cochran-Mantel-Haenszel test stratified by site. In cases of small counts, Fisher's Exact test may be used. For sensitivity analyses, appropriate regressions will also be performed using treatment group as a predictor and will control for relevant confounders.

The primary and secondary analyses will be compared in the following subgroups of interest:

- Subjects ABO group

- Sex
- Race/ethnicity
- Cardiac surgery severity score, defined as Surgical Complexity Score (adult) or O'Brien STAT Mortality Category (pediatric)
- Age group (<12, 12 to 64, ≥65 years of age)
- Volume of platelets transfused (< versus ≥ 15 mL/kg for under 12 years of age, < versus ≥10 mL/kg for 12 years or older)
- ABO compatibility of platelets between donor and recipient (compatible, major and minor incompatibility)
- Pre-operative antiplatelet agent use status

Regressions will be performed using an interaction between randomization arm and subgroup of interest to determine if effects differ by subgroup levels.

For analysis of TEG assays that will be obtained on a subset of enrolled subjects, we assume that the change in maximum amplitude (MA) in response to adenosine diphosphate (ADP) and arachidonic acid from the baseline to after receiving platelets between the 22°C and 4°C study arms would be 4, with a common standard deviation of 6. Targeting 90% power and a 5% Type I error rate, we need to collect TEG samples on at least 50 subjects in each arm to detect a difference between arms. Since TEG is not available at all study sites, we will aim to identify at least 4 sites that use TEG clinically to perform this analysis.

6.6 Testing for Heterogeneity

The primary analysis of the trial will pool the treatment effects for all platelet products of different manufacturing methods used in the trial (Trima, Amicus, in plasma or PAS, pathogen reduced), recognizing that only this pooled analysis will be highly powered. To address heterogeneity of treatment effect, we will employ a combination of directly testing for heterogeneity and an exploratory Bayesian hierarchical model analysis.

Heterogeneity will be tested using a linear model containing terms indicating differential treatment effect across platelet product manufacturing methods. If this collection of terms is significant, that will be evidence that at least one product differs from the others. If these terms collectively are not significant, that would be consistent with the hypothesis of a common treatment effect across products. This analysis, with p-values and confidence intervals for the collection and individual parameters, will be provided to the FDA.

Regardless of the result of the heterogeneity test, an exploratory Bayesian hierarchical model analysis will be performed, identical to those performed commonly in devices to describe site to site variability or in oncology to describe differences in treatment effect across different tumor types. This model will borrow information across different products but will allow for different treatment effects to be estimated for each product. The resulting point estimate and 95% credible intervals for each product will be presented, recognizing that the smaller sample sizes per product will be underpowered compared to the primary analysis.

7 STUDY PROCEDURES

7.1 Screening and Consent

The target population includes all adult and pediatric subjects (viable neonates ≥ 3 kg at enrollment or greater than 28 days and less than 85 years) undergoing complex cardiac surgery using cardiopulmonary bypass who are transfused platelets for active bleeding during surgery or in the first 24 hours following ICU admission post complex cardiac surgery. If approved by the IRB/EC, a phone script may be used to screen eligibility prior to obtaining consent in-person or (if IRB/EC approved) via electronic consent. Electronic consent may be used in lieu of or supplementary to traditional written consent if approved by the IRB/EC and is 21 CFR Part 11 compliant.

7.2 Randomization Workflow

After consent has been obtained, the site clinical coordinator (or designated staff) will randomize the subject using a web-based randomization system. Designated unblinded staff at the study site (blood bank staff or transfusionist [person transfusing study platelets] who may become unblinded) should not inform blinded research staff, surgical or anesthesia staff, or other individuals the study arm of the subject, as blinding of individuals outside the blood bank or transfusionist is important to avoid bias. Logistics for requesting and supplying cold-stored platelets will be made by designated unblinded personnel.

7.3 Blinding Procedures to Minimize Bias

Blood bank staff will not be blinded to the treatment arm to allow allocation of the correct study platelets. Blood bank staff will complete data collection and recording regarding platelet and other blood product characteristics (collection platform, storage solution, pathogen reduction, storage duration) and other blood components transfused during the study to maintain blinding. The product will be labeled in a manner that will maintain blinding of the clinical and research teams, as described in Section 7.4.3. Prior to initiation of the study, it was deemed not feasible to ensure blinding of clinicians involved in the administration of study platelets. The potential exists that the person transfusing study platelets (e.g., anesthesiologist in the operating room or nurse in the ICU) may be unblinded to the temperature of the study platelet unit by touching the unit. Clinical care staff that become unblinded due to touching the study platelet product will be instructed by the research team not to communicate to any other clinical care staff or research staff member which intervention was given to the study patient. Clinicians not involved in study platelet administration, patients, data collectors and outcome assessors will all be blinded.

Multiple methods for blinding will be permitted to blind information in the electronic medical record and on the platelet unit label. A sample of these methods are described in the blood bank manual of operations. The unblinded blood bank personnel or transfusion staff who may become unblinded must not disclose unblinding information to any blinded team member of the study.

7.4 Platelet - Related Procedures

It is estimated that platelets will not be transfused prior to 24 hours from collection due to the time it takes for procurement and processing. The 22°C platelet units will have a maximum of 5-day to 7-day storage duration per site regulations. The investigational 4°C platelet units will have maximum storage duration of 7 days, initially. If non-inferiority is demonstrated with the 7-day

duration, longer durations will be sequentially tested.

7.4.1 Acquisition of Platelets

Subjects will be randomized to receive either cold-stored (4°C) platelets or standard room temperature stored (22°C) platelets. Subjects should only be randomized if both component types are anticipated to be available on the day of surgery or if time between consent and surgery would permit for sufficient acquisition of study platelets. If both components are anticipated to be available by the date of surgery, then the site clinical coordinator may randomize the subject. If it is not anticipated that both cold and room temperature platelets will be available by the date of surgery, then the subject will not be randomized. Detailed instructions for randomization are provided in the manual of operations.

The standard issue platelet components and all other blood components will be supplied by the hospital blood bank in accordance with local and national regulations. Only pre-storage leukoreduced RBC and apheresis platelet units will be used in this trial. Irradiation of blood components to prevent transfusion associated graft versus host disease (TA-GVHD), other modifications of RBC units (e.g., washed, volume reduction, split into aliquots) or blood administration practices will be per the local practice of the clinical site. The use of ABO compatible platelets is preferred, but if not available, products will be given per each site's practice for ABO incompatible platelets. Blood product characteristics for all transfused products will also be recorded to minimally include collection methods, special processing, ABO type, storage solution and storage duration.

The trial design initially allows cold-stored platelets to be administered up to 7 days of storage at 4°C, and if non-inferiority is demonstrated, the maximum duration may be sequentially increased to a maximum of 21 days. The unblinded CCC will inform blood banks of any change in the maximum cold storage duration, as determined in Section 5 and approved by the DSMB and will ensure that the blood banks have implemented the adjusted storage duration.

It is important that cold-stored platelets be obtained sufficiently early that the age of administered platelets is as close to the maximum duration as possible when transfused to the subject in the trial.

7.4.2 Preparation, Storage, and Transport of Platelets

All blood product components will be prepared according to existing local and national standards. Each unit is produced in accordance to the applicable national regulations such that at least 90% of units contains at least 3 $x10^{11}$ platelets and at least 95% of units contain $<5 \times 10^6$ residual leukocytes in standard room temperature stored platelets. The following apheresis platelets leukocyte reduced products stored in the cold may be included in the trial:

- Apheresis Platelets Leukocyte Reduced collected on the AMICUS separator system (Fresenius Kabi) suspended in plasma or platelet additive solution (Intersol/PAS-C);
- TRIMA ACCEL system (Terumo BCT) suspended in plasma or platelet additive solution (Isoplate/PAS-F - U.S.; PAS-E - Australia);
- INTERCEPT Blood System (Cerus) pathogen reduced platelets collected on the AMICUS separator system suspended in PAS-C;
- INTERCEPT Blood System (Cerus) pathogen reduced platelets collected on the TRIMA

ACCEL system suspended in plasma.

For the prevention of TA-GVHD, units can be irradiated per the policy of the transfusing facility. Units must be irradiated with a minimum of 25 Gy (2500 cGy) delivered to the central portion of the container and the minimum dose at any point in the component is 15 Gy (1500 cGy) or an alternate method validated to be equivalent. Pathogen reduction treatment is an acceptable alternative to irradiation for the prevention of TA-GVHD.

All platelets for study sites in the U.S. will be collected by a U.S. FDA licensed or registered blood collection facility. The blood is collected from donors who are screened for risk factors for disease transmission. The unit indicates the donor's ABO group and Rh type. Blood from the donor is tested for human immunodeficiency virus I/II, hepatitis C virus, hepatitis B virus, human T lymphotropic virus, West Nile Virus on the current donation; Trypanosoma cruzi on at least the first donation; and Babesia testing per "Recommendations for Reducing the Risk of Transfusion-Transmitted Babesiosis: Guidance for Industry" U.S. DHHS, FDA CBER May 2019, and other applicable national regulations; and other applicable national regulations. Cold-stored units are not tested for bacteria since these will be placed in 1–6°C conditions within 8 hours of collection or within 24 hours for pathogen-reduced platelets.

For clinical sites outside of the U.S., the platelets are to be collected by apheresis, tested, and released in accordance with local national health authority requirements and guidance(s). The standard apheresis process and storage will conform to the requirements of this protocol and be documented in the site Trial Master File documents.

Cold-stored non-PRT platelets must be placed into 1-6ºC conditions within 8 hours of the end of collection; cold-stored PRT platelets must be placed into 1-6ºC conditions after completion of the pathogen-reduction process and within 24 hours of the end of collection. Units are transported to clinical sites at 1 to 10°C in devices designed and validated to ensure appropriate temperature is maintained and monitored. Additional details regarding the manufacturing and shipping of cold-stored platelets are in the manual of operations.

At hospital blood banks, cold-stored platelets will be stored at 1-6°C without gentle agitation. Additional details regarding the storage and handling of cold-stored platelets at hospital-based blood banks are in the blood bank manual of operations.

To conserve cold-stored platelets manufactured for CHIPS, cold-stored platelets may be transferred from one clinical study site to another. The transfer of cold-stored platelets will occur using the validated shipping containers and by a carrier that is licensed to transport blood products. Specific details on the process of transferring cold-stored platelets from one clinical study site to another are in the blood bank manual of operations.

7.4.3 Labeling of Platelets

Each platelet unit will have been labeled with the standard International Council for Commonality in Blood Banking Automation (ICCBBA) International Standard for Blood and Transplant (ISBT) label for apheresis platelets. Each unit will also include the appropriate donor classification status (volunteer or paid donor). The ISBT label will include the "FOR INVESTIGATIONAL USE

ONLY" label applied by the blood bank prior to issue and will not contain any visible unblinding information. FDA approved blinding stickers will be placed on all text on the label and forms that may be included with a study platelet that could unblind the research and clinical teams.

7.4.4 Study Platelet Administration

All platelet transfusions given to a subject during the intervention period should be the assigned study platelets up to a maximum of eight units or eight pediatric doses (one dose equals 10–15 mL/kg), per the randomization. The study intervention period is 24 hours after from the start of the first study platelet transfused. If the subject is in the cold-stored platelet arm, all subsequent platelet transfusions after the 24-hour intervention period should be platelets stored at room temperature. The decision to administer a platelet transfusion will be made by the treating clinicians based upon standard clinical practice since this is a pragmatic trial. Subjects are eligible to begin receiving study platelets either intra-operatively or up to 24 hours following ICU admission following complex cardiac surgery.

Thresholds for transfusion of blood components will not be protocolized but investigators will be encouraged to follow recent guidelines from the Society of Cardiovascular Anesthesiologists for the management of perioperative bleeding and hemostasis in cardiac surgery patients.[14–16] Other interventions that will not be protocolized but will monitored and recorded include, but are not limited to, total amount of all platelets, red cells, plasma, whole blood, cryoprecipitate products transfused, total amount of cell saver blood transfused, hemostatic agent use (e.g., prothrombin complex concentrate, recombinant Factor VIIa, antifibrinolytics), vasopressor support, post-operative anticoagulant and antiplatelet use, vasoactive agents and other supportive measures including extracorporeal membrane oxygenation (ECMO), balloon pumps, ventricular assist devices, plasma exchange, and renal replacement support.

The dose of the platelet transfusion is determined by the clinical team. A subject may be randomized if study platelets are anticipated to be available in the blood bank, based on the date of the scheduled surgery. If the subject is randomized to the cold-stored arm and cold-stored platelets are not available at the time requested for active bleeding, standard platelet products for the treating center will be issued in an unblinded fashion and the subject will be marked as not enrolled in CHIPS. If the subject requires more cold-stored platelets than are onsite, the blood bank should revert to the room temperature units; however, only up to eight units/doses should be blinded regardless of assigned treatment arm.

The maximum amount of cold-stored platelets that can be administered to subjects randomized to the cold-stored arm during the intervention period will be 8 platelet units or 8 pediatric doses (10–15 mL/kg). For the purposes of the trial, since the primary outcome is categorized into pediatric and adult scoring around the threshold of 50 kg, pediatric dosing limitations for all study subjects who weigh 50 kg or less will be used and adult dosing limitations will be used for all subjects who weigh more than 50 kg. To ensure subject safety, the DSMB will be notified of all subjects who, during the intervention period, receive more than 8 units or doses of cold study platelets within 7 days of the DCC becoming aware that this threshold has been met. This notification will also include aggregate summaries of safety events by arm for subjects who receive more than 8 study platelets (regardless of arm). The DSMB will review the clinical course and outcomes of all these subjects. If this review raises concern about the safety of cold platelets, the DSMB may recommend

reduction of the maximum units or doses of cold platelets that may be administered. Further, at each planned interim analysis, the DSMB will assess the overall safety profile of cold-stored platelets versus room temperature platelets and may recommend either a decrease or an increase in the maximum limit on the number of cold-stored platelets that may be administered. An additional report will be included at DSMB meetings to review data on all subjects who receive more than 8 units or doses of any platelet within the intervention period.

7.5 Discontinuing Study Intervention

It is possible that the clinical team or subject may make a decision to discontinue receiving study arm platelets during the 24-hour intervention period, though this is anticipated to be unlikely. If a decision to discontinue blinded study platelet administration during the intervention period is made, any subsequent platelet transfusions will be 22°C platelets, but the subject will be analyzed in both the modified intention to treat population as well as the safety population, and data collection will continue unchanged.

7.6 Withdrawal from Study

As noted in Section 7.5, withdrawal from the study is a distinct concept from stopping the study intervention. Withdrawal from the study should be exceedingly rare. If a subject (or subject's legally authorized representative (LAR)) says they want to stop being in the study, they USUALLY mean they want to stop the intervention rather than withdrawing consent for further participation. Efforts should be made to clarify and request the subject's consent to remain in the study (without intervention) to be able to continue to collect study data, and safety-related data through 28 days or death (whichever is earlier). Data that have been collected up to the time of the subject's withdrawal from the study must be retained, as safety data must be correlated with those data and reported to the FDA or other applicable regulatory authority.

8 DATA COLLECTION

8.1 Screening and Eligibility Data

Eligible subjects will be entered into the CHIPS database, the Electronic Data Capture (EDC) system if study inclusion criteria are met, unless the site PI does not anticipate the subject is likely to require a platelet transfusion or whom the site does not intend to approach for consent. As such, subjects who have met any exclusion criteria and are not eligible for participation may be entered into EDC. In addition to inclusion and exclusion criteria, the age, sex, race and ethnicity will be included in screening and eligibility data.

If an eligible subject is identified but is not approached to consent for participation in CHIPS, the reason for not approaching the subject will be recorded.

For subjects who are approached and consented for participation, the date of consent will be recorded. For subjects who decline to consent for participation, the reason for non-consent will be recorded, if possible.

For eligible subjects who have consented to participate in CHIPS, randomization will be done by the site research coordinator or designee. The date and time of randomization will be recorded. If randomization cannot be done because of known inability to obtain the necessary blood products prior to surgery, this will be recorded.

Reasons for not enrolling consented randomized subjects in CHIPS will be recorded.

8.2 Baseline Subject Data

Subject baseline data to be recorded will include, but is not limited to, weight (obtained within 14 days of surgery), height, ABO and Rh type, pre-operative morbidities (as defined in Appendix B), surgical history, ASA status (I–V) and cardiac diagnosis. The use of anticoagulants and antiplatelet agents in the seven days prior to surgery will also be recorded. Results for CBC, BUN, creatinine, INR, PTT, fibrinogen, lactate, troponin, and ALT *if collected clinically* within 72 hours prior to and including the date of cardiac surgery will be recorded. If a CBC is not done clinically within 72 hours of the date of cardiac surgery it must be performed as a research lab prior to the procedure start time.

8.3 Surgical Procedure Data

The following surgical procedure data will be collected:

- Surgical procedure(s) and complexity score for age
- Cardiac surgery severity score (STAT mortality score for children and surgical complexity score, based on number of procedures, for adults)
- Operative start and stop times
- CPB cannulation site(s)
- CPB data (total runs, run times, aortic cross-clamp time, cooling temperatures, temperature at separation)
- CPB medications or antifibrinolytics

- Heparinization & heparin reversal or alternative anticoagulation strategy
- Intra-operative cell recovery and reinfusion
- Hemodilution strategy (if any)
- Blood product exposures and volumes intra-operatively
- Perioperative Bleeding Score measured from the time of initiation of surgery to the time of starting the first platelet transfusion

8.4 Concomitant Medication (including Blood Product and Platelet Data)

A complete list of concomitant medications to be collected and the reporting timeframe is specified in Appendix C. Blood products administration (platelets, plasma, RBC, cryoprecipitate, whole blood) and hemostatic adjunct administration (e.g., antifibrinolytics, coagulation factor concentrates) will be recorded from the initiation of surgery (amount, date and time) until 72 hours after ICU admission or 72 hours after start of first platelet transfusion (whichever is later). The following additional data will be collected for each platelet unit ordered for the subject from the start time of first platelet transfusion until 72 hours after ICU admission or 72 hours after the start of the first platelet transfusion (whichever is later):

- Unit ID number (DIN)
- Product Code
  - Method of collection (Amicus or Trima)
  - Storage medium
  - Pathogen reduction use
- Blood group of unit (ABO and Rh)
- Irradiation status
- Date and time of product irradiation
- Re-issue status of platelet unit
- Expiration date
- Transfusion start time
- Transfusion volume

8.5 Outcome Data

Primary Outcome: Perioperative Bleeding Score

The determination of the Perioperative Bleeding Score will be assessed after the first platelet transfusion for subjects who are eligible to be in the trial. Eligibility includes a platelet transfusion that occurs either intra-operatively or within 24 hours of ICU admission post-operatively.

The primary outcome is the Perioperative Bleeding Score measured from the start of the first platelet transfusion to 24 hours after the initiation of the first platelet transfusion as detailed in Section 3.1. The score will be derived by the Data Coordinator Center after all relevant information has been entered into the database.

Secondary Outcome: Chest Tube Drainage

Chest tube output will begin to be measured from the time of ICU admission. If the first study platelet transfusion occurs in the operating room, then all of the chest tube output in the ICU will be included in in the post-intervention bleeding score. If study platelets are first administered in

the ICU rather than during surgery, the chest tube drainage from the time of ICU admission to administration of study platelets will be used for the pre-transfusion bleeding score, and the chest tube output after the intervention will be used for the post-transfusion bleeding score. A subset analysis will be performed for subjects transfused platelets in the OR and the ICU.

Research Laboratory Data

Complete blood count (CBC), INR, PTT, and fibrinogen should be measured within 30 minutes prior to first study platelet transfusion, after heparin reversal if possible, (whether administered in the OR or ICU). Repeat measurements should be obtained 6 (± 2) hours and 24 (±2) hours after the beginning of the first platelet transfusion. Thromboelastography (TEG) will be obtained in a subset of subjects (at least 100 subjects) at selected clinical sites on the same schedule.

Hospitalization Data

Outcome data will be collected as described in Sections 3.3 and 3.4. In addition, use of anticoagulants, antiplatelet agents, vasoactive agents, balloon pumps, ventricular assist devices, plasma exchange, and renal replacement support will be recorded daily for 7 days.

Protocol Deviations

Deviations of the protocol that have a significant or potentially significant impact to human subject protection or reliability of trial results, deviations that were intended to eliminate apparent hazard to a research participant, and deviations that resulted in an unanticipated problem with significant or potentially significant impact on human subject protection or reliability of trial results will be reported throughout the trial.

9 DATA MANAGEMENT

9.1 Clinical Site Data Management

Study files, either paper or electronic, will be maintained at each clinical site, and will include Essential Documents, worksheets, and consent documents. Research staff may choose to include selected copies of original medical records (shadow records) for critical data elements, which can facilitate site monitoring.

The DCC will provide an electronic regulatory platform for use by each site, and Essential Documents and source documents may be uploaded to this 21 CFR 11 compliant system for remote site monitoring.

9.2 Data Coordinating Center

9.2.1 Data Center Description

At the time of protocol preparation, the DCC at the University of Utah School of Medicine provides data coordination and management services for a variety of national research networks. Anchoring these services is a state-of-the-art, energy efficient data center. The data center facility supports more than 3500 users around the country and provides a secure, reliable, enterprise-wide infrastructure for delivering mission critical DCC systems and services.

The data center was built using high industry standards and energy efficient cooling solutions. The data center is cooled by an LCP (Liquid Cooling Package) inline cooling technology, providing efficiency, redundancy and modularity. Cooling is based upon a hot/cold aisle design that allows for even air distribution with minimal hot spots. The data center electrical power system contains an uninterruptible power system (UPS) with a diesel backup generator. The data center is protected with a FM200 fire suppression system to act as a secondary system to the smoke detectors. The data center provides enhanced security to safeguard the equipment and the data within in it. Security guards are on-site conducting access control 24/7/365. Entry into the data center is restricted by card access and layered security measures and controls. The data center and external building access points are monitored with video surveillance.

The data center has a virtualized environment. The virtual environment consists of more than 400 virtual servers and nearly 25 physical servers. The data center's virtualization solution provides key advantages:

1. High availability: in the event of hardware failure, virtual servers automatically go back online in a seamless process.
2. Flexible infrastructure: disk storage, memory and processor capacity can be increased or reallocated at any time.
3. Rapid deployment: servers can be provisioned on-demand.

Production servers running mission critical applications are clustered and configured for failover events. Servers are backed up with encryption through a dedicated backup server. Storage area networking (SAN) applications, clusters, and switch-to-switch links are on a 10-gigabit network.

Incremental backups occur Monday through Friday. A full system backup occurs weekly. Full backups are taken off site on a weekly basis to an off- site commercial storage facility. The data center currently manages over 150 terabytes of data.

The DCC information systems are available 24 hours a day, 7 days a week to all users, unless a scheduled maintenance interruption or mitigation of an unexpected event is required. If this occurs, all users of the relevant systems are notified, and data entry can be deferred until after the interruption is over. Critical systems availability exceeded 99.9% for the two years prior to initial preparation of this protocol.

9.2.2 Security and Confidentiality

The data center coordinates the network infrastructure and security with the Health Sciences Campus (HSC) information systems at the University of Utah. This provides effective firewall hardware, automatic network intrusion detection, and the expertise of dedicated security experts working at the University. Network equipment includes four high-speed switches. User authentication is centralized with two Windows 2012 domain servers. Communication over public networks is encrypted with virtual point-to-point sessions using transport layer security (TLS) or virtual private network (VPN) technologies, both of which provide at least 128-bit encryption. All Web-based systems use the TLS protocol to transmit data securely over the Internet. Direct access to data center machines is only available while physically located inside DCC offices, or via a VPN client.

All network traffic is monitored for intrusion attempts, security scans are regularly run on servers, and IT staff are notified of intrusion alerts. Security is maintained with Windows 2012 user/group domain-level security. Users are required to change their passwords every 90 days, and workstations time out after 5 minutes of inactivity. All files are protected at group and user levels; database security is handled in a similar manner with group-level access to databases, tables, and views in Microsoft SQL Server. Finally, all laptop computers in use in the DCC or in the Department of Pediatrics are whole-disk encrypted.

The data center uses control center tools to continuously monitor systems and failure alerts. Environmental and network systems are also monitored to ensure up time. Highly trained system administrators on staff are available to respond in high-risk emergency events.

All personnel involved with the DCC have signed confidentiality agreements concerning data encountered in the course of their daily work. All personnel (including administrative staff) have received Human Subjects Protection and Health Information Portability and Accountability Act (HIPAA) education. All users are required to sign specific agreements concerning security, confidentiality, and use of our information systems, before access is provided.

9.3 Electronic Data Capture System

The DCC will develop an electronic data capture system for this trial using a 21 CFR 11 validated instance of REDCap (i.e., REDCap Cloud). Each user at clinical sites, the CCC and the DCC will have an individual account and will receive training prior to accessing the system.

9.4 Study Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Study monitoring is critical to this process. Monitoring has been a very effective tool for maintaining data quality in previous studies and will be utilized to ensure excellent quality data in this study. This study utilizes risk-based methodology to identify and correct problems that may arise at sites. The risk-based approach to monitoring focuses on oversight activities and preventing or mitigating key risks to data quality, as well as to processes critical to human subject protection and integrity of the trial or study.

Study monitors must be provided with appropriate access to study materials and the medical records/source document records for study subjects. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the study monitor's review of data in the electronic medical record.

9.4.1 Site Monitoring Plan

A supplemental study-specific risk-based monitoring plan, separate from the protocol will be completed which outlines specific criteria for monitoring. This plan may include the number of planned site visits, criteria for focused visits, additional visits or remote monitoring, a plan for medical chart review and a follow up plan for non-compliance at sites. The monitoring plan also describes the type of monitoring that will take place (e.g., sample of all subjects within a site; key data or all data), the schedule of visits, how these are reported and a time frame to resolve any issues found.

9.4.2 Clinical Site Monitoring

Site monitoring visits will be performed by a trained site monitor during the study period to ensure regulatory compliance, subject safety, and to monitor the quality of data collected.

Essential document binders, regulatory documents and data collection forms may be reviewed. Interim visits will take place with the frequency being adjusted depending on site enrollment and compliance issues identified. The site monitor will provide visited sites with a written report of findings, and sites will be required to follow up on any deficiencies. It is anticipated that the study monitoring visits for this protocol will consist of a site initiation visit (prior to subject enrollment), interim visits, and a close out visit. The site initiation may take place as group training made up of site investigators and research assistants.

9.4.3 Remote Data Review

Remote data review supplements the clinical site monitoring with remote data management activities that involves detailed review of the data entered by the clinical site and consultations with the clinical site investigator and/or research coordinator to review safety and data quality. This may require uploading copies of medical records, subject study files, regulatory documentation, or other source documents for review.

9.4.4 Blood Bank Monitoring

The clinical site blood bank must maintain adequate records of all cold-stored platelets received and all dispensed study platelets. Each blood bank will be monitored and may be requested to send copies of these documents to the monitor. Only unblinded DCC or CCC study team members will receive unblinded records prior to database lock.

9.5 Record Access

The medical record and study files (including informed consent, permission, and assent documents) must be made available to authorized representatives of the DCC and CCC, upon request, for source verification of study documentation. In addition, medical information and data generated by this study must be available for inspection upon request by representatives (when applicable) of the Food and Drug Administration (FDA), Department of Defense, Therapeutic Goods Administration (TGA), other Federal funders or study sponsors, and the Institutional Review Board (IRB)/Ethics Committee (EC), as applicable, for each study site.

10 PROTECTION OF HUMAN SUBJECTS

10.1 Institutional Review Board Approval/Ethics Committee Approval

The DCC and each clinical center must obtain approval from their respective IRB/EC prior to participating in the study. The CCC will track IRB/EC approval status at all participating centers and will not permit subject recruitment without documentation of initial IRB approval and maintenance of that approval throughout subsequent years of the project. Washington University IRB will serve as the single IRB (sIRB) for U.S. study sites. Sites located in Australia will obtain local and national approvals, as appropriate, before commencing subject recruitment.

Additionally, the protocol, informed consent forms, recruitment materials and all subject materials must be reviewed and approved for compliance with Department of Defense (DoD) human subjects' protection requirements and approved by the Office of Human Research Oversight (OHRO). Approval from both the IRB/IEC and OHRO for the protocol and the consent forms must be obtained before any participants are recruited. Amendments to the protocol will require review and approval by both the IRB/IEC and OHRO before the changes are implemented in the study.

10.2 Informed Consent

10.2.1 Subject Consent

If a subject is 18 ≥ years of age or attains the age of 18 years during the study period, informed consent is required. Subjects who are capable of giving consent and who are alert and competent, will be asked, following an appropriate discussion of risks and benefits, to give consent to the study. For those with diminished mental capacity, a LAR will be used.

10.2.2 Parental Permission

Subjects under 18 years of age who are eligible for this study will be required to obtain written permission from a parent or legal guardian for participation as per clinical site requirements. After determining that a subject is eligible, the site investigator or designee will approach the parent or legal guardian to offer participation for their child in the study. The parent or legal guardian will be informed about the objectives of the study and the potential risks and benefits of participation. Subject will only be enrolled if their parent or legal guardian provides permission for their child to participate.

10.2.3 Child Assent

In accordance with the governing IRB/EC requirements, children who are capable of giving assent and who are alert and competent will be asked, following an age-appropriate discussion of risks and benefits, to give assent to the study or further study procedures. Assent will be waived if the child is too young, has a severely reduced mental age, decreased level of consciousness, psychological problems, or other legitimate reasons as judged by the IRB/EC.

10.3 Waivers Requested

Waiver from informed consent is requested for collection and recording of demographic and screening data (inclusion and exclusion criteria) prior to obtaining consent. These data are needed

for the trial CONSORT diagram.

10.4 Potential Risks

Subjects in this trial will be undergoing complex cardiac surgery with an expectation of bleeding requiring platelet transfusion. Available data suggest that cold-stored and room temperature platelets have equivalent safety, but this has yet to be studied. Thus, it is possible that cold-stored platelets will be inferior to room temperature platelets. All serious adverse events (SAEs) will be recorded for 28 days and specific adverse events will be recorded after the start of the first study platelet transfusion for up to 28 days after the intervention as illustrated in Appendix D. Particular attention will be focused on the study's safety endpoints, including:

1. Unplanned sternal closure delay
2. Re-exploration for bleeding within 24 hours of first study platelet transfused
3. Unplanned extracorporeal support post operatively within 48 hours of first study platelet transfused
4. Morbidities within 7 days of first study platelet transfused:
   (a) Acute respiratory distress syndrome
   (b) Need for and duration of renal support
   (c) Renal failure
   (d) Septic shock
5. Measures of end organ injury to include BUN, creatinine, lactate, troponin, alanine aminotransferase (ALT) within 48 hours of the start of the first platelet transfusion if drawn clinically.
6. Transfusion associated adverse events classified as per the Center for Disease Control and Prevention (CDC) National Healthcare Safety Network Biovigilance Component Hemovigilance Module Surveillance Protocol (www.cdc.gov/nhsn) within 7 days of first study platelet transfused by a blinded investigator (or designee) at the site.
7. Arterial thrombotic events (stroke, MI) within 7 days of first study platelet transfused.
8. Venous thrombotic events within 7 days of first study platelet transfused (deep vein thrombosis or pulmonary embolism confirmed by Doppler ultrasound, venography, perfusion scan, spiral CT, MRI or pulmonary angiogram).
9. 28-day all-cause mortality.

A telephone call will be made to confirm the vital status (mortality) of subjects who are discharged from the hospital prior to Day 28.

10.5 Protections Against Potential Risks

The CHIPS study is being conducted by experienced research teams and cardiac surgical teams in tertiary medical centers with excellent post-operative intensive care units staffed by cardiac surgery and critical care expert physicians and nurses. Loss of confidentiality of the subject is a

potential risk of the study; however, safeguards are in place to protect against this potential risk.

10.6 Potential Benefits

There are no known benefits anticipated from participation in this study, though we hypothesize that cold-stored platelets are superior to room temperature platelets. In the absence of direct benefit, however, there is potential benefit to others because of the knowledge that may be gained from this research.

11 DATA AND SAFETY MONITORING PLAN

11.1 Data Safety Monitoring Board (DSMB)

The study will have a Data Safety Monitoring Board (DSMB). The DSMB will have a charter, will approve the protocol prior to implementation, and will review interim analyses as applicable. The purpose of the DSMB is to advise the sponsor and Principal Investigator(s) regarding the continuing safety of study subjects and the continuing validity and scientific merit of the study. The DSMB is responsible for monitoring accrual of study subjects, adherence to the study protocol, assessments of data quality, performance of individual clinical centers, review of serious adverse events and other subject safety issues, and review of formal interim statistical analyses of treatment efficacy.

The DSMB will review all reported adverse events (not necessarily serious, unexpected, and study-related) during scheduled DSMB meetings. The DCC will prepare a Summary Report of Adverse Events for the DSMB meetings, classified with the MedDRA coding system. In addition, the DSMB will review events on subjects who receive more than 8 units or doses of study platelets as described in Section 7.4.4.

11.2 Adverse Event Reporting

The site investigator is responsible for ensuring evaluation of all adverse events at their site by a blinded investigator and appropriate reporting of adverse events. All Serious Adverse Events through Day 28 will be recorded. Non-serious adverse events that are commonly associated with platelet transfusions (as illustrated in Appendix D) will also be recorded. The nature of each adverse experience, date and time (where appropriate) of onset, outcome, course, and relationship to treatment will be documented.

11.2.1 Definition of Adverse Event and Serious Adverse Event

Adverse Event (AE): Adverse event means any untoward medical occurrence associated with participation in the CHIPS study, whether or not considered intervention-related (21 CFR 312.32 (a)). An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom.

Serious Adverse Event (SAE): A serious adverse event (SAE) for this population is an adverse event that:

- results in death; or
- is life-threatening (the subject was, in the view of the site investigator, in immediate danger of death from the event as it occurred); or
- requires inpatient hospitalization or prolongs an existing hospitalization; or
- results in persistent or significant disability or incapacity; or
- results in congenital anomaly/birth defect; or
- any other event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Notes:

a. The indication to transfuse platelets for active bleeding that commonly occurs with cardiac surgery or post operatively is not to be considered an adverse event for this study. Excessive

bleeding that is not expected based on the patient history or risk factors and procedure performed or bleeding unrelated to the cardiac procedure(s) would be considered an adverse event/serious adverse event.

b. The adverse event/serious adverse event term should not be defined as the intervention provided to treat the event. Transfusion is an intervention, it is not an adverse event.

11.2.2 Classification of an Adverse Event (Relatedness, Severity and Expectedness)

Relatedness: The suspected relationship between study interventions (i.e., administration of study platelets) and any adverse event will be determined by the site investigator using the criteria below. *Relatedness must be assessed by a blinded investigator and may **not** be assessed by a research coordinator.*

- **Not Related:** The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.
- **Possibly Related:** The event follows compatible temporal sequence from the time of beginning the assigned study intervention but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.
- **Probably Related:** The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention and *cannot be reasonably explained* by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Severity: The severity, which is a measure of intensity, of clinical adverse events and laboratory abnormalities will be recorded by the site investigator and categorized. The following guidelines will be used to describe severity. The Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 should be used as a reference guide to determine severity as outlined below.

- **Mild:** The event requires minimal or no treatment and does not interfere with the participant's daily activities. (e.g., Grade 1-2)
- **Moderate:** The event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning. (e.g., Grade 3)
- **Severe:** The event interrupts a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious". (e.g., Grade 4-5)

Expectedness of the Event: All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An AE/SAE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the

known risks and complications of complex cardiac surgery and platelet transfusion.

- **Expected:** An event is considered expected if it is known to be associated with any of the following: the cardiac procedure, the underlying condition, the study intervention (i.e., platelets), or is mentioned in the protocol, informed consent, or other study documents (e.g., Investigator Brochure). An event may be expected despite the study subject's clinical state immediately prior to the event.

- **Unexpected:** An event is considered unexpected if there are no prior data linking this event with the intervention under study (i.e., platelets) or an event that occurred unexpectedly in the course of treatment.

Treatment or Action Taken: For each reported adverse event, the site investigator will record whether an intervention was required:

- Medical or surgical procedure
- Concomitant medication: started, changed, or discontinued
- Other, specify
- No action taken

Outcome of Event: Finally, the site investigator will record the clinical outcome of each reported adverse event as follows:

- Death
- Recovered and the subject returned to baseline status
- Recovered with permanent sequelae
- Symptoms persist

11.2.3 Time Period for Adverse Events

For purposes of this study, all serious adverse events that occur following the start of the initial platelet transfusion through 28 days will be reported. Thrombotic, embolic and hemorrhagic non-serious adverse events that are commonly associated with platelet transfusions will be collected and reported through Day 28, as illustrated in Appendix D.

11.2.4 Data Collection Procedures for Adverse Events

Adverse events (including all serious adverse events) will be recorded according to relatedness, severity, and expectedness, as well as their duration and any treatment prescribed as illustrated in Appendix D. Any medical condition present at the time of platelet administration, recorded in the subject's baseline history at study entry, which remains unchanged or improves (unless the clinician feels it is clinically relevant), will not be recorded as an adverse event at subsequent evaluations. However, worsening of a medical condition that was present at the time of enrollment will be considered a new adverse event and reported in accordance with Appendix D.

Abnormal laboratory values that are deemed clinically significant by the treating medical staff will be recorded as adverse events and the site investigator will assess the severity and relationship to the study in accordance with Appendix D. Laboratory values that are abnormal at the time of

enrollment and that do not worsen will not be recorded as adverse events.

Adverse events will be reported locally as described in this protocol and the manual of operations. Final adverse event coding will be performed using the Medical Dictionary for Regulatory Activities (MedDRA). Coding will be done centrally at the DCC.

11.2.5 Unanticipated Problems (UP)

Unanticipated problems (UP) are defined as incidents, experiences, or outcomes that are unexpected, related or possibly to participation in the study, and suggest that the research places subjects at a greater risk of harm than was previously known or recognized. The site investigator will report unanticipated problems via EDC within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent within 3 working days of the event. After receipt of the complete report, the report of these unanticipated problems will be sent to the funding sponsor representatives from the Department of Defense USAMMDA in an expedited manner (as close to 24 hours as possible). In accordance with local IRB/EC requirements, the site investigator may be required to report such unanticipated problems to the local and sIRB/EC. In addition, the study team will also notify the medical monitor, DoD OHRO, DSMB, and the national regulatory authorities (i.e., FDA and TGA), as appropriate. If the event warrants emergent suspension of enrollment in the trial, the principal study investigators will notify site investigators to cease enrollment, accordingly. Resumption of enrollment will not occur without consent of the Department of Defense USAMMDA staff and after discussion with the DSMB.

11.2.6 Monitoring Serious Adverse Events

A qualified physician, Dr. Frank Booth, will be designated to fulfill the function of the medical monitor for this study. Site investigators and/or research coordinators will report serious adverse events via EDC (e.g., REDCap Cloud) within 24 hours of becoming aware of the event. A detailed completed report will be required to be submitted within 3 days of the event, and Dr. Booth will assess all serious adverse events reported from site investigators.

For each serious adverse event, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. Dr. Booth will approve each SAE report after review. All SAE reports will be retained in the EDC and the Trial Master File (TMF), and all SAE reports will be available for review by DSMB members. The SAE reporting process may be incorporated into the EDC System in use for the study.

In the unlikely event that Dr. Booth believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, Department of Defense USAMMDA staff and the DSMB chairperson will be immediately consulted. If these individuals concur with the judgment of Dr. Booth, or if the Department of Defense USAMMDA staff and the DSMB chairperson cannot be reached expeditiously, the study investigators (Drs. Spinella, Steiner and Zantek) will be notified and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the Department of Defense USAMMDA staff after discussion with the DSMB. In accordance with local IRB/EC requirements, the site investigator may be required to report such events to the IRB/EC in addition to notifying the DCC.

After notification of the Department of Defense USAMMDA representatives and the DSMB

chairperson of *serious, unexpected, and study-related* adverse events (SUSAR) or unanticipated problems (UP), decisions will be made whether to continue the study without change, and whether to convene the entire DSMB for an emergent meeting. If a decision is made to suspend enrollment in the trial, this will be reported to the study investigators (Drs. Spinella, Steiner and Zantek) and all clinical investigators, who will be instructed to report this suspension of enrollment to their local IRB.

11.2.7 Adverse Event Reporting to Regulatory Authorities

Adverse events that are serious, unexpected, and with a reasonable possibility of relatedness to participation in this study will be reported to the FDA and other appropriate regulatory authorities and all investigative sites by the CCC in accordance with 21 CFR 312.32(c)(1) and other national regulations. The Australian sites are responsible for filing any required reports with the TGA.

11.2.8 Follow-up of Serious, Unexpected and Related Adverse Events

All serious, unexpected, and related adverse events that are unresolved at the time of the subject's termination from the study or discharge from the hospital will be followed by the site investigator until the events are resolved, subject is lost to follow-up, the adverse event is otherwise explained or has stabilized, or 12 months have passed from the time of last study platelet transfused.

12 STUDY TRAINING

A formal training program for investigators and research staff will be held prior to the start of enrollment. The training program will cover regulatory topics and Good Clinical Practice (ICH GCP). The training will also provide in-depth explanations regarding study procedures, adverse event reporting, data entry procedures, quality assurance, site monitoring, and the informed consent process. A manual of operations will be provided to each investigator prior to the start of enrollment. The manual will detail specific information about the study procedures, regulatory information, safety reporting, and other necessary information. Updates and revisions to the manual will be made available electronically. The CCC, in collaboration with the study investigators (Drs. Spinella, Steiner and Zantek), will be the main contact for study questions.

13 REGULATORY CONSIDERATIONS

13.1 Food and Drug Administration

This trial is being conducted under an Investigational New Drug application (Investigational New Drug application #19538). The clinical investigator at each participating site will complete a Form FDA 1572, "Statement of Investigator."

This trial, using this protocol, is also being conducted at selected sites outside the US. The study will be registered with and conducted under the rules set forth by the local and national regulatory authorities and Human Ethics Committees (EC) and GCP as outlined by ICH E6. Each site outside the U.S. understands that the data may be reviewed by FDA investigators if needed for approval of the cold-stored platelet process. Full documentation of the regulatory and IRB/EC review and approval of the protocol will be supplied to the CCC for inclusion in the TMF.

13.2 Health Insurance Portability and Accountability Act

Data elements include identifiers such as dates of birth and procedures, race, ethnicity, gender, and site of clinical care. Written authorization for disclosure of these data, as well as information from the medical record, will be obtained as part of the informed consent procedures for patients who are enrolled in CHIPS. Data that are incorporated into public use datasets prepared from the study will be fully deidentified.

Waiver of authorization has been requested for collection of screening and eligibility data, as well as race, ethnicity and gender. These demographic data are being collected to delineate subject accrual by race, ethnicity, and gender. Remaining screening information (inclusion and exclusion criteria) are necessary to properly report results of the study. For patients who are not eligible for study participation, no authorization will be signed since those patients will not be approached to discuss the study and request consent. Similarly, for patients who elect not to participate in the study, there will not be any request for them to sign authorization documents. For these patients, who are not enrolled in CHIPS, the dates of birth and screening will be deidentified, and the age of the patients will be calculated by the DCC. Screening data for patients who are not enrolled will thereby be de-identified, and these data will not be included in public use datasets prepared from the study.

For purposes of the DCC handling potential protected health information (PHI), including accessing medical records and verifying source documents, the University of Pittsburgh will enter a data use agreement with the University of Utah on behalf of all clinical sites. This agreement will include providing the assurances required by Business Associate Agreements normally used for compliance with HIPAA. Copies of the fully executed agreement will be maintained at the DCC and CCC. Sites outside the U.S. will comply with data protections required by local and national law.

13.3 Inclusion of Women and Minorities

There will be no exclusion of patients based on gender, race, or ethnicity.

13.4 ClinicalTrials.gov Requirements

This trial will be registered at ClinicalTrials.gov in accordance with Federal regulations.

13.5 Retention of Records

For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3 years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research also entails completion of all publications relating to the research. Longer retention may be required if CHIPS data are submitted to the FDA or other regulatory authorities. All records shall be accessible for inspection and copying by authorized representatives of the regulatory authorities at reasonable times and in a reasonable manner [45 CFR 46.115(b)].

13.6 Public Use Data Set

After subject enrollment and follow up have been completed, the DCC will prepare a final study database for analysis. A releasable database will be produced and completely de-identified in accordance with the definitions provided in the Health Insurance Portability and Accountability Act (HIPAA) and with other applicable national regulations. Namely, all identifiers specified in HIPAA will be recoded in a manner that will make it impossible to deduce or impute the specific identity of any patient. The database will not contain any institutional identifiers.

The DCC will also prepare a data dictionary that provides a concise definition of every data element included in the database. If specific data elements have idiosyncrasies that might affect interpretation or analysis, this will be discussed in the dictionary document. In accordance with policies determined by the investigators and funding sponsors, the releasable database will be provided to users in electronic form.

14 BIBLIOGRAPHY

[1] Prajeeda M Nair, Heather F Pidcoke, Andrew P Cap, and Anand K Ramasubramanian. Effect of cold storage on shear-induced platelet aggregation and clot strength. *J Trauma Acute Care Surg*, 77(3 Suppl 2):S88–93, Sep 2014.

[2] Robbie K Montgomery, Kristin M Reddoch, Shankar J Evani, Andrew P Cap, and Anand K Ramasubramanian. Enhanced shear-induced platelet aggregation due to low-temperature storage. *Transfusion*, 53(7):1520–30, Jul 2013.

[3] Kristin M Reddoch, Heather F Pidcoke, Robbie K Montgomery, Chriselda G Fedyk, James K Aden, Anand K Ramasubramanian, and Andrew P Cap. Hemostatic function of apheresis platelets stored at 4 °*C* and 22 °*C*. *Shock*, 41 Suppl 1:54–61, May 2014.

[4] Prajeeda M Nair, Shaunak G Pandya, Shatha F Dallo, Kristin M Reddoch, Robbie K Montgomery, Heather F Pidcoke, Andrew P Cap, and Anand K Ramasubramanian. Platelets stored at 4 °*C* contribute to superior clot properties compared to current standard-of-care through fibrin-crosslinking. *Br J Haematol*, 178(1):119–129, 07 2017.

[5] Heather F Pidcoke, Philip C Spinella, Anand K Ramasubramanian, Geir Strandenes, Tor Hervig, Paul M Ness, and Andrew P Cap. Refrigerated platelets for the treatment of acute bleeding: a review of the literature and reexamination of current standards. *Shock*, 41 Suppl 1:51–3, May 2014.

[6] Todd M Getz, Robbie K Montgomery, James A Bynum, James K Aden, Heather F Pidcoke, and Andrew P Cap. Storage of platelets at 4°*C* in platelet additive solutions prevents aggregate formation and preserves platelet functional responses. *Transfusion*, 56(6):1320–8, 06 2016.

[7] G A Becker, M Tuccelli, T Kunicki, M K Chalos, and R H Aster. Studies of platelet concentrates stored at 22 °*C* and 4 °*C*. *Transfusion*, 13(2):61–8, Mar 1973.

[8] Lacey Johnson, Shereen Tan, Ben Wood, April Davis, and Denese C Marks. Refrigeration and cryopreservation of platelets differentially affect platelet metabolism and function: a comparison with conventional platelet storage conditions. *Transfusion*, 56(7):1807–18, 07 2016.

[9] C R Valeri. Hemostatic effectiveness of liquid-preserved and previously frozen human platelets. *N Engl J Med*, 290(7):353–8, Feb 1974.

[10] Geir Strandenes, Joar Sivertsen, Christopher K. Bjerkvig, Theodor K. Fosse, Andrew P. Cap, Deborah J. Del Junco, Einar Klboe Kristoffersen, Rune Haaverstad, Venny Kval- heim, Hanne Braathen, Turid Helen Felli Lunde, Tor Hervig, Karl Ove Hufthammer, Philip C. Spinella, and Torunn Oveland Apelseth. A pilot trial of platelets stored cold versus at room temperature for complex cardiothoracic surgery. *Anesthesiology*, 133:1173–1183, December 2020.

[11] C S Manno, K W Hedberg, H C Kim, G R Bunin, S Nicolson, D Jobes, E Schwartz, and W I Norwood. Comparison of the hemostatic effects of fresh whole blood, stored whole blood,

and components after open heart surgery in children. *Blood*, 77(5):930–6, Mar 1991.

[12] Cornelius Dyke, Solomon Aronson, Wulf Dietrich, Axel Hofmann, Keyvan Karkouti, Marcel Levi, Gavin J Murphy, Frank W Sellke, Linda Shore-Lesserson, Christian von Heymann, and Marco Ranucci. Universal definition of perioperative bleeding in adult cardiac surgery. *J Thorac Cardiovasc Surg*, 147(5):1458–1463.e1, May 2014.

[13] Gösta B Pettersson, Derlis Martino, Eugene H Blackstone, Edward R Nowicki, Penny L Houghtaling, Joseph F Sabik, 3rd, and Bruce W Lytle. Advising complex patients who require complex heart operations. *J Thorac Cardiovasc Surg*, 145(5):1159–1169.e3, May 2013.

[14] Jacob Raphael, C David Mazer, Sudhakar Subramani, Andrew Schroeder, Mohamed Abdalla, Renata Ferreira, Philip E Roman, Nichlesh Patel, Ian Welsby, Philip E Greilich, Reed Harvey, Marco Ranucci, Lori B Heller, Christa Boer, Andrew Wilkey, Steven E Hill, Gregory A Nuttall, Raja R Palvadi, Prakash A Patel, Barbara Wilkey, Brantley Gaitan, Shanna S Hill, Jenny Kwak, John Klick, Bruce A Bollen, Linda Shore-Lesserson, James Abernathy, Nanette Schwann, and W Travis Lau. Society of cardiovascular anesthesiologists clinical practice improvement advisory for management of perioperative bleeding and hemostasis in cardiac surgery patients. *Anesth Analg*, 129(5):1209–1221, Nov 2019.

APPENDICES

Appendix A: Schedule of Events

	Screening				**Intervention**				**Follow-up**			
	Up to -180 Days	**Within -72 Hours**	**Within -24 Hours**	**Within -30 Min**	**Start of Surgery**	**Time 0[h, m]**	**Time +6 Hours (±2 Hours)**	**Time +24 Hours (±2 Hours)**	**Within Time +48 Hours**	**Time +72 Hours**	**Time +7 Days**	**Time +28 Days**
Obtain informed consent / assent	X											
Assess **study** eligibility criteria	X											
Randomization	X											
Confirm **treatment** eligibility criteria[a]			X									
Record Demographic & baseline characteristics[b]	X	X										
Collect CBC (if not available clinically)[c]		X		X			X	X				
Collect INR, PTT, fibrinogen (if not available clinically)[c]				X			X	X				
Record BUN, creatinine, troponin, lactate, ALT (if available clinically)[d]		X				X						
TEG (select sites only) [c]				X			X	X				
Record surgical procedure data[e]					X							
Record blood products administration[f, h]					X							
Transfuse study platelets (4˚C or 22˚C platelets)[h]					X							
Record components of Perioperative Bleeding Score[g]					X							
Record chest tube output[i]					X							

	Screening				**Intervention**				**Follow-up**			
	Up to -180 Days	**Within -72 Hours**	**Within -24 Hours**	**Within -30 Min**	**Start of Surgery**	**Time 0[h, m]**	**Time +6 Hours (±2 Hours)**	**Time +24 Hours (±2 Hours)**	**Within Time +48 Hours**	**Time +72 Hours**	**Time +7 Days**	**Time +28 Days**
Record hemostatic adjuncts[j]					X							
Concomitant medications or interventions[k, l]					X							
Record morbidities after ICU admission[n]					X							
Record Thrombotic Events, Transfusion Reactions, other AEs[o]						X						
Record ICU, hospital durations												X
Record time on mechanical vent												X
Record vital status (mortality)[q]												X
All SAEs[p]						X						

Notes:

a. Confirm subject treatment eligibility labs (platelet count), which should be completed within 72 hours prior to day of surgery. Confirm anticipated availability of study platelets (room temperature and cold platelets) and that subject has not had a platelet transfusion of any type within 24 hours prior to the date of surgery nor has pre-operative thrombocytopenia ($<75x10^9$/L, per most recent pre-operative labs completed within 72 hours prior to the date of surgery).

b. Record weight (within 14 days of surgery), height, ABO and Rh type; pre-operative morbidities and surgical history (see Appendix B), ASA status (I-V); cardiac diagnosis; use of anticoagulants & antiplatelet agents within 7 days prior to surgery.

c. Record lab results for CBC, INR, PTT, and fibrinogen. If a CBC is not done within 72 hours of the date of cardiac surgery, it must be performed as a research lab. CBC, INR, PTT, and fibrinogen should be collected, *as research labs*, up to 30 minutes prior to first platelet transfusion and 6 (±2) hours and 24 (±2) hours after the first study platelet transfusion. TEG assessments should be collected at the same times (e.g., -30 minutes, +6 hours, +24 hours).

d. Record BUN, creatinine, lactate, troponin, and ALT, *if collected clinically*, within 72 hours prior to date of cardiac surgery. *If collected clinically*, record the worst value within +48 hours following the start of the first platelet transfusion as measure of end organ injury.

e. Surgical procedure(s) and complexity score for age; cardiac severity score (STAT mortality score for children and Surgical Complexity Score for adults); operative start & stop times; CPB cannulation site(s); CPB data (total runs, run times, aortic cross-clamp time, cooling temperatures, temperature at separation); CPB medications or antifibrinolytics; heparinization and heparin reversal or alternative anticoagulation strategy; intraoperative cell recovery and reinfusion; hemodilution strategy (if any); Perioperative Bleeding Score measured from the time of initiation of surgery to the time of starting the 1st platelet transfusion.

f. Record total amount of platelets, plasma, RBCs, cryoprecipitate, and whole blood administered.

g. Perioperative Bleeding Score measured from the time of starting the 1st platelet transfusion.
h. All platelet transfusions administered during the surgical procedure through +24 hours post ICU admission (or post 1st platelet transfusion, if administered in the ICU rather than intra-operatively) should be the assigned study platelet (e.g., 4˚C or 22˚C) up to a maximum of 8 units or 8 pediatric doses (one dose = 10-15 mL/kg). Record platelet unit information including: unit ID number, source of unit (blood supplier), method of collection (Amicus or Trima), storage medium, pathogen reduction use, blood group of unit (ABO and Rh), irradiation status, date and time of product irradiation, re-issue status of platelet unit and expiration date.
i. Record chest tube output from the time of ICU admission. If the first platelet transfusion occurred in the operating room, document the amount of chest tube drainage for 24 hours after ICU admission. If study platelets are administered in the ICU, document amount of chest tube drainage after 1st platelet transfusion for the next 24 hours.
j. See Appendix C for list of hemostatic agents to record.
k. See Appendix C for list of concomitant medications to record. Additional concomitant medications may be recorded if SAEs are reported.
l. Record interventions including unplanned sternal closure delay, re-exploration for bleeding within 24 hours of first study platelet transfusion, repeat surgical procedures, unplanned extracorporeal support within 48 hours of first study platelet transfusion, and use of balloon pumps, ventricular assist devices, plasma exchange and renal replacement support daily until 7 days after surgery, discharge from hospital or death (whichever comes first).
m. Time 0 = time of the start of the first study platelet transfusion, which may occur intra-operatively or within 24 hours after ICU admission.
n. Morbidities include: acute respiratory distress syndrome, need for and duration of renal support, renal failure and septic shock.
o. Thrombotic events include arterial (stroke, MI) and venous (deep vein thrombosis or pulmonary embolism confirmed by Doppler, venography, perfusion scan, spiral CT, MRI or pulmonary angiogram) from time of first study platelet transfusion. See Appendix D for AEs to record.
p. All SAEs through 28 days after the first study platelet transfusion will be collected.
q. Includes all causes of mortality.

Appendix B: Pre-operative Comorbidities

The following pre-operative comorbidities will be collected.

Comorbidity	Reporting Period
Obesity (BMI > 30)	On day of surgery
Diabetes	Any history/ongoing
Hypertension	Any history/ongoing
Smoking	Any history/ongoing
STAT mortality score (children) or Surgical Complexity Score (adults)	On day of surgery (collected as surgical procedure data)
NY AHA class 1–4 (≥18 years of age)	On day of surgery
Hemodialysis or continuous renal replacement therapy or peritoneal dialysis	Any prior to surgery but not supported currently
History of previous cardiac surgery	Any history
Anticoagulant use	Within 7 days of surgery
Antiplatelet therapy	Within 7 days of surgery
Organ support (e.g., prior ECMO, LVAD)	Any prior to surgery but not supported currently
Mechanical ventilation for respiratory failure	Within 7 days of surgery (Note: intubated only for the surgery or for a procedure that precedes the surgery do not count as requiring mechanical ventilation.
History of thrombotic/embolic event	Any history
History of bleeding with surgery	Any history
Liver disease	Any/ongoing
Prematurity (for patients < 1 year)	Less than 34 weeks gestational age
History of myocardial infarction	Any history
History of stroke	Any history
History of intracranial hemorrhage or trauma	Any history
Major Surgery	Within 6 weeks of surgery
Other invasive procedure, including cardiac catheterization	Within 4 weeks of surgery
GI bleed	Within 4 weeks of surgery

Appendix C: Concomitant Medications to be Collected

The following concomitant medications will be recorded. Additional concomitant medications may be recorded for subjects who experience a serious adverse event to meet regulatory reporting guidelines.

Drug Type	Drug	Reporting Timeframe		
		Pre-Operative	**Intraoperative**	**In ICU**
Anti-Platelet Agents	Abciximab Aspirin Cangrelor Clopidogrel Dipyridamole Eptifibatide Prasugrel Ticagrelor Ticlopidine Tirofiban Vorapaxar	Within 7 days of first study platelet transfusion	In the OR/PACU before arrival to ICU	Through 7 days following surgery
Anticoagulants	Apixaban Argatroban Bivalirudin Dabigatran Edoxaban Fondaparinux Heparin Low molecular weight heparin Rivaroxaban Warfarin Protamine	Within 7 days of first study platelet transfusion	In the OR/PACU before arrival to ICU	Through 7 days following surgery
Vasoactive agents	Dopamine Dobutamine Epinephrine Giapreza (Angiotensin II) Milrinone Norepinephrine Phenylepherine Vasopressin Other	Immediately prior to start of surgery	In the OR/PACU before arrival to ICU	Through 7 days following surgery
ACE-inhibitors	Benazepril Captopril Enalapril Fosinopril Lisinoproil Moexioril Perindopril Quinapril Ramipril Zofenopril	Last dose within 24 hours	NA	Through 24 hours in ICU or 24 hours after start of first platelet transfusion (whichever is later)

Drug Type	Drug	Reporting Timeframe		
		Pre-Operative	**Intraoperative**	**In ICU**
Pulmonary vasodilators	Amlodipine Apresoline Epoprostenol Iloprost Milrinone Nitric oxide Nitroglycerine Nifedipine Selexipag Sildenafil Tadalafil Treprostinil Other	Immediately prior to start of surgery	In the OR/PACU before arrival to ICU	Through 24 hours in ICU or 24 hours after start of first platelet transfusion (whichever is later)
Hemostatic agents/ antifibrinolytics	Anti-inhibitor coagulant complex (FEIBA) DDAVP Epsilon-amino-caproic acid Factor XIII concentrate Factor concentrate (other) Fibrinogen concentrate Prothrombin complex concentrate (3 factor) Prothrombin complex concentrate (4 factor) Recombinant factor VIIa (rFVIIa) Tranexamic acid Von Willebrand factor concentrate Other	Immediately prior to surgery	In the OR/PACU before arrival to ICU	Through 72 hours in ICU or 72 hours after start of first platelet transfusion (whichever is later)

Drug Type	Drug	Reporting Timeframe		
		Pre-Operative	**Intraoperative**	**In ICU**
Blood Products	RBC Plasma (FFP, FP24, thawed, SD, dried, liquid) Cryoprecipitate Whole blood Platelets (other than study platelets)	NA	Blood Products will be recorded from the initiation of surgery (exact start time of each product is required) through 72 hours following ICU admission or after the first platelet transfusion (whichever is later).	Blood Products will be recorded from the initiation of surgery (exact start time of each product is required) through 72 hours following ICU admission or after the first platelet transfusion (whichever is later).

Appendix D: Adverse Events to be Collected

All SAEs through 28 days after the first study platelet transfusion will be collected and reported in the EDC. Only thrombotic, embolic and hemorrhagic non-serious adverse events that are commonly associated with platelet transfusions will be collected and reported for a maximum of 28 days following ICU admission in the EDC, as illustrated below.

Type	Description	Reporting Period*
Thrombotic or embolic adverse events	Bypass cannula site thrombus (arterial) Bypass cannula site thrombus (venous) Catheter-associated thrombus (arterial) Catheter-associated thrombus (venous) Intracranial stroke Conduit thrombus Deep vein thrombosis (not CAT) Myocardial infarction Patch thrombus Pulmonary embolism Shunt thrombus Valve thrombus (site) Other thrombotic event (location)	28 days following time of 1st study platelet transfusion

Type	Description	Reporting Period*
Transfusion-related adverse events	Acute hemolytic transfusion reaction Allergic reaction Anaphylaxis Bilirubinuria Chills/Rigors Citrate toxicity Cough Delayed hemolytic transfusion reaction Delayed serologic transfusion reaction Disseminated intravascular coagulation (DIC) Dyspnea Febrile non-hemolytic transfusion reaction Fever Hemoglobinuria Hemolysis Hyperbilirubinemia Hyperkalemia Hyperphosphatemia Hypertension Hypocalcemia Hypotension Hypotensive reaction Hypothermia Hypoxia Pain (chest, flank, IV access) Post transfusion purpura (PTP)	7 days following time of 1st study platelet transfusion

Type	Description	Reporting Period
Transfusion-related adverse events (*continued)*	Pulmonary Hypertension Rash/Pruritis Transfusion related acute lung injury (TRALI) Transfusion associated circulatory overload (TACO) Transfusion associated dyspnea (TAD) Transfusion associated graft versus host disease (TA-GVHD) Transfusion transmitted infection (bacterial, viral, other) Urticaria Wheezing	7 days following time of 1st study platelet transfusion
Bleeding events (other than surgical bleeding)	Catheter-associated hemorrhage Epistaxis Gastrointestinal bleeding Hematuria Hemoptysis Intracranial hemorrhage Joint bleeding Oropharyngeal bleeding Petechiae/purpura Retinal bleeding Subdural hemorrhage Esophageal variceal bleeding Other bleeding (location)	28 days following time of 1st study platelet transfusion
Other	Acute liver failure Renal replacement support Unplanned extracorporeal membrane oxygenation (ECMO)	7 days following time of 1st study platelet transfusion

*Events which are, by definition, less than 7 days (i.e., TRALI) will be reported accordingly.