A Randomized, Multi-center, Controlled, In Vivo Study to Assess the Recovery and Survival of Radiolabeled Autologous Apheresis Platelet Components Suspended in 100% Plasma Treated with the INTERCEPT Blood System for Platelets with LED Illuminator Stored for 5 Days

Protocol CLI 00183, Version 2.0
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PROTOCOL

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SPONSOR:	Cerus Corporation 1220 Concord Ave Suite 600 Concord, CA 94520 (925) 288-6000
PROTOCOL NUMBER:	CLI 00183
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PHASE:	PHASE II
MEDICAL OFFICER & MEDICAL MONITOR:	Richard J. Benjamin MD, PhD, FRCPath Chief Medical Officer Cerus Corporation (925) 288-6020
MANAGER:	Margaret Hennig, PhD Early Clinical Development Program Manager Hematology and Early Clinical Development Cerus Corporation (925) 288-6178

1.0 SYNOPSIS

Title of Study:	CLI 00183: A Randomized, Multi-center, Controlled, *In Vivo* Study to Assess the Recovery and Survival of Radiolabeled Autologous Apheresis Platelet Components Suspended in 100% Plasma Treated with the INTERCEPT Blood System for Platelets with LED Illuminator Stored for 5 Days
Sponsor	Cerus Corporation
Protocol Number	CLI 00183
IDE Number	BB-IDE 6200
Investigators (Study Sites):	Bethany Brown, PhD, MSCS (American Red Cross Research Laboratory) Moritz Stolla, MD, PhD (Bloodworks Northwest Research Institute)
Phase of Development	Phase II

Objectives:

The objective of this study is to assess the post-infusion recovery and survival of INTERCEPT with LED Illuminator treated platelets in 100% plasma stored for 5 days after apheresis collection. The post-infusion recovery and survival of autologous radiolabeled 5-day INTERCEPT platelets (Test) stored in 100% plasma will be measured in comparison to fresh autologous radiolabeled platelets (Control).

Methodology

Study Design

This trial is a single arm design. INTERCEPT Blood System with LED Illuminator processed platelets (Test) from a minimum of 24 healthy subjects, stored for 5 days, will be prepared for radiolabeling following the Variant 1 of BEST 4.2.1 (Variant 1) methodology. The recovery and survival for Test platelets will be compared against the fresh Control platelets.

The study population will consist of healthy subjects who meet the FDA, AABB, and site-specific research donor eligibility criteria for allogeneic and plateletpheresis donor qualifications. Apheresis platelets will be collected in 100% plasma on the Trima Accel® Automated Blood Collection system.

Test platelets

Test apheresis collections will be processed using the INTERCEPT Blood System for Platelets with the LED Illuminator. Platelet components containing 4.0 to 5.2 $\times 10^{11}$ platelets in 300 to 390 mL of plasma will be processed using the INTERCEPT LV processing set. The INTERCEPT process will begin on either the day of collection (Day 0) or the day following collection (Day 1); illumination must start within 24 hours after the end of collection. Test platelet components will be stored for 5 days, from day of collection, in 100% plasma.

Samples for *in vitro* platelet testing will be collected prior to INTERCEPT treatment (Day 0/1), post INTERCEPT treatment (Day 1/2), and at the end of storage (Day 5; see *In vitro* evaluation of platelets below).

At the end of storage, an aliquot of Test platelets will also be aseptically removed from each subject's INTERCEPT platelet storage container for preparation of samples for radiolabeling using Variant 1 methodology.

Control platelets

On Day 5 post- collection, healthy subjects will return to the site, and whole blood (WB) will be drawn into a syringe containing Anticoagulant Citrate Dextrose Solution, Formula A (ACD-A). WB will be used to prepare fresh Control platelets following the Variant 1 of BEST 4.2.1 methodology.

Radiolabeling

Test and Control platelets will be radiolabeled with either ^{51}Chromium (^{51}Cr, approximately 10 to 30 µCi) as sodium chromate ($Na_2{}^{51}CrO_4$) or ^{111}Indium (^{111}In, approximately 10 to 30 µCi) as Indium Oxine, depending upon the randomization assignment. After radiolabeling, the autologous Control and Test platelet samples will be simultaneously infused into the subject (approximately 5 to 30 mL). A negative pregnancy test for females of childbearing potential is required before infusion.

Blood samples will be drawn immediately before infusion and for radioactivity measurements at 2 hours ±15 min post-infusion (Day 0), and 6 more samples will be drawn at 1 (within ±4 hours from time of infusion), 2, 3, 5±1, 7/8, and 11±1 days post-infusion (DPI)). The exact time of each sample draw will be recorded.

Safety

Subjects will be monitored for safety (adverse events including transfusion reactions) from the time of the apheresis procedure until 24 hours after the last DPI blood sample is drawn.

Radioactivity measurements

Samples will be obtained from the radiolabeled Control and Test platelets before infusion and used as a radioactive standard. The total dose of radioactivity infused will be calculated based on the volume of labeled platelets infused. *In vitro* elution of the label from the transfused platelets, as well as the *in vivo* elution of radioactivity from the serial blood samples obtained post-infusion will be determined by the BEST elution assessment method.

The radioactivity of the samples will be determined by use of a gamma counter with correction for isotope spill over. Duplicate WB samples will be counted for each time point. Sample dilutions for enumeration of radioactivity of the standards will be calibrated to ensure that measured values are at least 10-fold above background values. The subject's WB samples will be corrected for the activity in the plasma fraction determined by simple centrifugation and cellular but non-platelet residual activity using the 11 ±1 DPI results. Data points greater than 20 hours post-infusion will be used to calculate the post infusion recovery after all radioactive corrections have been made. A multiple-hit model will be used to estimate the survival of the radioactively labeled platelets.

***In vitro* evaluation of Test platelets**

In vitro platelet function of the platelet component will be evaluated on pre-treatment (Day 0/1), post-INTERCEPT treatment (Day 1/2), and at end of storage (Day 5). The physical and metabolic characteristics to be evaluated at each timepoint are listed in Table S-1.

Table S-1 *In Vitro* Platelet Function Assays to Evaluate INTERCEPT Platelet Components

Assay	Input (Day 0/1)	Post-INT (Day 1/2)	End of Storage (Day 5)
Component weight (g) [a]	X	X	X
Platelet count ($\times 10^3/\mu L$)	X	X	X
Platelet dose ($\times 10^{11}$ cells/unit) [a]	X	X	X
Mean platelet volume (MPV) (fL)	X	X	X
$pH_{22°C}$	X	X	X

$pO_{2\ (37°C)}$ (mm Hg) [c]	X	X	X
$pCO_{2\ (37°C)}$ (mm Hg) [c]	X	X	X
$HCO_3^{-}{}_{(37°C)}$ (mmol/L) [c]	X	X	X
Supernatant glucose (mmol/L) [c]	X	X	X
Supernatant lactate (mmol/L) [c]	X	X	X
ATP (µmol/dL) [c]	X	X	X
Supernatant LDH (U/L) [c]	X	X	X
Total LDH (U/L) [d]	X	X	X
LDH as a % of total LDH [b]	X	X	X
CD62P (% P-selectin expression)	X	X	X
Bacterial culture	-	1 - 3 days before end of storage	-

[a] Volume is calculated from component weight. Platelet dose is calculated from the platelet count and volume.
[b] Calculated from ratio of LDH activity in 1 mL of platelet concentrate supernatant to the total LDH activity in 1 mL of Triton X-100 lysed platelet concentrate. This value will also be corrected for baseline (Day 0/1) LDH.
[c] The values for these parameters will also be normalized for platelet count.
[d] Acquired from the Triton X-100 lysed platelet concentrate.

Number of subjects:

A minimum of 24 subjects with evaluable (Test and Control) recovery and survival data may be obtained. Randomized subjects who do not have both paired (Test and Control) recovery and paired survival data will be replaced until the enrollment goal is met. Up to 60 subjects may be enrolled to obtain a sufficient number of evaluable subjects.

Main criteria for inclusion:

Inclusion criteria:

- Age ≥18 years
- Normal health status (as determined by Investigator review of medical history and blood donor physical exam)
- Complete blood count (CBC) and serum chemistry values within normal limits or outside of normal reference range but determined by the Investigator, and consultation with the Sponsor, to be not clinically significant.
- Meet FDA, AABB, or institutional guidelines for allogeneic and plateletpheresis donor qualifications with the following exceptions:
    - Deferrals due to travel, tattoos/piercings, male to male sexual contact as institutional policies allow
- All routine infectious disease testing must be negative or non-reactive (during screening)
- Subjects of childbearing potential must agree to use a medically acceptable (as per the Investigator) method of contraception throughout the study
- Signed and dated informed consent form

Exclusion criteria:

- Clinically significant acute or chronic disease (as determined by the Investigator)
- Treatment with aspirin or aspirin-containing medications within 7 days of apheresis or treatment with non-steroidal anti-inflammatory drugs (NSAID), anti-platelet agents or other drugs affecting platelet viability within 3 days of apheresis (e.g., ibuprofen or other NSAIDs)
- Subject received platelet inhibitors within 14 days of donation (e.g., clopidogrel, ticlopidine, amphetamines (e.g., Adderall, Dexedrine)
- Immunosuppressive therapy (e.g., oral or IV prednisone) within the past 28 days
- Treatment with any medication known to affect platelet viability
- Pregnant or nursing females
- Received an investigational drug within the past 28 days or current participation in another clinical interventional study.
- Non study blood component donation throughout the study
- Subjects with positive cocaine and/or amphetamine results from urine drug screen
- Splenectomized subjects
- History of known hypersensitivity to ^{51}Chromium or ^{111}Indium

Test product and mode of administration:

Apheresis platelet components in 100% plasma collected using the Trima separator, prepared with the INTERCEPT Blood System for Platelets (Test Platelets) using the LED Illuminator and stored for 5 days at 20 to 24°C with continuous agitation. Samples from the Test component will be processed with the Variant 1 method. The radiolabeled autologous Test and Control platelets, approximately 5 to 30 mL, will be simultaneously administered intravenously into the subject.

Duration of treatment:

Subjects will be screened for eligibility within 15 days prior to participating. For each qualified subject, study participation will include 15 to 17 days following the apheresis platelet procedure depending on the day of the last blood sample collection with an initial screening window of up to 15 days, for a total participation of approximately 31 to 33 days.

Reference therapy and mode of administration:

Autologous fresh, whole blood derived platelets isolated from platelet rich plasma (Control platelets) processed and radiolabeled using the Variant 1 of BEST method will be administered simultaneously with the radiolabeled Test platelets into the subject.

Criteria for evaluation:

Efficacy

Primary efficacy endpoints

- Post-infusion recovery after 5 days of storage
- Post-infusion survival after 5 days of storage

Using the FDA guidance on platelet viability by radiolabeling (i.e., recovery and survival compared to a fresh autologous platelet sample as control, drawn and prepared on day of reinfusion of radiolabeled test samples), the acceptance criteria are:

- Lower bound of a two-sided 95% confidence interval (CI) for the mean treatment difference (Test - 0.66 × Control) in 24-hour post-infusion recovery is greater than or equal to zero.
- Lower bound of a two-sided 95% CI for the mean treatment difference (Test - 0.58 × Control) in post-infusion survival is greater than or equal to zero.

Secondary efficacy endpoints (product parameter evaluation of platelet components)

The secondary efficacy endpoints will be summarized descriptively and are based on product parameters post-INTERCEPT treatment and at end of storage.

Product Parameters at the End of INTERCEPT Treatment

- Platelet dose ($\geq 3.0 \times 10^{11}$ platelets)
- Platelet yield retention ($\geq 80\%$)

Product Parameters at Day 5

- $pH_{22°C}$ (≥ 6.2)

Additional efficacy endpoints

Additional analyses will include the *in vitro* function parameters of the stored INTERCEPT platelet component after INTERCEPT treatment and at the end of storage:

Assessments of INTERCEPT platelet components post-INTERCEPT treatment and at End of Storage

Product Parameters	Biochemical	Functional
• Component volume	• Glucose & normalized consumption per platelet count	• CD62P (P-selectin) expression
• Platelet count	• Lactate & normalized production per platelet count	
• Platelet dose	• pO_2 & normalized consumption per platelet count	
• Mean platelet volume	• pCO_2 & normalized production per platelet count	
	• Bicarbonate & normalized production per platelet count	
	• Lactate dehydrogenase (LDH) (supernatant and total from Triton X-100 lysate) & normalized supernatant LDH per platelet count	
	• LDH as a % of total LDH and baseline (Day 0/1) adjusted LDH as a % of total LDH	
	• ATP & normalized per platelet count	

Safety

Subjects' vital signs, hematology and chemistry evaluations, and adverse events (AE) will be collected from the initiation of apheresis collection through 24 hours after the last DPI blood sample is drawn.

Statistical methods:

Primary efficacy endpoints

Recovery and survival data with 5-day platelet storage will be assessed and compared with its paired control descriptively:

(1) For post-infusion recovery:

H_0: Test – 0.66 × Control < 0 vs. H_1: Test – 0.66 × Control ≥ 0

The null hypothesis will be rejected if the lower bound of a two-sided 95% CI for the mean treatment difference (Test – 0.66 × Control) is greater than or equal to zero.

(2) For post-infusion survival:

H_0: Test – 0.58 × Control < 0 vs. H_1: Test – 0.58 × Control ≥ 0

The null hypothesis will be rejected if the lower bound a two-sided 95% CI for the mean treatment difference (Test – 0.58 × Control) is greater than or equal to zero.

The primary analysis population for the primary efficacy endpoints will only include subjects who have both paired (Test and Control) recovery and paired survival data. The two-sided CIs will be computed using the variance estimated from a paired t test.

If non-inferiority cannot be declared for the primary endpoints with a minimum of 24 evaluable subjects, additional exploratory analyses may be conducted to quantify the proportions of subjects whose recovery and survival data meet the 0.66 and 0.58 cutoff, respectively (e.g., platelet recovery from a Test platelet component greater than or equal to 66% of platelet recovery from the paired fresh Control platelets).

Secondary efficacy endpoints

Data will be summarized descriptively. The proportions of Test platelet components with platelet dose ≥ 3.0×10^{11} platelets (post-INTERCEPT treatment), platelet yield retention ≥ 80% (post-INTERCEPT treatment), and $pH_{22°C}$ ≥ 6.2 (end of storage) will be summarized, with the corresponding lower bound of a one-sided 95% CI for the proportion provided.

Correlations between the primary and secondary efficacy endpoints may also be explored using graphs or regression analysis.

Additional efficacy endpoints

Additional analyses will include the *in vitro* function parameters of the Test INTERCEPT platelet component and the platelet samples at the end of INTERCEPT treatment and at the end of storage.

Analysis Sets

Consented Set

- All consented subjects.

Intention to Treat (ITT) (Safety Analysis Set):

- All subjects who successfully initiate an apheresis collection.

Modified Intention to Treat (mITT):

- Subjects who have data for recovery and survival data and/or other *in vitro* characteristics.

Per-Protocol Set (PPS):

- All randomized and infused subjects who have both paired (Test and Control) recovery and paired survival data, Test recovery and/or survival less than Control recovery and/or survival, and otherwise complied with the protocol without any protocol deviation potentially affecting the primary efficacy outcome.

The primary and secondary efficacy analysis will be based on the PPS. As a sensitivity analysis, primary and secondary efficacy endpoints will be conducted on mITT as well. *In vitro* function and physical parameters will be assessed on ITT, mITT, and PPS. Safety data will be summarized for the ITT. Consented Subjects will be assessed only for eligibility, study completion, and deviations. All other summaries will be presented using the ITT unless stated otherwise.

Statistical methods:

Data will be summarized descriptively by mean, standard deviation, median, and range (minimum, maximum) for continuous parameters, and by frequencies and percentages for categorical data variables. Summaries will be presented across all study sites and within each study site.

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2.0 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AABB	Association for the Advancement of Blood & Biotherapies
ACD-A	Anticoagulant Citrate Dextrose Solution-Formula A, USP (2.13% free citrate ion)
AE	Adverse Event (defined in **Section 9.3.1**)
ATP	Adenosine 5'-Triphosphate
BEST	Biomedical Excellence for Safer Transfusion
BMI	Body Mass Index
CAD	Compound Adsorption Device
CBC	Complete Blood Count
CD62P	P-selectin
CI	Confidence Interval
^{51}Chromium, ^{51}Cr	An isotope of chromium with a radioactive half-life of 27.7 days
Control	Fresh autologous platelets
Device Malfunction	The failure of an investigational medical device to function according to its requirements.
DPI	Days post-infusion of autologous radiolabeled platelets
eCRF	Electronic Case Report Form
EDTA	Ethylenediaminetetraacetic acid
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
^{111}Indium, ^{111}In	An isotope of indium with a radioactive half-life of 2.80 days
INT200	INTERCEPT LED Illuminator commercial configuration (Also described as LED Illuminator or INTERCEPT LED Illuminator).

INTERCEPT Platelet or Platelet Component	Apheresis platelets suspended in in 100% plasma and treated with the INTERCEPT Blood System for Platelets using LED Illuminator
INTERCEPT Blood System® for Platelets	A medical device that inactivates infectious pathogens (viruses, bacteria, and protozoa) and leukocytes using amotosalen and UVA light in platelet components.
IRB	Institutional Review Board
LED	Light-emitting diode
LV	Large Volume (one storage container)
LDH	Lactate dehydrogenase
Min	Minute
MPV	Mean Platelet Volume
mrem	Millirem
NSAID	Non-steroidal anti-inflammatory drug
PC	Platelet Component
PPP	Platelet Poor Plasma
Product Complaint	The failure of a medical device to function according to its requirements.
PRP	Platelet Rich Plasma
QA	Quality Assurance
RBC	Red Blood Cell
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
Sponsor	Cerus Corporation
TACO	Transfusion-Associated Circulatory Overload
TA-GVHD	Transfusion-Associated Graft Versus Host Disease
Test	Autologous INTERCEPT platelet
TRALI	Transfusion-Related Acute Lung Injury
TTI	Transfusion-Transmitted Infection
UVA	Ultraviolet A light
WB	Whole blood

3.0 BACKGROUND INFORMATION

The INTERCEPT Blood System for Platelets is intended to be used for *ex vivo* preparation of pathogen-reduced apheresis platelet components (PCs) to reduce the risk of transfusion-transmitted infection (TTI), including sepsis, and as an alternate to gamma irradiation for prevention of transfusion-associated graft-versus-host disease (TA-GVHD).

The INTERCEPT Blood System for Platelets is a Class III medical device consisting of plastic disposable sets and an illumination device (INTERCEPT Illuminator), which provides a controlled dose of ultraviolet A (UVA) light to the PC. The final product is either transfused or stored according to blood center standard operating procedures (SOP), US Food and Drug Administration (FDA), and Association for the Advancement of Blood & Biotherapies (AABB) Guidelines.

The INTERCEPT Blood System for Platelets was approved by the FDA in December 2014 for treatment of platelets collected in a commercially available platelet additive solution (BP140143). In March 2016, the FDA approved the use of the INTERCEPT System for treatment of platelets suspended in 100% plasma (BP140143/8). The current maximal expiration dating period for INTERCEPT platelets in the U.S. is 5 days. In March 2024, FDA approved the post-approval commitment to complete a recovery and survival of apheresis platelets suspended in 100% plasma and treated with the INTERCEPT Blood System for Platelets (CLI 00164). This study demonstrated that 5-day stored INTERCEPT platelets met the FDA criterion for non-inferiority for both *in vivo* recovery and survival. Additionally, 5-day INTERCEPT platelets retained critical *in vitro* and functional characteristics and demonstrate *in vivo* viability.

The current commercial INTERCEPT INT100 Illuminator was designed to deliver a controlled amount of UVA light emitted by fluorescent light bulbs. In recent years, the lighting industry has experienced a shift from fluorescent lighting technology to more energy efficient light-emitting diode (LED) technologies. As a result, the procurement of fluorescent light bulbs, for manufacturing new Illuminators and for preventative maintenance of existing Illuminators, has become challenging. It is expected that within the next few years, LED technology will become the dominant lighting technology and fluorescent bulbs will be phased out.

To address this technology change, Cerus has a development project to update the current commercial INTERCEPT INT100 Illuminator. The INTERCEPT LED Illuminator was developed to primarily address and mitigate ongoing hardware obsolescence regarding the UVA light source in the instrument. There is no change to the intended use of the INTERCEPT Illuminator.

This study will compare the *in vivo* post-infusion radiolabeled recovery and survival of INTERCEPT platelets (Test) suspended in 100% plasma and stored for 5 days to fresh autologous platelets (Control) prepared from platelet rich plasma (PRP) isolated from whole blood (WB). The results of this study are intended to support the registration of the INTERCEPT Blood System for Platelets with the INTERCEPT LED Illuminator for apheresis-derived platelets and will be conducted using currently approved INTERCEPT platelet processing sets [Large Volume (LV)] and LED Illuminator.

3.1 Name and Description of the Study Platelet Product and Investigational Device

The INTERCEPT Blood System for Platelets is a medical device (Table 1, Figure 1) consisting of a single-use processing set (INTERCEPT Processing Set for platelets) and an illumination device (INTERCEPT LED Illuminator), which provides a controlled dose of UVA light to the platelet component(s). The apheresis-derived platelet component (PC), prepared with the INTERCEPT Blood System for Platelets (INTERCEPT platelet component) and the radiolabeled Control platelets are investigational study platelet products. There is no change in the intended use of the INTERCEPT LED Illuminator.

Table 1 Components of the INTERCEPT Platelet Processing Sets

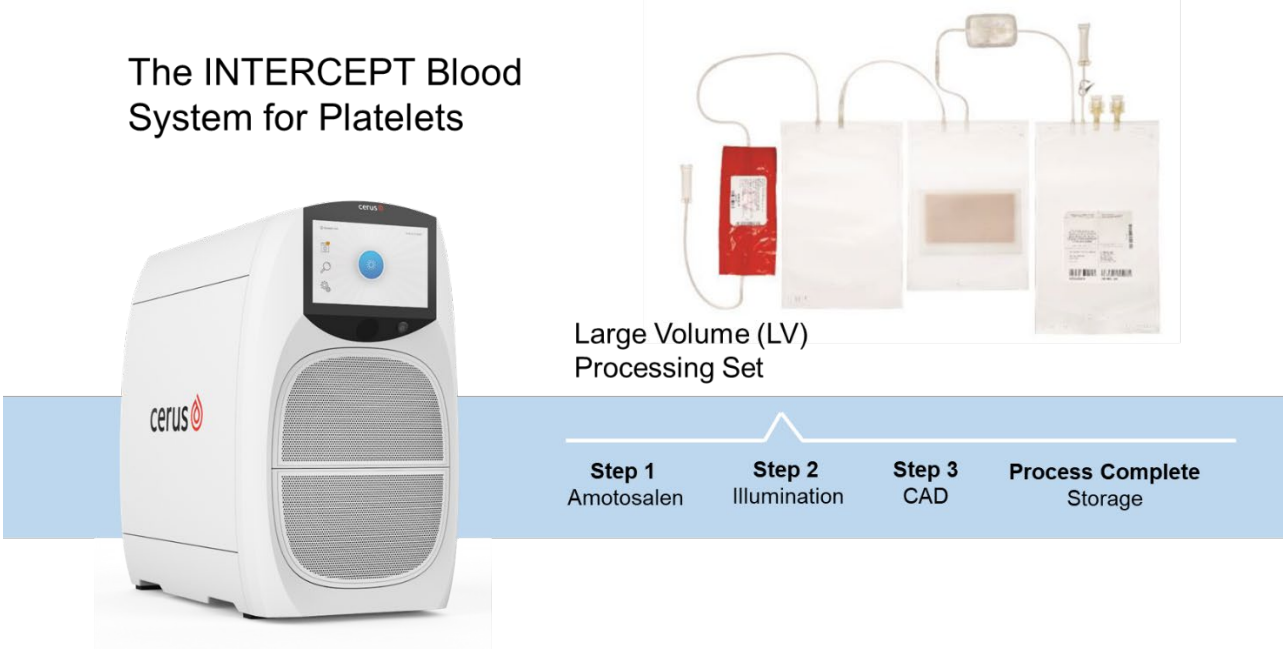
Component	Description
Amotosalen (S-59, psoralen derivative) solution container	**Platelet SV Processing Set (INT21):** 15 mL, 3 mM amotosalen in 0.924% NaCl packaged in a 20 mL, flexible, heat-sealed plastic container, with light protective sleeve **Platelet LV and DS Processing Sets (INT22 and INT25):** 17.5 mL, 3 mM amotosalen in 0.924% NaCl packaged in a 20 mL, flexible, heat-sealed plastic container, with light protective sleeve
Illumination container	Heat-sealed, plastic bag
Compound Adsorption Device (CAD)	Immobilized beads (wafer) in mesh pouch
CAD container	**Platelet SV and LV Processing Sets (INT21 and INT22):** 1000 mL capacity **Platelet DS Processing Set (INT25):** 1300 mL capacity
Platelet storage containers	**Platelet SV and LV Processing Sets (INT21 and INT22):** One platelet storage bag – 1300 mL storage capacity **Platelet DS Processing Set (INT25):** Two platelet storage bags – 1300 mL storage capacity each

The LV (INT22) processing sets will be used in this study. The LV set consists of the following sequentially integrated components: 17.5 mL of 3 mM amotosalen solution, a 1-liter illumination container, a 1-liter compound adsorption device (CAD,), an in-line filter, and one 1.3-liter storage container.

The intended commercial LED Illuminator (INT200) will be used in this study. The INTERCEPT LED Illuminator is a device designed to deliver a pre-defined amount of UVA light to the illumination container of the INTERCEPT Processing Set using an arrangement of light sources and intensity-monitoring photodiodes. The INTERCEPT LED Illuminator will be used in conjunction with platelets in an INTERCEPT Processing Set and is the Investigational Device for this study. The processing set allows blood products to be handled in a functionally closed system. The INTERCEPT LED Illuminator has the capability of illuminating two units simultaneously. Each platelet unit rests on a UVA-transparent tray that undergoes horizontal agitation during the illumination process. The INTERCEPT LED Illuminator delivers a target dose of 3.3 Joules/cm^2 (J/cm^2) UVA treatment to each PC through two opposing light engine components (LECs) mounted above and below each illumination tray. The INTERCEPT LED Illuminator maintains records of blood products, processes, and transfers these records to a printer or external computer.

The INTERCEPT LED Illuminator is intended only for use in the pathogen reduction process to deliver UVA light for the photochemical treatment of blood products. The INTERCEPT LED Illuminator should be used only by personnel trained to perform the INTERCEPT Blood System process.

Figure 1 INTERCEPT Blood System for Platelets with LED Illuminator



3.2 Summary of Nonclinical and Clinical Studies

The nonclinical and clinical studies are summarized in the Investigator's Brochure (INB 00002, Investigator Brochure INTERCEPT Blood System for Platelets) and package insert (SPC 00698-AW INTERCEPT Blood System for Platelets – Large Volume (LV) Processing set). The following paragraphs provide additional background for the proposed study.

3.3 Summary of Potential Risks and Benefits

3.3.1 Benefits of Study Participation

There are no direct benefits to the subjects in this study.

3.3.2 Risks of Study Participation

A summary of the safety experience with INTERCEPT platelets using the INT100 is provided in the Package Insert (SPC 00698-AW). The cumulative experience suggests no excess treatment-related morbidity associated with the transfusion of INTERCEPT PCs compared to conventional PCs. There is no anticipation of additional risk with the INT200 LED Illuminator as there is no change in the intended use of this new INTERCEPT LED Illuminator. In addition, development studies have shown that the light dose delivered from the INT200 is sufficient to provide similar pathogen reduction as the INT100. A recent study reported a prototype LED illuminator had no impact on platelet metabolism, spontaneous activation, apoptosis, or viability, or on the *in vitro* function of buffy coat platelet components stored for 7 days compared to illumination with the INT100 Illuminator (Brouard 2023). With the limited exposure to autologous platelets in the study proposed herein (approximately 5 to 30 mL infusion), systemic risks are considered unlikely.

3.3.2.1 Risk of Receiving an Incorrectly Labeled Platelet Component

PCs will be prepared, labeled, and tracked using site SOPs to ensure that appropriate identification of study components is always maintained. Verification of each subject's platelet component will occur prior to infusion as an additional check on component identity.

3.3.2.2 Risk of Transfusion-Transmitted Infectious Disease

The risk of transfusion-transmitted infections (TTI) is considered minimal for study subjects who will receive approximately 5 to 30 mL of washed radiolabeled autologous Test and Control platelets. All standard viral pathogen testing performed routinely by the blood centers will be performed. Since subjects will be receiving autologous platelets that have been screened for TTIs, the risk of infection transmission is minimal from Test PCs. All Test PCs will be treated with the INTERCEPT Blood System with the LED Illuminator which has demonstrated prominent levels of bacterial inactivation efficacy in equivalent to inactivation

with illumination using the INT100 Illuminator in verification studies. Additionally, the light dose received by the platelets is similar between the INT100 Illuminator and the INT200 Illuminator.

Bacterial contamination is possible during radiolabeling due to required use of an open system and due to the possible use of reagent grade Chromium. The radiolabeling occurs in a biosafety cabinet and in a narrow time frame to help prevent contamination. Sterile filtration and endotoxin testing will be performed when reagent grade Chromium is used. In addition to minimizing risk of bacterial contamination, only a small aliquot of radiolabeled platelets will be re-infused shortly after labeling, thus the risk of bacterial proliferation due to contamination during the radiolabeling procedure is reduced. Based on verification studies demonstrating inactivation of viruses and bacteria, the infectious disease risk to recipients of Test platelets is reduced compared with that of conventional PC (INTERCEPT package insert, 2019) and is comparable to INT100 illuminated components.

3.3.2.3 Risk of Transfusion Reaction

Transfusion reactions known to occur following transfusion of conventional platelets may occur following infusion of study platelets (e.g., fever, chills, itching, rash, hives, bronchospasm, nausea/vomiting, hypertension/hypotension, TRALI, TACO, and anaphylaxis). The risk of experiencing a transfusion reaction is considered minimal from the small autologous infusions used in this study (approximately 5 to 30 mL).

3.3.2.4 Risk of Apheresis Collection

Risks associated with the apheresis collection of platelets include blood pressure changes, a temporary decrease in platelets, a small loss of white blood cells, problems with vein access (including pain, bleeding, and infection at the catheter site), effects of the anticoagulant on the donor's calcium level (including paresthesias from citrate-induced hypocalcemia), and allergic reactions to the anticoagulant or other products used, which may be life-threatening. On very rare occasions, risks associated with the apheresis collection include nerve damage, serious problems, and death. Allergic reaction due to iodine allergy is possible if an iodine solution is used to cleanse the phlebotomy site to meet standards in 21 CFR 640.4(f). Blood pressure changes can sometimes cause nausea, fatigue, and dizziness. Platelet loss may increase the risk of bleeding from cuts or wounds. Venous access problems can cause bruising referred to as a hematoma. While donating, a supply of calcium antacid tablets is usually kept close by to replenish the calcium lost. Because the anticoagulant works by binding to the calcium in the blood, a donor's levels of calcium, and especially of active calcium ions, decrease during the donation process. The lips may begin to tingle or there may be a metallic taste; since calcium enables the function of the nervous system, nerve-ending-dense areas (such as the lips) are susceptible, at least during the donation process. Unusually low calcium can cause more serious problems such as fainting, nerve irritation and short-duration tetany. Such an acute hypocalcemia is usually due to low calcium levels prior to donation, aggravated by the anticoagulant. Hypocalcemia can be curtailed by modestly increasing dietary calcium intake in the days prior to donation. Nerve damage, which is more rarely observed, may result in numbness, pain, or paralysis. Serious problems are extremely rare and may include heart attack and stroke. The apheresis procedure can also result in a need for extended medical treatment and can be fatal. Apheresis donors are routinely monitored during the long donation process.

3.3.2.5 Risk Related to Whole Blood Collection

WB collection may be associated with hematoma formation, local irritation, or infection. Use of experienced phlebotomists will reduce the likelihood of these types of injuries. Local care can be given to prevent hematoma formation, according to standard medical practice.

3.3.2.6 Risk Related to Infusion Procedure

The infusion procedure may be associated with hematoma formation, local irritation, or infection. Local care can be given to prevent hematoma formation, according to standard medical practice. The use of a heparin lock during reinfusion may cause low platelet counts and thrombosis in subjects receiving larger doses.

3.3.2.7 Risk Related to Radiation Exposure

^{111}Indium Oxine (^{111}In) and sodium chromate ($Na_2{}^{51}CrO_4$; ^{51}Cr) are routinely used for radiolabeling and infusion of fresh or stored platelets in humans. The amount of radiation that each subject will receive in the study infusion is estimated to be up to approximately 60 mrem, below the annual maximum limits typically considered to be safe.

No known risks are associated with ^{51}Cr administration at the level of ^{51}Cr exposure used in this study. Allergic dermatological reactions have been reported in association with ^{111}In.

4.0 TRIAL OBJECTIVES

The objective of this study is to assess the post-infusion recovery and survival of INTERCEPT with LED Illuminator treated platelets in 100% plasma stored for 5 days after apheresis collection. The post-infusion recovery and survival of autologous radiolabeled 5-day INTERCEPT platelets (Test) stored in 100% plasma will be measured in comparison to fresh autologous radiolabeled platelets (Control). In addition, *in vitro* function will also be evaluated for the stored Test INTERCEPT platelets.

5.0 TRIAL DESIGN

5.1 Overall Trial Design and Plan

The study population will consist of healthy subjects who meet the FDA, AABB, and site-specific research donor eligibility criteria for an apheresis platelet collection.

Apheresis platelets will be collected in 100% plasma on the Trima Accel® Automated Blood Collection system. Each study apheresis collection will be processed using the INTERCEPT Blood System for Platelets; apheresis platelets containing a platelet dose of 4.0 to 5.2 $\times 10^{11}$ platelets in 300 to 390 mL of plasma will be processed using the INTERCEPT LV processing set (Table 2). The INTERCEPT process will begin on either the day of collection (Day 0) or the day following collection (Day 1); illumination on the LED Illuminator must start within 24 hours after the end of collection. Test platelet components will be stored for 5 days, after collection, in 100% plasma. *In vitro* platelet function will be evaluated on pre-treatment (Day 0/1), at the end of INTERCEPT treatment (Day 1/2), and at end of storage (Day 5) (**Section 8.2.2**, Table 7).

Table 2 Platelet Input Study Specifications

Platelet Processing Step	Study Requirements
Apheresis Platform	Trima Accel® Automated Blood Collection System
Anticoagulant	ACD
Collection and Storage Medium	100% plasma
Input RBC Content	$< 4 \times 10^6$ RBC/mL
Input Dose ($\times 10^{11}$ platelets)	4.0 – 5.2
Input Platelet Count ($\times 10^6/\mu L$)	900 - 1700
Input Volume (mL)	300 - 390
Storage	Continuous gentle agitation at 22°C ±2°C for 5 days.

After 5 days of storage, Test platelets will be aseptically removed from each subject's INTERCEPT platelet storage container and prepared for radiolabeling using Variant 1 BEST version 4.2.1 method (**Appendix B**). The method for collection and radiolabeling of the fresh Control platelets in Variant 1 of BEST 4.2.1 contains the same steps as published in the BEST 4.2.1 method (BEST).

The recovery and survival for Test platelets will be compared against the fresh platelet Control. Recovery and survival of INTERCEPT platelets will be assessed after 5 days of storage for 24 evaluable subjects (target 12 subjects, with a minimum of 8, at each site).

A minimum of 24 subjects with evaluable (Test and Control) recovery and survival data may be obtained. Randomized subjects who do not have both paired (Test and Control) recovery and paired survival data will be replaced until the enrollment goal is met.

On Day 5 of storage for the Test component, healthy subjects will return to the site, and 43 (±2) mL of WB will be drawn into a syringe containing 9 mL of Anticoagulant Citrate Dextrose Solution, Formula A (ACD-A). The sample will be used to prepare fresh Control platelets from platelet rich plasma following the Variant 1 of BEST methodology (**Appendix B**). In addition, prior to infusion a blood sample in EDTA will be drawn for use in determination of radiolabel elution.

Test and Control will be randomly radiolabeled with either ^{51}Cr as sodium chromate ($Na_2{}^{51}CrO_4$) or ^{111}In as indium oxine, depending upon randomization assignment (see **Section 5.2**). Subjects will be randomized with equal probability to the radiolabeling sequences ($^{111}In/^{51}Cr$ vs. $^{51}Cr/^{111}In$) for Test/Control. After radiolabeling, the autologous Control and Test platelet samples will be simultaneously infused into the subject.

Blood samples will be drawn immediately before infusion and for radioactivity measurements at 2 hours ± 15 min post-infusion (Day 0 post-infusion), and 6 more samples will be drawn at 1 (within ±4 hours from the time of radiolabeled platelet infusion), 2, 3, 5±1, 7/8, and 11±1 days post-infusion (DPI).

Subjects will be monitored for safety [adverse events (AE)] from the start of the apheresis procedure until 24 hours after the last DPI blood sample is drawn.

The duration of the study for each qualified subject is approximately 31 to 33 days.

5.2 Randomization and Blinding

Cerus will generate the randomization scheme to be executed by the study sites. Subjects will be randomized across radiolabeling sequences ($^{111}In/^{51}Cr$ or $^{51}Cr/^{111}In$ for Test/Control). In addition, an equal number of subjects will be targeted at each study site (12 subjects with a minimum of 8 at a single site). Subjects will receive both Test and Control platelets at the same time. Randomized subjects who do not have both paired (Test and Control) recovery and paired survival data will be replaced until the enrollment goal is met.

In this open-label study, the sites and subjects will not be blinded to the randomization assignment.

5.3 Treatment and Dose

5.3.1 Administration of Study Product

The study platelets (approximately 5 to 30 mL of washed radiolabeled fresh [Control] and 5-day stored autologous radiolabeled platelets [Test]) are administered by intravenous infusion in a peripheral vein.

5.3.2 Preparation of the Test Platelet Component

5.3.2.1 Apheresis Platelet Collection

Study subjects who qualify to participate in the study will provide one apheresis platelet collection as specified in **Section 5.3.6.2**. All study apheresis donations will be collected in 100% plasma on the Trima Accel® automated blood collection system according to blood center SOPs and the manufacturer's instructions, with leukocyte reduction by centrifugation as part of the collection process and with anticoagulant ACD, using FDA approved collection sets for used on the Tima device; the collection set product codes will be reported.

A small donor blood sample will be acquired to obtain the subject's platelet count. The subject's platelet count at screening and/or on the day of apheresis, and body mass prior to the donation will determine if a single or double-dose collection will be performed.

After collection, the platelet components will be handled following the manufacturer's instructions which includes static rest followed by continuous agitation at 20°C to 24°C. Platelets must have an RBC content $< 4 \times 10^6$/mL and be free of visible aggregates prior to INTERCEPT treatment. Platelet components with an RBC content $\geq 4 \times 10^6$/mL will be excluded from the study.

At several different steps in the preparation of the Test component, the component volume will be measured by weight. The tare weight for each of the different platelet containers (empty dry container with standardized tube length) will be used to determine the net product weight. Tare weights will be reported.

Platelet volume (mL) will be determined using the following equation:

- [Gross weight (g) of the component – tare weight of the container (g)] ÷ specific gravity of platelets suspended in 100% plasma]
- The specific gravity of platelets suspended in plasma used for calculations for this protocol will be: 1.03 g/mL

5.3.2.2 Preparation of Test Platelets

Platelet input components will be sampled and evaluated for parameters described in Table 7.

Treatment with INTERCEPT including illumination (Figure 1) will begin within 24 hours of collection. The processing steps described in the instruction for use (SPC 00770-AW) will be followed, unless superseded by instructions presented in this protocol (i.e., sampling of components and extended storage durations).

Each Test input component will contain 4.0 to 5.2×10^{11} platelets in 300 to 390 mL in 100% plasma after input sample collection. If the input platelet component volume is greater than 390 mL, the volume will be adjusted to 300 to 390 mL by sterile connection to an empty container and removing the excess volume, which will be discarded.

Each Test input component will be connected to the tubing of the INT22 LV Processing Set using a sterile connection device (SCD).

Amotosalen will be added to the component by passing the entire contents of the Test input component through the amotosalen container and into the illumination container. After the component is mixed and air has been returned to the input container, the tubing connecting the amotosalen container to the illumination container will be sealed and the amotosalen container and the original platelet container will be detached.

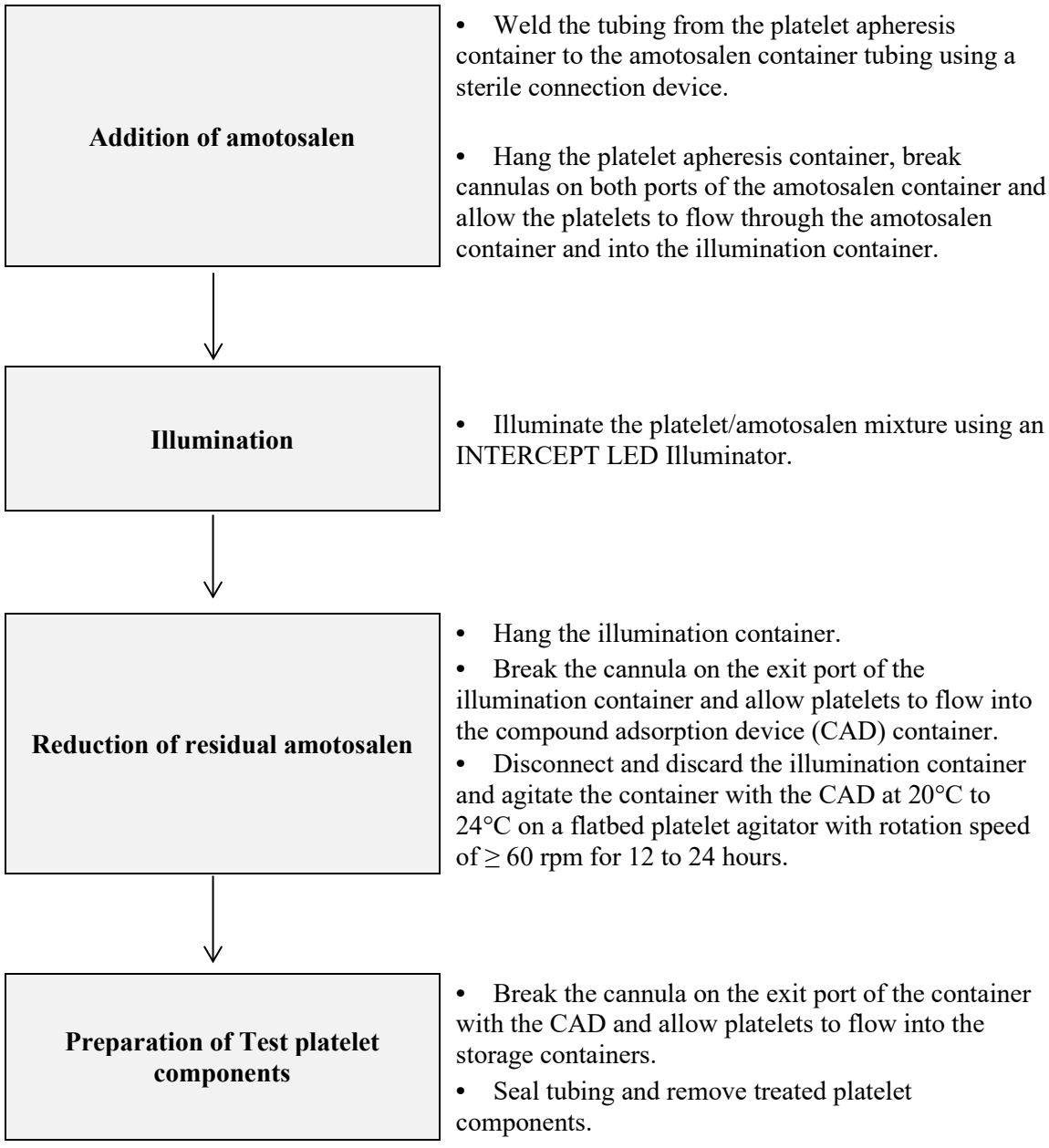
In-process test platelet components will then be illuminated with a target dose of 3.3 J/cm^2 UVA treatment on the INTERCEPT LED Illuminator (INT200).

Following illumination, in-process test platelets will be transferred to the container with the CAD, the illumination container will be detached, and the platelets will be placed in an incubator at 20°C to 24°C on a flatbed platelet agitator with rotation speed of ≥ 60 rpm. The time the platelets are transferred to the container with the CAD and the time the CAD is placed into agitated storage will be recorded.

The duration of agitated storage with the CAD will be 12 to 24 hours. The time when the container with the CAD is removed from agitated storage will be recorded. The container with the CAD will be hung and platelets will be transferred by gravity into the storage containers and any air returned to the empty container with the CAD. The INTERCEPT storage containers and connected tubing will be weighed; the net weight will be used to determine the total post INTERCEPT volume. The empty container with the CAD will be detached and discarded using a heat seal technique to preserve sterility. The individual INTERCEPT storage container will be weighed for calculation of post-treatment volume and the post INTERCEPT sample will be removed as described in **Section 5.3.2.1** for assessment of the indices described in Table 7.

All INTERCEPT platelets (Test) will be stored under standard conditions of continuous agitation using platelet agitator and temperature control (20°C to 24°C) until the time of radiolabeling on Day 5 post-collection.

Figure 2 Flow Diagram of the INTERCEPT Process for Platelets



5.3.2.3 Labeling

5.3.2.3.1 *Test Platelet Components*

During apheresis collection, processing, and Test platelet storage and infusion of autologous platelets, the platelet components or samples, and the whole blood sample will be properly labeled to definitively identify the subject and ensure correct infusion of autologous platelets. Minimal information to be provided on the label will include:

- IDE Number (BB-IDE 6200)
- Protocol number (CLI 00183)
- Site/Subject ID
- Date of collection
- End of Storage Date (based on 5-day storage duration)

All study platelet components will contain the label:

Caution: Investigational Product - Limited by United States Law to Investigational Use

5.3.2.3.2 *Test Platelet and Whole Blood Samples*

The minimal information to be provided on the label of study samples will include:

- Protocol Number (CLI 00183)
- Site/Subject ID
- Treatment group (Test or Control), if applicable
- For test platelet sample, timepoint and date of sample collection (Input, Post-INTERCEPT, Day 5)
- For whole blood samples, date of sample collection (Day 5 or DPI)

5.3.2.4 Radiolabeling of Test Platelets

On the day of scheduled radiolabeling and infusion (Day 5 post-collection), samples will be aseptically removed from the component for radiolabeling and *in vitro* platelet function testing. The Test platelets are prepared for radiolabeling using the Variant 1 method (**Appendix B**).

5.3.2.5 Preparation of Control Platelets – Fresh Platelets

On the scheduled day of radiolabeling/infusion approximately 43 (±2) mL of whole blood is collected from the subject in a syringe containing 9 mL ACD-A to prepare the Control platelets (fresh platelets). The fresh platelets are separated from the WB by centrifugation to prepare platelet rich plasma (PRP). The Control platelets will be labeled with the alternate radionuclide to the one used for the paired Test platelets. The Control platelets are radiolabeled according to the Variant 1 of BEST version 4.2.1 method (**Appendix B**).

5.3.2.6 Study Platelet Infusion

The study platelets (approximately 5 to 30 mL of washed radiolabeled fresh [Control] and 5-day stored, washed, radiolabeled autologous platelets [Test]) are administered by intravenous infusion in a peripheral vein.

5.3.3 Duration of Subject Participation

Within 15 days prior to participating, subjects will be screened for eligibility. For each qualified subject, study participation will include 15 to 17 days following the apheresis platelet collection, depending on the day of the last blood sample for radioactivity. The total duration of subject participation will be approximately 31 to 33 days.

The study schematic design is displayed in Table 3.

Table 3 Study Schema

Screening	Apheresis Collection Trima collector (Day of collection)	INTERCEPT Treatment (Day post collection)	Sampling, Radio-labeling, Infusion, Subject Blood Sampling (Day post collection)	Subject Blood Sampling (Day Post Infusion)					
Day -15 to 0	Day 0	Day 0/1	Day 5 / DPI 0 [a]	DPI 1	DPI 2	DPI 3	DPI 5±1	DPI 7/8	DPI 11±1

[a] Samples will be acquired pre infusion and approximately 2-hours post infusion on DPI 0.

5.3.4 Schedule of Assessments and Procedures

The study procedures and assessments are displayed in Table 4. The following assessments should be completed at the designated time(s).

Table 4 Study Assessments

SCREENING AND ENROLLMENT	
Day -15 to 0 **Subject Screening**	• Sign informed consent form • Medical history and demographics • Blood donor physical exam (including height, weight, and vital signs: temperature, sitting blood pressure and heart rate) • Concomitant medications (including drugs of abuse) and nicotine and cannabis inquiry • Urine drug screening test • Blood sample for hematology and chemistry panels (see list of specific parameters in Table 6) • Blood donor screening panel (including ABO, Rh type, and routine infectious disease testing) • Urine or blood pregnancy test (for females of child-bearing potential) • Review of inclusion/exclusion criteria
TEST PLATELET APHERESIS COLLECTION AND STORAGE	
Day 0	**Subject Procedures Prior to Apheresis Collection** • Vital signs • Blood sample(s) for donor screening panel (including subject platelet count) • Concomitant medications and nicotine and cannabis review • Update baseline medical history • Confirm subject meets eligibility criteria **Apheresis collection** • Autologous single or double apheresis platelet collection and concurrent plasma using the Trima cell separator collected and suspended in 100% plasma • Collect AEs
Day 0-1	**Platelet Component Procedures - Prior to INTERCEPT Treatment** • Visual inspection to confirm absence of platelet aggregates • Confirm RBC content < 4×10^6 RBCs • Component weight pre- and post-sampling (for volume calculation) • Obtain sample for *in vitro* assessments (Table 7) **Platelet Component - Initiate INTERCEPT Treatment**

Day 1-2	**Platelet Component Procedures - Post-INTERCEPT Treatment** • Component weight • Component platelet count • Obtain sample for *in vitro* assessments (Table 7) • Randomize subject to Test/Control radiolabeling sequence assignment
BACTERIAL TESTING	
Day 2, 3, or 4	A sample for bacterial screening is withdrawn 1 to 3 days before the scheduled radiolabeling/infusion, although bacterial testing is not routinely required for INTERCEPT PCs stored for 5 days (see Table 7).
RADIOLABELING AND INFUSION OF STUDY PLATELET ALIQUOTS	
Day 5	**Subject Procedures on the Day of Infusion (Prior to Infusion)** • Vital Signs • Urine or blood pregnancy test for females of childbearing potential • Blood sample for hematology and chemistry panels (see list of specific parameters in Table 6) • Collect blood sample for preparation of fresh platelets (Control) • Collect blood sample in EDTA for determination of radiolabel elution (or can occur prior to study platelet infusion) • Concomitant medications, nicotine, and cannabis review • Collect AEs **Test Platelet Component Procedure Prior to Radiolabeling** • Obtain aliquot for radiolabeling • Platelet component must pass all release criteria (Table 5) to be infused **Test Platelet Component - *In Vitro* Platelet Assessments** • Obtain sample for *in vitro* assessments (Table 7) **Radiolabeling of Test Platelet Aliquots** • Prepare sample for radiolabeling following Variant 1 **Radiolabeling Control Platelet Aliquots** • Prepare fresh platelet sample (Control) • Radiolabel Control platelets using Variant 1 of BEST 4.2.1 method and labeling assignments based on randomization **Radioactive Standard** • Reserve samples of radiolabeled combined injectate before infusion to be used as a radioactive standard (pre-infusion) **Subject Procedures - Infusion of Study Platelets** • Infuse radiolabeled platelet aliquot from both the autologous apheresis collection treated with INTERCEPT Blood System for Platelets with LED Illuminator and stored 5 days (Test) and autologous fresh whole blood derived platelets (Control) • Pre-infusion and at 2 hours ± 15 min post-infusion: o Vital Signs o Blood sample to measure radioactivity o Concomitant medications and nicotine and cannabis review

	○ Collect AEs ○ Collect blood sample in EDTA for determination of radiolabel elution (if has not already been collected) • Discharge
DPI 1 (within ±4h of the infusion), 2, 3, 5±1, and 7/8	**Subject Procedures – Post-Infusion** • Blood sample to measure radioactivity to calculate recovery and survival • Vital Signs • Concomitant medications and nicotine and cannabis review • Collect AEs
DPI 11±1	**Subject Procedures – Post Infusion Final Visit** • Vital signs: temperature, sitting blood pressure, and heart rate • Blood sample to measure radioactivity to calculate recovery and survival • Blood sample for hematology and chemistry panels (see list of specific parameters in Table 6) • Urine or blood pregnancy test (for females of childbearing potential) • Concomitant medications and nicotine and cannabis review • Collect AEs

5.3.5 Subject Screening

5.3.5.1 Prior to apheresis (Day -15 to 0)

Prior to participating in the study, healthy volunteer subjects will be screened for eligibility. Subject screening procedures will be performed within 15 days preceding the apheresis collection. Screening procedures will include the following:

- Obtain signed informed consent
- Obtain demographics and relevant, self-reported medical history
- Blood donor physical exam (including height, weight, and vital signs: temperature, sitting blood pressure, and heart rate)
- Concomitant medications (including drugs of abuse), and nicotine and cannabis inquiry within the past 28 days
- Urine drug screening test
- Blood sample (~13 mL) collection for laboratory tests: hematology and chemistry panels (see list of specific parameters in Table 6)

- Blood sample (~24 mL) collection for donor screening panel (including ABO, Rh type, and routine infectious disease testing)
- Urine or blood pregnancy test (for females of child-bearing potential)
- Review of inclusion/exclusion criteria

Subjects who meet all inclusion and none of the exclusion criteria will visit the site as specified in the Schedule of Assessments (Table 4) and Study Schema (Table 3).

5.3.6 Test Platelet Apheresis Collection and Storage

5.3.6.1 Day 0: Prior to Apheresis Collection

Prior to apheresis collection, the subject vital signs will be measured and recorded. A blood sample(s) will be taken for donor screening panel (including subject platelet count). Subjects will be asked about concomitant medications, nicotine and cannabis use, and updates to baseline medical history. Subject eligibility will be confirmed.

5.3.6.2 Day 0: Apheresis Collection

Study subjects who qualify to participate will provide one single or double apheresis platelet collections as described in **Section 5.3.2.1**.

Subjects will be asked about AEs (serious and non-serious) that may have taken place during or following the collection, and these will be recorded.

5.3.6.3 Day 0-1: Post Apheresis Collection Input Sample

A sample (approximately 10 to 20 mL) will be aseptically removed from the component for *in vitro* platelet assessments (Table 7), and INTERCEPT treatment qualification.

5.3.6.4 Day 0-1: Platelet Pathogen Reduction with the INTERCEPT Blood System for Platelets with LED Illuminator

INTERCEPT treatment (Figure 1) will begin within 24 hours of collection (Day 0) following the steps outlined in **Section 5.3.2.2**. The INTERCEPT platelets will be weighed for calculation of post-treatment volume.

Following the INTERCEPT treatment process, the INTERCEPT storage container will be weighed for calculation of post-treatment volume. A post-INTERCEPT sample will be removed using the integrated sample bulb (approximately 10 to 20 mL from the component for *in vitro* platelet assessments (Table 7)). Following INTERCEPT treatment, the radiolabeling sequence for Test and Control platelets will be randomized per the randomization scheme.

5.3.7 Bacterial Testing

A sample (approximately 8 to 20 mL) of the Test platelets will be withdrawn 1, 2 or 3 days prior to the end of storage for bacterial contamination screening. Testing for bacterial contamination will utilize an approved system for aerobic and/or anaerobic culture of potential viable bacteria based on site procedures. Bacterial detection will be used in this study because the study subjects are healthy who will derive no benefit from platelet reinfusion. Bacterial detection testing will provide an extra measure of safety for these healthy subjects. In addition, if any subject experiences a post transfusion febrile reaction, bacterial detection samples will be useful to evaluate the cause of the febrile reaction.

5.3.8 Platelet Storage: Days 1 to 5

All INTERCEPT platelets will be stored under standard conditions of continuous agitation with approved devices and temperature control (20°C to 24°C) until the end of Day 5 post-collection.

5.3.9 Study Platelet Radiolabeling and Infusion, Day 5 (0 DPI)

5.3.9.1 Subject Procedures Prior to Infusion

On the scheduled infusion day (DPI 0 or Day 5 of component storage), subjects will return to the site. Subject vital signs will be measured, urine, or blood pregnancy test will be performed (if applicable subject is female of childbearing potential), and a blood sample (approximately 13 mL) for hematology and chemistry panels (see list of specific parameters in Table 6) will be taken. Subjects will be asked about concomitant medications, nicotine and cannabis use, and AEs (serious or non-serious) that may have taken place.

5.3.9.2 Collection of Whole Blood

5.3.9.2.1 *Fresh Platelets (Control)*

On the day of scheduled infusion fresh platelets will be prepared from the 43 (±2) mL sample according to the following procedure: collect 43 (±2) mL of WB into a syringe containing 9 mL of ACD-A and transfer into 50 mL conical screw-top tubes and rest for 30 minutes (alternatively, 43 mL of whole blood can be collected with 7 mL of ACD-A followed by a 1 to 2 hour holding period). The tubes will be centrifuged at 200 ×g for 15 minutes to prepare platelet-rich plasma (PRP), and the PRP from each tube will be transferred to another conical tube. ACD-A equal to 15% of the PRP volume will be added.

5.3.9.2.2 *Collection of Whole Blood in EDTA*

In addition, approximately 10 mL of the subject's blood will be collected in an EDTA tube to determine the radiolabel elution for each isotope using the Variant 1 of BEST 4.2.1 method (**Appendix B**).

5.3.9.3 Release Criteria of Test platelets

At the end of storage (Day 5), prior to radiolabeling, Test platelet sample must fulfill the release criteria, as detailed in Table 5. Test components that do not fulfill the release criteria will not be transfused and hence will not be included in the primary efficacy analysis for the platelet recovery and survival. To confirm viability of the platelets prior to infusion, a sample (approximately 1 mL) from the Test platelets will be taken to confirm pH is ≥6.2. This sample can be obtained at the same time as the stored platelets are sampled for radiolabeling.

Table 5 Release Criteria on Day of Radiolabeling/Infusion

Attribute	Method	Specification
Description	Visual Inspection	Semi-opaque liquid in plastic container
Labeling	Visual Inspection	Affixed and completed
Product integrity	Visual Inspection	No evidence of clots, fibrin strands, aggregates, or compromised container integrity
Bacterial contamination	BacT/ALERT	Negative to infusion date
pH	pH analyzer (manual or blood gas analyzer)	≥ 6.2
Records review	Data capture sheets	Complete and reviewed
Chromium (for reagent grade only)	Endotoxin Assay	Negative result prior to infusion [a]

[a.] Per study site SOPs, a negative result is endotoxin < 0.125 EU/mL.

5.3.9.4 Preparation of Test Platelets for radiolabeling– Test Platelets Stored for 5 Days

On the day of scheduled radiolabeling and infusion (DPI 0 or Day 5 of component storage), 10 to 30 mL of the platelet unit will be aseptically removed from the Test storage container for radiolabeling. The sample for radiolabeling will be removed before or at the same time the unit is sampled for *in vitro* platelet function testing. The final storage containers will be weighed to determine platelet component volume after sampling.

The Test platelets are prepared for radiolabeling using the Variant 1 method (**Appendix B**).

5.3.9.5 Test and Control Platelet Radiolabeling

Previously stored Test platelets and fresh PRP Control platelets will be radiolabeled according to randomization assignment for that subject with either ^{51}Cr as sodium chromate ($Na_2{}^{51}CrO_4$), or ^{111}In as indium oxine, following the labeling and washing procedures outlined the radiolabeling method in **Appendix B**. The isotope labels will be determined by a random number table such that equal numbers of fresh platelets (Control) and stored Test platelets will be labeled with each isotope.

For both Control and Test platelets, after resuspension in a small volume of ACD-A/saline, either 50–100 µCi ^{51}Cr or 50–60 µCi of ^{111}In is added to the platelets and incubated for 20+2 minutes at room temperature to achieve radioactive labeling. The platelets are washed to remove excess radioactivity and then resuspended in autologous platelet-poor plasma. If reagent grade ^{51}Cr is used for radiolabeling, the ^{51}Cr will be sterile filtered and have a negative endotoxin result prior to infusion.

5.3.9.5.1 *Radiolabeling Efficiency*

Efficiency of labeling will be measured for each radioisotope using the Variant 1 of BEST methodology as described in **Appendix B**.

5.3.9.5.2 *Elution of Radioactivity from Labeled Platelets*

Radiolabel spontaneously dissociates (elution) from platelets both *in vitro* and *in vivo* (Holme, et al. 1993, Snyder, et al. 2004). To correct for elution before calculation of recovery and survival, the mean elution of radioisotope into the plasma after radiolabeling will be measured *in vitro* and modeled using an assay to estimate *in vivo* elution using the BEST elution assessment method (as described in the Variant 1 of BEST 4.2.1 method in **Appendix B**).

5.3.9.6 Stored Test Platelet *In Vitro* Function Testing

An aliquot (10 to 20 mL) of the stored Test platelets will be aseptically removed from the test platelet container on the scheduled day of infusion; *in vitro* platelet function assessments will be performed (Table 7). The pH of the stored Test platelet will be taken prior to or at the same time as the sample for radiolabeling (**Section 5.3.9.3**).

The sample for radiolabeling will be removed prior to or at the same time the unit is sampled for *in vitro* platelet function testing.

5.3.9.7 Retention of Test Platelet Component

Once all end of storage sampling is complete, the Test components will be placed in refrigerated storage and reserved for future bacterial culture in the event of a febrile transfusion reaction indicative of sepsis (temperature elevation > 2°C from pre-infusion or greater than 1°C with rigors). After three days post-infusion, the Test components will be discarded following institutional practices.

5.3.9.8 Infusion of Test and Control Aliquots and Post-Infusion Subject Procedures

After radiolabeling, and a negative pregnancy test (if subject is female of childbearing potential), the autologous fresh PRP Control platelets and stored radiolabeled INTERCEPT platelets (Test) will be combined and infused into the subject (approximately 5 to 30 mL). Subject vital signs will be measured prior to infusion and a sample will be obtained from the combined radiolabeled fresh and stored platelets before infusion and used as a radioactive standard. If not already collected, a sample for elution (approximately 10 mL) may also be collected before infusion.

Blood samples (approximately 10 mL) will be drawn from the subject pre-infusion and for radioactivity measurements at 2 hours ±15 min from the end of infusion. For details on radioactivity measurements, see **Section 8.2.1**.

Record the subject vital signs, concomitant medications, and AEs (non-serious and serious), and when completed, the subject will be discharged.

5.3.10 Subject Procedures: DPI 1 to 7/8

On days corresponding to 1 (±4h from the time of infusion), 2, 3, 5±1, and 7/8 DPI, subjects will return to the research unit. Blood samples (~10 mL) will be drawn to measure radioactivity. The exact times of sampling will be recorded.

At each blood sampling time, subject vital signs, concomitant medications, nicotine and cannabis use, and AEs (non-serious and serious) will be recorded, and when completed, the subject will be discharged.

5.3.11 Subject Procedures – End of Study

On the day corresponding to 11±1 days post-infusion, subjects will return to the research unit. Blood samples (approximately 23 mL) to measure radioactivity will be drawn as well as for hematology and chemistry panels (see list of specific parameters in Table 6). The exact time of sampling will be recorded. Subject vital signs will be recorded, and subjects will be questioned about concomitant medications, and nicotine and cannabis use and AEs. A pregnancy test will

be done for females of childbearing potential. Self-reported AEs occurring within 24 hours after the last blood draw will be recorded.

5.3.12 Study Procedure Definitions

The following list is a description of the procedures to be completed during the study:

5.3.12.1 Informed Consent

An institutional review board (IRB) approved informed consent must be signed and dated by each study subject prior to any study procedures being performed, and a copy must be given to the subject.

5.3.12.2 Inclusion and Exclusion Criteria Assessment

All subjects must qualify based on the inclusion and exclusion criteria specified in **Section 6.1** and **Section 6.2**, respectively.

5.3.12.3 Medical History, Demographics, and Prior Medications

A complete medical history will be obtained including a review of all major organ systems. The medical history, demographics, concomitant medications (including drugs of abuse), and nicotine and cannabis inquiry within the past 28 days will be taken at screening. Demographics will include sex, date of birth and age at time of screening, race, and ethnicity. Concomitant medications will include all medications taken throughout the study (from the time of signing the informed consent to completion of the study). In addition, a brief medical history, and a review of concomitant medications including drugs of abuse, and nicotine and cannabis use will be performed on Day 0.

5.3.12.4 Blood Donor Physical Examination

A blood donor physical examination will be performed at screening, where applicable, or upon early withdrawal if the subject was infused. The examination will include height (screening only), weight (screening only), and vital signs: temperature, sitting blood pressure and heart rate. Personnel performing the blood donor physical exams must be qualified by training.

5.3.12.5 Vital signs

Vital signs will be obtained at screening, on Day 0 (prior to the apheresis collection), on 0 DPI, the day of scheduled infusion (two times pre-infusion and at 2 hours ±15 min post-infusion), and on all follow up days, or upon early withdrawal if subject was infused. Study vital signs will include temperature, sitting blood pressure (to be taken after 5 minutes of sitting), and heart rate.

5.3.12.6 Weight, Height, and Body Mass Index

Subjects will be weighed in street clothes after removing shoes and coat or jacket. Subject height will be measured without shoes. Weight will be measured at screening. Height will be measured at screening only. Subjects body mass index (BMI) at screening will be calculated using the following formula: BMI (kg/m^2) = weight (kg)/height (m^2). Blood volume will be calculated using the BEST method (as described in Variant 1 of BEST 4.2.1 method in **Appendix B**).

5.3.12.7 Blood Sampling

Subject blood samples will be collected at each time indicated in the Schedule of Assessments (Table 4). The date and time of sample collection, as well as the date and time of dose administration, will be recorded. Approximately 24 mL will be collected for blood donor screening and infectious disease screening. Approximately 13 mL will be collected at each of the protocol-specified time points for hematology and/or chemistry panels. Approximately 10 mL will be collected for the radioactivity measurements at each of the protocol-required time points. In addition, blood samples will be collected to prepare Control platelets (approximately 43 mL) and the radiolabeling elution assessments (up to 10 mL). A total of approximately 200 mL of subject blood will be obtained during study for screening, hematology, chemistry, and radioactivity measurements. Additional blood sampling may be required per site procedures.

5.3.12.8 Laboratory Tests

The local laboratory will perform the subject hematology and chemistry panel tests specified in Table 6.

Table 6 Subject Clinical Laboratory Tests

Hematology	Serum Chemistry
Hematocrit Hemoglobin Red Blood Cell Count Platelet Count White Blood Cell Count (*with Differential*)	Blood Urea Nitrogen Calcium Carbon Dioxide Chloride Creatinine Glucose Potassium Sodium

Samples (approximately 13 mL) for clinical laboratory tests will be taken at screening, at the end of the storage/day of scheduled infusion, and at the last scheduled blood draw (11±1 DPI), or upon early withdrawal if the subject was infused.

All samples will be collected in accordance with acceptable laboratory procedures and processed according to the local laboratory standard procedures. Copies of the laboratory certificates and procedure, or reference to the procedure, will be filed in the study records. Laboratory values that are out of range will be identified and may be repeated at the Investigator's discretion after notification of the Sponsor. Out-of-range laboratory values that are determined by the Investigator to be clinically significant must be recorded on the Medical History or AE electronic case report form (eCRF). The Investigator may refer the subject for medical treatment, as appropriate, if this occurs.

5.3.12.9 Radioactivity Measurements

See **Section 8.2.1**.

5.3.12.10 *In Vitro* Platelet Function Testing

See **Section 8.2.2**, Table 7 for a list of *in vitro* platelet function assays to be performed for the evaluation of INTERCEPT PCs.

5.3.12.11 Pregnancy Test

A urine or blood pregnancy test will be performed on all female subjects of childbearing potential at screening, prior to infusion, and on the last day of the study, or upon early withdrawal if the subject was infused.

5.3.12.12 Drug Screening Test

A urine drug screening test will be performed on all subjects during screening.

5.4 Accountability of Study Platelet Products

The study platelet products will be produced by each participating blood center according to instructions for use or as specified by the protocol. Accountability of the apheresis collection products, and the whole blood collection products, will be maintained by the Investigator or designee.

5.4.1 Handling and Disposal

The study platelet product produced at the site will be handled and disposed of according to the site’s SOP.

6.0 SELECTION AND WITHDRAWAL OF SUBJECTS

The study population consists of healthy volunteer subjects who meet the FDA, AABB and institutional eligibility criteria for allogeneic and plateletpheresis donor qualifications and the study inclusion/exclusion criteria detailed below. Subjects who do not have both paired (Test and Control) recovery and paired survival data may be replaced, and a sufficient number of subjects will be enrolled to provide a minimum of 24 subjects with evaluable *in vivo* recovery and survival data.

6.1 Subject Inclusion Criteria

- Age ≥ 18 years
- Normal health status (as determined by the Investigator review of medical history and blood donor physical exam)
- Complete blood count (CBC) and serum chemistry values within normal limits or outside of normal reference range but determined by the Investigator, and consultation with the Sponsor, to be not clinically significant.
- Meet FDA, AABB or institutional guidelines for allogeneic and plateletpheresis donor qualifications with the following exceptions:
    - Deferrals due to travel, tattoos/piercings, male to male sexual contact as institutional policies allow
- All routine infectious disease testing must be negative or non-reactive (during screening)
- Subjects of childbearing potential must agree to use a medically acceptable (as per the Investigator) method of contraception throughout the study
- Signed and dated informed consent form

6.2 Subject Exclusion Criteria

- Clinically significant acute or chronic disease (as determined by the Investigator)
- Treatment with aspirin or aspirin-containing medications within 7 days of apheresis or treatment with non-steroidal anti-inflammatory drugs (NSAID), anti-platelet agents or other drugs affecting platelet viability within 3 days of apheresis (e.g., ibuprofen or other NSAIDs)
- Subject received platelet inhibitors within 14 days of donation (e.g., clopidogrel, ticlopidine, amphetamines (e.g., Adderall, Dexedrine))
- Immunosuppressive therapy (e.g., oral or IV prednisone) within the past 28 days

- Treatment with any medication known to affect platelet viability
- Pregnant or nursing females
- Received an investigational drug within the past 28 days or current participation in another clinical interventional study
- Non study blood component donation throughout the study
- Subjects with positive cocaine and/or amphetamine result from urine drug screen.
- Splenectomized subjects
- History of known hypersensitivity to ^{51}Chromium or ^{111}Indium

6.3 Subject Withdrawal Criteria

Subjects may withdraw from the study at any time. The Investigator may remove a subject for reasons of safety or non-compliance.

The Investigator must determine the primary reason for withdrawal and record it on the eCRF. The Investigator or the Medical Monitor may exercise his/her medical judgment to terminate a subject's participation in the study due to clinically significant changes in any clinical or laboratory parameters. The study sponsor also reserves the right to terminate the study at any time. All data normally collected at completion of the study must be collected either at the time of the subject's withdrawal, or before or during the scheduled study close-out.

The primary reason for withdrawal should be noted in the eCRF using the following categories:

- Adverse Event: The subject has experienced an AE that, in the opinion of the subject or Investigator, leads to an early termination (withdrawal). The Investigator must complete an AE eCRF for each AE. If a subject is withdrawn from the study due to an AE, the Investigator is required to follow the subject until the event is resolved or when it becomes stable.
- Significant Protocol Deviation.
- Screen Failure
- Withdrawal of Consent: The subject (or other responsible individual) wishes to withdraw from the study for non-medical reasons, as determined by the Investigator.
- Death: Provide the underlying cause that led to the death as an AE and complete the AE eCRF

- Investigator Discretion: In the Investigator's judgment, it is in the subject's best interest to be withdrawn from the study.
- Study Termination: The Sponsor, IRB, or FDA terminates the study.
- Lost to Follow-Up
- Other

6.4 Stopping Rules

There are no pre-specified stopping rules for this study. The Sponsor may stop the study for any reason.

7.0 TREATMENT OF SUBJECTS

7.1 Treatments

Each subject will provide one apheresis platelet component and receive one infusion of autologous radiolabeled fresh Control platelets combined with Test platelets stored for 5 days (approximately 5 to 30 mL). Platelets are administered by intravenous infusion in a peripheral vein.

Procedures will be as follows: On Day 0 each healthy volunteer subject provides a single- or double-dose apheresis platelet collection.

On Day 5 (0 DPI), subjects will return to the site, and 43 (±2) mL of blood will be drawn into a syringe containing 9 mL of ACD-A, USP (2.13% free citrate ion) (alternatively, 43 mL of WB can be collected with 7 mL of ACD-A followed by a 1 to 2 hour holding period) and held for 30 minutes. Fresh platelets will be prepared from this sample for radiolabeling according to the Variant 1 of BEST 4.2.1 method (**Appendix B**). One aliquot (10 to 30 mL) of the stored autologous Test platelets will be aseptically removed from each subject's platelet storage container and prepared for radiolabeling according to the Variant 1 method (**Appendix B**). Previously stored (Test) and fresh platelets (Control) will be radiolabeled with either ^{51}Cr as sodium chromate ($Na_2{}^{51}CrO_4$), or ^{111}In as indium oxine, depending upon randomization order, following the labeling, and washing of the Variant 1 method. The radioisotope labels will be determined by a randomization scheme such that equal numbers of fresh platelets and stored INTERCEPT platelets will be labeled with each isotope. After radiolabeling, the autologous fresh and stored platelets will be simultaneously infused into the subject (approximately 5 to 30 mL). The pregnancy test for females of childbearing potential is required to be negative before infusion.

Subject blood samples for radioactivity measurements will be drawn as follows: pre-infusion, and 2 hours ±15 min post-infusion, and on 1, 2, 3, 5±1, 7/8, and 11±1 DPI (see schedule in **Table 4** and **Section 5.3.12.7**).

7.1.1 Subject Confinement and Restrictions

Subjects will visit the study site on Day 0 to have single or double apheresis platelets collected. On day of scheduled infusion, subjects will return to the study site, and blood samples will be drawn to prepare a fresh platelet sample (Control) and to assess for radiolabel elution. Fresh platelets and stored INTERCEPT platelets will be radiolabeled and infused to the subjects (autologous infusion). Subjects will have to stay in or return to the study site or facility where blood is being drawn until the 2 hours ±15 min post-infusion blood sample is drawn. Thereafter, subjects will visit the study site on 1 (within ±4 hours of the initial infusion time), 2, 3, 5 ±1, 7/8, and 11 ±1 DPI.

Subjects will be instructed to abstain from treatment with aspirin or aspirin-containing medications within 7 days of apheresis or treatment with non-steroidal anti-inflammatory drugs (NSAID), anti-platelet agents, or other drugs affecting platelet viability within 3 days of apheresis (e.g., ibuprofen or other NSAIDs).

Subjects will be asked to not engage in any strenuous activity while confined to the research unit and will follow the rules governing their activities, as set forth by the study site.

7.2 Concomitant and Excluded Therapy

Concomitant medications (including drugs of abuse) within the past 28 days will be recorded during study participation. Subjects taking medications that preclude allogeneic and plateletpheresis donor qualifications per AABB reference standards and subjects with positive amphetamine and/or cocaine test results are excluded from participating in the study.

Treatment with aspirin or aspirin-containing medications within 7 days of apheresis or treatment with non-steroidal anti-inflammatory drugs (NSAID), anti-platelet agents, or other drugs affecting platelet viability within 3 days of apheresis (e.g., ibuprofen or other NSAIDs) will preclude apheresis.

7.3 Subject Compliance

Healthy subjects who understand the study commitments and sign the informed consent will be enrolled in the study.

Treatment compliance will be tracked by the Investigator or designee, recorded on the eCRF, and monitored by the Sponsor.

8.0 ASSESSMENT OF EFFICACY

8.1 Efficacy Parameters

8.1.1 Primary Efficacy Endpoints

- Post-infusion recovery of Test platelets at Day 5
- Post-infusion survival of Test platelets at Day 5

Post-infusion platelet recovery and survival will be estimated by using radiolabeled platelets with a computerized multiple hit gamma function analysis with a curve fitting algorithm. Post-infusion blood samples will be corrected for plasma associated radioisotope and the spontaneous *in vitro* and *vivo* dissociation (elution) of radiolabels using the method described in the BEST method (as described in Variant 1 of BEST 4.2.1 method in **Appendix B**).

Results will be compared as described in the statistical plan (**Section 10.0**).

8.1.2 Secondary Efficacy Endpoints

- Product parameters at end of INTERCEPT treatment:
  - Platelet dose ($\geq 3.0 \times 10^{11}$ platelets)
  - Platelet yield retention ($\geq 80\%$)
- Test Product Parameters at (Day 5)
  - $pH_{22°C}$ (≥ 6.2)

8.1.3 Additional Endpoints

Additional analyses will include the *in vitro* function parameters of the INTERCEPT platelet component at the end of INTERCEPT treatment and from a platelet sample at the end of storage.

- End of INTERCEPT treatment (Day 1 or 2) and End of Storage (Day 5):
  - *Product parameters:* Platelet count, mean platelet volume, platelet dose, and component volume.
  - *Biochemical assessments:* glucose, lactate, pO_2, pCO_2, bicarbonate, LDH (supernatant and total from Triton X-100 lysate), LDH as a % of total LDH (for each timepoint: LDH activity in supernatant / total LDH activity in Triton × 100), baseline (Day 0/1) adjusted LDH as a % of total LDH (Day 5 supernatant LDH – baseline supernatant LDH / total Day 5 LDH activity in Triton × 100), ,

and ATP. Normalized O_2 consumption, normalized CO_2 production, normalized glucose consumption, normalized lactate production, normalized ATP consumption, and normalized supernatant LDH values will be calculated per platelet.

- *Functional assessments:* CD62P (P-selectin expression)

8.2 Methods and Timing of Efficacy Parameters

8.2.1 Radioactivity Measurements and Recovery and Survival Estimation

Samples will be obtained from the radiolabeled Control and stored Test platelets before infusion and used as a radioactive standard. By measuring the volume infused, the total dose of radioactivity infused will be calculated. The radioactivity of the samples will be determined by use of a gamma counter. Sample dilutions for enumeration of radioactivity of the standards will be calibrated to ensure that measured values are at least 10-fold background values. Duplicates of the subject's WB will be measured for radioactivity while singletons of all other samples will be evaluated (unless otherwise specified in **Appendix B**). Post-infusion blood samples will also be corrected for plasma associated radioisotope and the spontaneous *in vitro* and *vivo* dissociation (elution) of radiolabels using the BEST elution assessment method (**Appendix B**).

8.2.2 *In Vitro* Platelet Assessments

Samples for analysis of pH need to be analyzed within 30 minutes of preparation. The remaining indices listed in Table 7 should be evaluated or prepared for testing following site SOPs. Samples for assessment of ATP will be prepared by the site and sent to a centralized lab for analysis. Samples for assessment of CD62P, LDH, glucose, and lactate can be prepared and stored for analysis later per site specific SOPs or sent to a centralized lab for analysis.

Table 7 *In Vitro* Platelet Function Assays to Evaluate INTERCEPT Platelet Components

Assay	Input (Day 0-1)	Post INTERCEPT (Day 1-2)	End of Storage (Day 5)
Component weight (g) [a]	X	X	X
Platelet count ($\times 10^3/\mu L$)	X	X	X
Platelet dose ($\times 10^{11}$ cells/unit) [a]	X	X	X
Mean platelet volume (MPV) (fL)	X	X	X
$pH_{22°C}$	X	X	X
pO_2 (37°C) (mmHg) [c]	X	X	X
pCO_2 (37°C) (mmHg) [c]	X	X	X
HCO_3^- (37°C) (mmol/L) [c]	X	X	X
Supernatant glucose (mmol/L) [c]	X	X	X
Supernatant lactate (mmol/L) [c]	X	X	X
ATP (µmol/dL) [c]	X	X	X
Supernatant LDH (U/L) [c]	X	X	X
Total LDH (U/L) [d]	X	X	X
LDH as a % of total LDH (%) [b]	X	X	X
CD62P (% P-selectin expression)	X	X	X
Bacterial culture	-	1-3 days before end of storage	-

[a] Volume is calculated from component weight. Platelet dose is calculated from the platelet count and volume.
[b] Calculated from ratio of LDH activity in 1 mL of platelet concentrate supernatant to the total LDH activity in 1 mL of Triton X-100 lysed platelet concentrate. This value will also be corrected for baseline (Day 0/1) LDH
[c] The values for these parameters will also be normalized for platelet count.
[d] Acquired from the Triton X-100 lysed platelet concentrate.

Specific assays and equipment for the assessment of the *in vitro* parameters will be detailed in the clinical study report.

9.0 ASSEMENT OF SAFETY

9.1 Safety Parameters

9.1.1 Safety Endpoints

- Adverse events (AE)
- Vital signs
- Hematological profile
- Serum chemistry profile

9.2 Methods and Timing of Safety Parameters

Subjects will be actively monitored for AEs (serious and non-serious) starting with apheresis collection and during the radiolabeled platelet infusion, until they are discharged from the study site. All AEs reported by the subject to the study staff will be recorded on the eCRF. AE) data may be collected by phone or in person by study staff.

Vital signs are collected at screening, prior to apheresis collection (Day 0), on the day of infusion (two times pre-infusion and approximately 2-hours post-infusion), and on 1, 2, 3, 5±1, 7/8, and 11±1 DPI. Clinically significant changes in vital signs as assessed by the study Investigator will be recorded as AEs.

Blood samples for hematology and chemistry panels will be obtained from each subject at screening, prior to platelet infusion (Day 5), and at the last scheduled blood draw (11±1 DPI). Clinically significant laboratory findings which occur after screening will be recorded as AEs. The subject laboratory assessments are identified in Table 6.

9.3 Recording and Reporting Adverse Events by Study Site to the Sponsor

All AEs that occur following the start of the apheresis collection through 24 hours after the last DPI blood sample draw will be reported. The Investigator will assess all AEs which will be recorded on the eCRF.

9.3.1 Definition of Adverse Event

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs, including an abnormal laboratory finding, in subjects, users or other persons, in the context of a clinical investigation whether or not related to the investigational device/drug. Additionally, an adverse event is any untoward medical occurrence in a patient administered a medicinal product in routine clinical/commercial use which does not necessarily have to have a causal relationship with this treatment.

9.3.2 Definition of Serious Adverse Event

Any adverse event that led to any of the following:

- Death
- Serious deterioration in the health of the subject, which resulted in any of the following:
    - Life-threatening illness or injury,
    - Permanent impairment of a body structure or a body function,
    - Hospitalization or prolongation of patient hospitalization,
    - Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
    - Chronic disease,
- Fetal distress, fetal death or a congenital physical or mental impairment or birth defect.
- Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (taken from ICH E2A).

9.3.3 Definition of Unanticipated Adverse Device Effects

Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

9.3.4 Assessing Relationship of the Adverse Event to the Study Procedure

The Investigator is required to assess whether there is a reasonable possibility that the study procedures (apheresis platelet collection and/or study infusion of radiolabeled study platelets) caused or contributed to an AE, per Table 8. Determination of whether there is a reasonable possibility that the study apheresis platelet collection or the infusion of radiolabeled study platelets caused or contributed to an AE includes assessing temporal relationships, biologic plausibility, dechallenge/rechallenge information (if available), association (or lack of association) with underlying disease, and presence (or absence) of a more likely cause.

Table 8 Causality Assessment Scale

Imputability level	Explanation
Excluded	When there is conclusive evidence beyond reasonable doubt for attributing the adverse event to alternative causes other than apheresis platelet donation, investigational product, or the infusion procedure of radiolabeled study platelets.
Possible	When the evidence is indeterminate for attributing adverse event either to apheresis platelet donation or the infusion of the radiolabeled study platelets to alternative causes.
Probable	When the evidence is clearly in favor of attributing the adverse event to apheresis platelet donation or the infusion of the radiolabeled study platelets.
Certain	When there is conclusive evidence beyond reasonable doubt for attributing the adverse event to apheresis platelet donation or the infusion of radiolabeled study platelets.

9.3.5 Assessing Severity of Adverse Event

The severity (intensity) of each AE is assessed using the standard severity grading scale provided in Table 9.

Table 9 Severity Grading Scale

Grade	Definition
Grade 1	Mild: the event required no medical intervention.
Grade 2	Moderate: the event may have required medical intervention (e.g., symptomatic treatment) but lack of such would not result in permanent damage or impairment of a bodily function
Grade 3	Severe: signs and symptoms requiring in-patient hospitalization or prolongation of hospitalization directly attributable to the event; and/or the event results in persistent or significant disability or incapacity; or the event necessitated medical or surgical intervention to preclude permanent damage or impairment of a bodily function.

Grade	Definition
Grade 4	Life-threatening: unstable vital signs with major treatment intervention required to prevent death (e.g., vasopressors, intubation, transfer to intensive care).
Grade 5	Death: adverse event resulting in death.

9.3.6 Assessing Seriousness of an Adverse Event

The Investigator is required to assess the seriousness of an AE. Refer to **Section 9.3.2** for definition of an SAE.

9.3.7 Reporting Adverse Events to the Sponsor

All AEs that occur following the start of the apheresis collection through 24 hours after the last DPI blood sample draw must be reported to the Sponsor via the eCRF. Non-serious AEs must be reported to the Sponsor within 14 days of becoming aware of the event. The Sponsor contact information is provided in Table 10.

Table 10 Sponsor Contact Information

Name Title	Phone	E-mail
Cerus Global Product Safety Team	Office: +1 925-288-6014	ProductSafetyInBox@cerus.com
Richard Benjamin, MD, PhD, FRCPath Chief Medical Officer (Medical Monitor)	Office: +1 925-288-6020	rbenjamin@cerus.com
Marissa Li, MD Senior Director of Clinical Development and Global Product Safety (Backup Medical Monitor)	Mobile: +1 310-980-2780	mli@cerus.com

9.3.7.1 Reporting Serious Adverse Events to the Sponsor

All SAEs must be reported to the Sponsor within 24 hours of becoming aware of the event. The notification may be in the form of data entry in the eCRF or notification to the Cerus Global Product Safety Team. Sponsor contact information is provided in Table 10.

9.4 Serious Adverse Event Follow up

The Sponsor reviews each SAE and assesses for expedited reportability to FDA. The Sponsor may request additional follow-up information to write a comprehensive narrative.

The Investigators agree to promptly provide follow-up information requested by the Sponsor. All SAEs are followed until resolution, or when the Investigator judges the event to be chronic or stable.

9.5 Reporting Product Complaints and Device Malfunctions

Study sites are required to report any product complaints or device malfunctions of the INTERCEPT Processing set and/or INTERCEPT Illuminator device on the eCRF. If a product complaint or device malfunction impacts the health of a subject is also considered an AE. The Investigator must notify Cerus Global Product Safety per Table 10.

Any product complaint or device malfunction occurring during study conduct will be documented in the Electronic Data Capture System. If a product or investigational device fails to perform in the expected manner at any time, the Sponsor will be notified, and the event will be appropriately reported to Cerus Quality Assurance Group.

10.0 STATISTICS

10.1 Analysis Sets

The analysis sets will be defined as follows:

Consented Set

All consented subjects.

Intention to Treat Set (ITT) (Safety Analysis Set):

All subjects who successfully initiate an apheresis collection.

Modified Intention to Treat (mITT):

Subjects who have data for recovery and survival data and/or other *in vitro* characteristics.

Per-Protocol Set (PPS):

All randomized and infused subjects who have both paired (Test and Control) recovery and paired survival data, Test recovery and/or survival less than Control recovery and/or survival, and otherwise complied with the protocol without any protocol deviation potentially affecting the primary efficacy outcome.

The primary and secondary efficacy analysis will be based on the PPS. As a sensitivity analysis, primary and secondary efficacy endpoints will be conducted on mITT as well. *In vitro* function and physical parameters will be assessed on ITT, mITT, and PPS. Safety data will be summarized for the ITT. Consented Subjects will be assessed only for eligibility, study completion, and deviations. All other summaries will be presented using the ITT unless stated otherwise.

10.2 Statistical Methods

This study utilizes a paired design to characterize the recovery and survival of autologous apheresis INTERCEPT Test platelet components stored for 5 days compared to their untreated fresh Control platelet components.

Data will be summarized descriptively by mean, standard deviation, median, and range (minimum, maximum) for continuous parameters and by frequencies and percentages for categorical data variables. Summaries will be presented by radiolabeling method across all study sites and within each study site.

Correlations between the primary and secondary efficacy endpoints may also be explored using graphs or regression analysis.

10.2.1 Efficacy Analyses

10.2.1.1 Primary Endpoints

Primary efficacy endpoints are the post infusion recovery and survival of Test platelets at end of storage. Post-infusion recovery and survival of platelets will be calculated based on the corrected radioactive counts observed from the six post-infusion sampling time points at 1, 2, 3, 5±1, 7/8, and 11±1 days post infusion. The following acceptance criteria for recovery and survival will be used to demonstrate non-inferiority of stored Test platelets against fresh Control platelets:

1. Post-infusion recovery:
   H_0: Test – 0.66 × Control < 0 vs. H_1: Test – 0.66 × Control ≥ 0

The null hypothesis of inferiority will be rejected in favor of the alternative hypothesis of non-inferiority at the two-sided 0.05 significance level if the lower bound of a two-sided 95% CI for the mean treatment difference (Test – 0.66 × Control) is greater than or equal to zero. For any platelet recovery values that are more than 100%, they will be capped at 100% for the data analysis.

2. For survival:
   H_0: Test – 0.58 × Control < 0 vs. H_1: Test – 0.58 × Control ≥ 0

The null hypothesis of inferiority will be rejected in favor of the alternative hypothesis of non-inferiority at the two-sided 0.05 significance level if the lower bound a two-sided 95% CI for the mean treatment difference (Test – 0.58 × Control) is greater than or equal to zero.

The two-sided 95% CI will be constructed using the variance estimated from a paired t test.

The primary analysis population for the primary efficacy endpoints will only include subjects who have both paired (Test and Control) recovery and paired survival data for the relevant radiolabeling method. Subjects who do not have both paired recovery and paired survival data may be replaced to ensure sufficient number of subjects is reached for the primary analysis. Reasons for absence of primary endpoint measurement(s) for any subject will be listed in the study report.

If non-inferiority cannot be declared for the primary endpoints with a minimum of 24 evaluable subjects, additional exploratory analyses may be conducted to quantify the proportions of subjects whose recovery and survival data meet the 0.66 and 0.58 cutoff,

respectively (e.g., platelet recovery from a Test PC greater than or equal to 66% of platelet recovery from the paired fresh Control).

10.2.1.2 Secondary Endpoints

Data will be summarized descriptively. The proportions of Test platelet components with platelet dose $\geq 3.0 \times 10^{11}$ platelets (end of INTERCEPT treatment), platelet yield retention $\geq$ 80% (end of INTERCEPT treatment), and $pH_{22°C} \geq 6.2$ (end of storage) will be summarized, with the corresponding lower bound of a one-sided 95% CI for the proportion provided.

10.2.2 Additional Endpoints

10.2.2.1 *In Vitro* Evaluation of Stored INTERCEPT Platelet Components

In vitro parameters of stored Test platelet components at the end of storage will be summarized descriptively.

10.2.3 Safety Analyses

Treatment-emergent AEs (defined as AEs with onset on or after the initiation of apheresis platelet collection) will be summarized descriptively. Other safety data (e.g., vital signs, lab data) will be provided in the data listings.

10.3 Determination of Sample Size

A sufficient number of subjects will be enrolled to provide a minimum of 24 evaluable subjects with both paired (Test and Control) recovery and paired survival data. The sample size of 24 is chosen to provide reasonable estimates of the primary efficacy endpoints.

10.4 Level of Significance

Unless stated otherwise, statistical significance will be set at the two-sided 0.05 significance level. Note that given the nature of this study, adjustments for multiple hypothesis testing will not be made.

10.5 Criteria for Termination of the Study

There are no pre-specified stopping rules for this study. The Sponsor may stop the study for any reason.

10.6 Procedure for Missing, Unused, and Spurious Data

Missing data will be noted as such. Procedures for imputing data will not be undertaken.

10.7 Deviations from the Statistical Plan

Any changes in the planned analyses instituted after commencement of the study will be documented. The reasons for the modifications and when the changes were made will also be documented.

10.8 Selection of Subjects to be Analyzed

The primary analysis population for the primary efficacy endpoints will be PPS.

11.0 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Prior to participating in the trial, the Investigator(s)/institution(s) agrees to provide direct access to and copies of source data/documents for collection of baseline data, study data, monitoring, audits, IRB review, and regulatory inspection.

All study-related documents and records are subject to inspection and audit by the Sponsor (or designee), and by the FDA, IRB, or other relevant regulatory bodies. The Investigator/institution guarantees access to source documents by the study Sponsor or its designee, the FDA, other regulatory bodies, and the IRB. If the FDA or local health authority should schedule an inspection, Sponsor should be advised prior to the time this inspection is to occur.

The subjects' apheresis collection records and medical records are considered source data. Data capture sheets used during the preparation of study platelet components will be considered source data for processing study platelet components. Analyzer reports or assay results from the laboratory will also be considered source data. The study data will be recorded on the eCRF.

12.0 QUALITY CONTROL AND QUALITY ASSURANCE

The platelet components will be prepared at each site following the institutional procedures and INTERCEPT treated at each study site following the INTERCEPT Instructions for Use (SPC 00770-AW). Analysis for the *in vitro* parameters will be performed at the site, designated external laboratories, or at Cerus Corporation according to institutional standard operating procedures at the applicable testing facility.

Collection of accurate, consistent, and reliable data will be ensured with written standard practices and procedures. Clinical study monitors will ensure that eCRFs are completed correctly, and that complete information has been provided. The Cerus Corporation Department of Quality Assurance (QA) will review the clinical study report for this trial. At the discretion of the Sponsor, in addition to the study monitor, a QA representative(s) of the Sponsor may visit a site to ensure proper conduct of the trial in terms of collection and recording of data and maintenance of records. The clinical site may also be audited by a regulatory agency.

12.1 Study Monitoring

The clinical site will be monitored routinely to ensure Good Clinical Practice (GCP) compliance and data quality. The CRF will be verified against the source document and any queries will be resolved/reconciled to assure data integrity. The clinical site may be audited by the Sponsor or Sponsor designee for quality control and quality assurance purposes, as specified in the Sponsor's SOPs for GCP compliance. The clinical site may also be audited by a regulatory agency.

The study will be monitored by the Sponsor's representatives at all stages of study conduct from inception to completion in accordance with current GCPs. This monitoring will include both centralized and on-site review and source document verification of the data. Centralized monitoring will include remote review of the data (data capture sheets, and eCRFs) that will be provided to Cerus on a regular basis. On-site monitoring involves the visit of a monitor to the investigational site. The Sponsor's monitor or representative will notify the Investigator prior to conducting any investigational site visit. It is important that the Investigator and other study personnel are available during the monitoring visits, and that sufficient time is devoted to the process. The frequency of these visits will depend upon the progress of the study and will encompass all aspects of the study conduct (i.e., review of all study related procedures, tour of the facilities, inspection of equipment and appropriate records, completion of eCRFs, recruiting methods, record-keeping, protocol adherence, data collection, AE reporting, etc.). Frequency of on-site monitoring may also be decreased due to institutional restrictions on site access or research activities due to disasters and public health emergencies (FDA 2023).

13.0 ETHICS

13.1 Ethical Conduct of the Study

This study will be conducted in compliance with the protocol, the International Council on Harmonization (ICH) E6(R2) Good Clinical Practice, *Guidance for Industry and FDA Review Staff: Collection of Platelets by Automated Methods*, December 2007), US Regulations governing the protection of human subjects (21 CFR Part 50); Institutional Review Boards (21 CFR Part 56), Financial Disclosure by Clinical Investigators (21 CFR Part 54); and local IRB requirements.

Investigators are encouraged to discuss any ethical issues that arise prior to or during the conduct of the study with the Sponsor. The Protocol and Informed Consent Form will be approved by the Investigator and the Investigator's IRB before the study is initiated.

13.2 Informed Consent

The informed consent form must be agreed to by Cerus and comply with all applicable US Regulations (21 CFR Part 50; 21 CFR Part 640), and the Health Insurance Portability and Accountability Act (HIPAA) as well as regional regulatory and legal requirements. The informed consent form used in this trial, and any changes made during the trial, must be prospectively approved by both the IRB and Cerus before use.

The risks and hazards of study participation will be explained to the potential study subjects, under the supervision of a qualified, licensed medical professional. Written informed consent and HIPAA authorization will be obtained from all study subjects prior to any tests or evaluations. A copy of the signed informed consent will be provided to each subject and will also be maintained in the subject’s donor record.

13.3 Institutional Review Board (IRB)

This protocol will be submitted to an appropriate central or local IRB and its written unconditional approval obtained and submitted to Cerus or its designee before enrollment of the first subject.

Cerus will supply relevant data for Investigators to submit to the IRB for the protocol’s review and approval. Written verification of IRB unconditional approval of the protocol and the subject Informed Consent Form (ICF) will be transmitted to Cerus or its designee prior to granting access to the eCRF to study sites. This approval must refer to the study by exact protocol title and number (including version), identify documents reviewed, and state the date of approval.

The Investigator must not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects. The Investigator must promptly report to the IRB all changes in the research activity and all unanticipated problems involving risk to human subjects or others. This includes all SAEs that have resulted in an expedited safety report to the FDA (serious, unexpected AEs possibly related to investigational product). Concurrently, the Investigator must send the study Sponsor documentation of such IRB notification Participant Recruitment

If an Investigator chooses to advertise for subjects, whether in professional or consumer publications, radio, or television, all advertising must be approved by Cerus and the IRB prior to initiation.

13.4 Confidentiality

Individual subject medical information obtained as a result of this study is considered confidential, and disclosure to third parties other than those noted below is prohibited. Subject confidentiality will be further assured by utilizing subject identification code numbers to correspond to treatment data in the computer files.

However, such medical information may be given to the subject's personal physician, or to other appropriate medical personnel responsible for the subject's welfare.

In addition, data generated as a result of this study are to be available for inspection upon request by FDA or local health authority auditors, the Sponsor's monitors, or by the IRB. Therefore, absolute confidentiality cannot be guaranteed.

14.0 DATA HANDLING AND RECORD KEEPING

14.1 Case Report Forms

The Investigator will maintain appropriate subject records. Clinical information gathered during this study will be recorded on eCRFs. An eCRF is required and should be completed for each included subject. The completed original eCRFs are the sole property of Cerus and should not be made available in any form to third parties, except for authorized representatives of Cerus or appropriate regulatory authorities, without written permission from Cerus.

A CRF casebook is required and should be completed for each screened subject. Prior to the study database lock, all CRFs must be electronically signed by the investigator or by a delegated staff member. These signatures serve to attest that the information contained on the CRFs is true. At all times, the Investigator has final personal responsibility for the accuracy and authenticity of all clinical and laboratory data entered on the CRFs. Subject source documents are maintained at the trial site. Record Retention

Investigators/institutions and the Sponsor shall maintain all study records required by subpart 21 CFR 812.140 during the investigation and for a period of 2 years after the latter of the following two dates: the date on which the investigation is terminated or completed, or the date that the records are no longer required for purposes of supporting a premarket approval application or a notice of completion of a product development protocol. Investigator/institution shall not destroy any such records prior to obtaining written permission from Cerus.

15.0 FINANCING AND INSURANCE

Study financial and insurance records are maintained as separate documents.

16.0 PUBLICATION POLICY

Investigational plans, protocols, and data related to the study will be treated as confidential information. Sponsor personnel will share authorship with Investigators for all publications that may result from this study. No Investigator may publish data derived from studies performed in his/her institution alone; all publications will incorporate data derived from all centers participating in the study.

17.0 APPENDICES

List of Appendices

Appendix A Literature References

1. AABB, Standards for Blood Banks and Transfusion Services, 34th edition, April 2024.
2. Brouard N, Pissenem-Rudwill F, et. al. 2023 Biochemical and functional characteristics of stored (double-dose) buffy-coat platelet concentrates treated with amotosalen and a prototype UVA light-emitting diode illuminator. Transfusion 63(10):1937-1950.
3. The Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Platelet radiolabeling procedure. Transfusion 2006;46 Suppl:59S-66S.
4. Code of Federal Regulations (CFR).. Title 21, Part 50, Protection of Human Subjects.
5. Code of Federal Regulations (CFR). Title 21, Part 54, Financial Disclosure by Clinical Investigators.
6. Code of Federal Regulations (CFR). Title 21, Part 56, Institutional Review Boards.
7. Code of Federal Regulations (CFR). Title 21, Part 312.32, Investigational New Drug Application, IND Safety Reporting.
8. Code of Federal Regulations (CFR). Title 21, Part 640, Additional Standards for Human Blood and Blood Products.
9. Code of Federal Regulations (CFR). Title 21, Part 812.3, Investigational Device Exemptions, Definitions.
10. Dumont LJ, Cancelas JA et al. 2013. A randomized controlled trial evaluating recovery and survival of 6% dimethyl-sulfoxide-frozen autologous platelets in healthy volunteers. Transfusion 53(1):128-137.
11. FDA Guidance for Industry, Collection of Platelets by Automated Methods. 2007.
12. FDA Guidance for Industry, Investigators, and Institutional Review Boards: Considerations for the Conduct of Clinical Trials of Medical Products During Major Disruptions Due to Disasters and Public Health Emergencies, FDA, September 2023.
13. Holme S, Heaton A, et al. 1993. Concurrent elution method allows accurate evaluation of platelet viability of stored platelet concentrates. Br J Haematol 84: 717-723.
14. International Conference on Harmonisation (ICH) Guidance for Industry, E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1). March 2018.
15. International Conference on Harmonisation (ICH), E2A, Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. ICH October 1994.

16. Lötter MG, Rabe WL, et al. 1988. A computer program in compiled BASIC for the IBM personal computer to calculate mean platelet survival time with the multiple-hit and weighted mean methods. Comput Biol Med 18:305-15.

17. Snyder E, Raife T, et al. 2004. Recovery and Lifespan of 111 Indium radiolabeled platelets treated with pathogen inactivation using amotosalen HCl (S-59) and UVA light. Transfusion 44:1732-1440.

Cerus References

1. CLI 00127: A Randomized, Multi-Center, Multi-Stage, Controlled, In Vivo Study to Assess the Recovery and Survival of Radiolabeled Autologous INTERCEPT Apheresis Platelet Components Suspended in 100% Plasma Stored for up to 7 Days: Clinical Study Report, April 15, 2021.
2. CLI 00164: A Randomized, Multi-center, Controlled, In Vivo Study to Assess the Recovery and Survival of Radiolabeled Autologous INTERCEPT Apheresis Platelet Components Suspended in 100% Plasma Stored for 5 Days: Clinical Study Report.
3. INB 00002, Investigator Brochure INTERCEPT Blood System for Platelets
4. SPC 00698-AW, INTERCEPT Blood System for Platelets – Large Volume (LV) Processing Set
5. SPC 00770-AW, INTERCEPT Blood System for Platelets – Large Volume (LV) Processing Set, For Investigational Use – Instructions for Use

Appendix B Radiolabeling Procedure for Platelets (Variant 1)

VARIANT 1 OF BEST 4.2.1 METHOD FOR PLATELET RADIOLABELING

INTRODUCTION

This appendix contains the Variant 1 of BEST 4.2.1 for method for the radiolabeling of both stored and fresh platelets and their autologous reinfusion for the determination of recovery and survival modified from the BEST 4.2.1 method published November 2006 in a supplement to *Transfusion* (BEST 2006).

Variant 1 of BEST 4.2.1 method (Variant 1), was qualified in the Cerus study CLI 00127: A Randomized, Multi-Center, Multi-Stage, Controlled, In Vivo Study to Assess the Recovery and Survival of Radiolabeled Autologous INTERCEPT Apheresis Platelet Components Suspended in 100% Plasma Stored for up to 7 Days. The method for collection and radiolabeling of the fresh Control platelets in Variant 1 of BEST 4.2.1 contains the same steps as published in the BEST 4.2.1 method (BEST).

PROCEDURE

1.0 Purpose: To describe a technique for radioisotopic labeling and infusion of fresh or stored platelets with ^{111}In Oxine (^{111}Indium) and $Na_2{}^{51}CrO_4$ (^{51}Chromium).

1.1 While the measurement of platelet recovery and survival can be used for a variety of purposes, this procedure is intended for assessment of the *in vivo* viability of platelets that have been stored and/or treated in comparison with fresh platelets from the same subject

1.2 This procedure includes the basic calculations for determining platelet recovery and survival. The recommended manner of comparing the results between control and test infusions is through comparison of the paired differences and calculation of upper confidence intervals as detailed by Dumont in this supplement (See 2.6.)

2.0 Applicable documents:

2.1 Snyder EL, Moroff G, Simon T, Heaton A, and Members of the Ad Hoc Platelet Radiolabeling Study Group. Recommended methods for conducting radiolabeled platelet survival studies. Transfusion 1986; 26:37-42

2.2 Panel Report, International Committee for Standardization in Hematology. Recommended methods for radioisotopic platelet survival studies. Blood 1977; 50:1137-1144

2.3 Holme S., Heaton A., Roodt J. Concurrent label method with ^{111}In and ^{51}Cr allows accurate evaluation of platelet viability of stored platelet concentrates. Brit J Haematol 1993; 84:717-723.

2.4 Nadler SB, Hidalgo JU, Bloch T. Prediction of blood volume in normal human adults. Surgery 1962; 51:224-232

2.5 Lötter MG, Rabe WL, Van Zyl JM, et al. A computer program in compiled BASIC for the IBM personal computer to calculate the mean platelet survival time with the multiple-hit and weighted mean methods. Comput Biol Med 1988; 18:305-15

2.6 Dumont LJ. Analysis and reporting of platelet kinetic studies. Transfusion 2006;46 (Suppl) 67S-73S

2.7 BEST. Platelet Radiolabeling Procedure. Transfusion 2006; 46:59S-66S

3.0 Equipment/Supplies/Reagents

3.1 Calibrated, temperature-controlled, variable speed, swinging bucket centrifuge.

3.2 Calibrated electronic balance, accurate to ±0.0002 g.

3.3 Gamma counter with 3-inch sodium chloride crystal

3.4 Laminar flow hood.

3.5 Sterile transfer pipettes (3.2 mL draw)

3.6 15 mL and 50 mL sterile conical polypropylene plastic tubes (Note: Although some procedural steps specify exact sizes, an alternative appropriate size may be substituted.)

3.7 Evacuated sample tubes with EDTA (powdered or dry "lavender tops").

3.8 ACD-A: Anticoagulant Citrate Dextrose Solution - Formula A

3.9 ACD-A/ Saline Solution: mix 1-part ACD-A to 7 parts sterile (0.9%) Normal Saline, adjusted to pH 6.5 to 6.8 with 1 N NaOH; sterilize by filtration. [Stable for one week when stored in refrigerator; warm to room temperature (20-24°C) before use.]

3.10 ^{111}Indium Oxine in buffer containing approximately 1-2 mCi per vial at the time of manufacturer's calibration. Use within 7 days for platelet labeling. (Changes in buffer/oxine ratio over time may result in changes in platelet function.)

3.10.1 1 mCi ^{111}Indium Oxine Labeling solution: if not premixed, aseptically add 4.0 mL sterile ACD-A/saline solution to the vial of ^{111}Indium Oxine to make 5.0 mL of sterile ^{111}In-Oxine solution for labeling. This mixing may also occur in a sterile syringe.

3.10.2 2mCi ^{111}Indium Oxine labeling solution: if not premixed, aseptically add 2 mL oxine suspension buffer to the vial of 2 mCi ^{111}Indium Oxine to make 3 mL ^{111}Indium oxine suspension. Aseptically mix approximately 83 µL of the ^{111}Indium Oxine suspension with 1 mL of ACD-A/saline to create approximately 50-60 µCi labeling solution. Mix well. (Stable for one week or until expiration of stock ^{111}In oxine solution, not to exceed expiration of the stock solution.) This mixing may also occur in a sterile syringe.

3.11 $Na_2{}^{51}CrO_4$, ^{51}Chromium

3.11.1 If reagent grade ^{51}Chromium is used, the ^{51}Chromium will be sterile filtered and assessed for endotoxin.

3.12 Assorted sterile syringes and needles (Note: Although some procedural steps specify exact sizes, any appropriate size may be substituted.)

3.13 Positive displacement pipettes for whole blood aliquots.

3.14 Assorted size pipettes and tips

3.15 SDS (Sodium Dodecyl Sulfate) 20%; Dissolve 20 g sodium dodecyl sulfate in 100 mL of distilled water. (Store at room temperature for up to one year.).

NOTE: All vessels holding platelets or plasma that will be reinfused must be properly labeled to definitively identify the subject

4.0 Preparation of Platelets for Labeling with ^{111}Indium Oxine

4.1 Preparation of freshly collected platelets (Control)

4.1.1 Using a 19-gauge needle, collect 43 mL (± 2 mL) of venous blood into a 60 mL sterile plastic syringe containing 9 mL of ACD-A. Avoid a traumatic venipuncture at the start of blood drawing. Care should be taken to avoid excessive negative pressure in the syringe during draw to prevent activation of the platelets. (Alternatively, 43 mL of whole blood can be collected with 7 mL of ACD-A followed by a 1-2 hour holding period in Step 4.1.3.)

4.1.2 At the same time as Step 4.1.1 or during Step 7.3, collect one 5, 7, or 10 mL blood sample from the subject into an EDTA tube. Mix well. Reserve for determination of radiolabel elution (see Step 9.0).

4.1.3 Using sterile technique, in a laminar flow hood, remove the needle and express the contents of the syringe into two sterile 50 mL screw-cap conical centrifuge tubes. A hold period of 30 minutes for the ACD-A/blood mixture before further processing should be utilized.

4.1.4 Centrifuge (soft spin) the conical tubes at 180 to 200 × g for 15 minutes at 20 to 24°C with the brake off to produce red cell-poor platelet–rich plasma (PRP)

4.1.5 Remove the PRP from both tubes with a sterile transfer pipette or spinal needle and syringe and place into a single 50 mL sterile conical plastic centrifuge tube. Avoid aspirating any contaminating red cells.

4.1.6 Add a volume of sterile ACD-A equal to 15% of the volume of PRP to the PRP. Cap the tube and mix gently by inversion. Continue with Step 5.3

4.2 Preparation of aliquot from stored platelet unit (Test)

4.2.1 Ensure unit is thoroughly mixed and platelets are completely suspended.

4.2.2 Using sterile technique, in a laminar flow hood, gently remove a sample of approximately 10 mL (±2 mL) from the stored platelet unit using a 20 mL syringe and 16 ga needle.

4.2.3 Remove the needle and express the 10 mL (±2 mL) a 15- or 50-mL screw-cap conical labeled centrifuge tubes Cap and mix gently by inversion or swirling.

4.3 Centrifuge (hard spin) the ACD-PRP (fresh sample) or stored platelet sample at 1500-2000 ×g for 15 minutes at 20 to 24ºC with brake off or brake minimum**.**

4.4 Remove and save the PPP produced as completely as possible and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 ×g for 15 minutes with brake off or brake minimum to allow for addition of PPP to platelet sample within 20 minutes of platelet labeling (Step 4.6.1)), discard pellet and

reserve supernatant PPP in a sterile tube for later use (Steps 4.6.2 and 4.6.5) (this spin may be done during radiolabeling).

4.5 Using a sterile pipet, resuspend the harvested platelet pellet with 2 mL of ACD-A/saline solution. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting to radiolabel. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.)

4.6 Platelet Labeling with ^{111}Indium-Oxine

4.6.1 Add 50 to 60 µCi of ^{111}Indium-Oxine labeling solution (Step 3.10) to the washed platelet suspension. Incubate at 20 to 24°C for 20+2 minutes to achieve labeling. (Note: 1 Ci = 3.7×10^{10} Bq)

4.6.2 At the end of incubation, add 3.5 mL of ACD-A/saline and 0.5 mL of the reserved PPP (from Step 4.4) directly to the incubation tube containing the platelets in ACD-A/saline and ^{111}In.

4.6.3 Centrifuge the tube at 1200-1250 ×g at 20 to 24°C for 10 minutes with brake off.

4.6.4 Remove the supernatant and retain in a separate test tube. Determine the activity of this supernatant and the labeled pellet in a dose calibrator. Calculate and note the efficiency of labeling by dividing the activity of the pellet by the combined activities of the pellet and supernatant.

4.6.5 Gently resuspend the platelet-pellet in 2 mL of PPP reserved previously (from Step 4.4). (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure). After complete resuspension, add an additional 4 mL of PPP.

5.0 Preparation of Platelets for Labeling with $Na_2{}^{51}CrO_4$

5.1 Labeling of fresh platelets (Control)

5.1.1 Using a 19-gauge needle, collect 43 mL (± 2 mL) of venous blood into a 60 mL sterile plastic syringe containing 9 mL of ACD-A. Avoid a traumatic venipuncture at the start of blood drawing. Care should be taken to avoid excessive negative pressure in the syringe during draw to prevent activation of the platelets. (Alternatively, 43 mL of whole blood can be collected with 7 mL of ACD-A followed by a 1-2 h holding period in Step 5.1.3).

5.1.2 At the same time as Step 5.1.1 or during Step 7.3, collect one 5, 7, or 10 mL blood sample from the subject into an EDTA tube. Mix well. Reserve for determination of radiolabel elution (see Step 9.0).

5.1.3 Using sterile technique, in a laminar flow hood, remove the needle and express the contents of the syringe into two sterile 50 mL screw-cap conical centrifuge tubes. A hold period of 30 minutes for the ACD-A/blood mixture before further processing should be utilized

5.1.4 Centrifuge (soft spin) the conical tubes at 180 to 200 ×g for 15 minutes at 20 to 24°C with the brake off to produce red cell poor PRP.

5.1.5 Remove the PRP from both tubes with a sterile transfer pipette or spinal needle and syringe and place into a single 50 mL sterile conical plastic centrifuge tube. Avoid aspirating any contaminating red cells.

5.1.6 Add a volume of sterile ACD-A equal to 15% of the volume of PRP to the PRP. Cap the tube and mix gently by inversion. Continue with Step 5.3

5.2 Preparation of aliquot from stored platelet unit (Test)

5.2.1 Ensure unit is thoroughly mixed and platelets are completely suspended.

5.2.2 Using sterile technique, in a laminar flow hood, gently remove approximately 20 (±2 mL) mL from the stored platelet unit using a 30 mL syringe and 16 ga needle.

5.2.3 Remove the needle and express 20 (±2 mL) into two 15-mL (10±1 mL each) or one 50-mL screw-cap conical labeled centrifuge tubes. Cap and mix gently by inversion or swirling.

5.3 Centrifuge (hard spin) the conical tubes containing the ACD-PRP (fresh sample) or stored platelet sample at 1500-2000 ×g for 15 minutes at 20 to 24ºC with brake off

5.4 Remove and save the PPP produced as completely as possible and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 ×g for 15 minutes with brake off or brake minimum to allow for addition of PPP to platelet sample within 20 minutes of platelet labeling (Step 5.6.1)), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 5.6.2 and 5.6.5) (this spin may be done during radiolabeling).

5.5 Resuspend the harvested platelet pellet in one tube with 3 mL of ACD-A/saline solution. Using a sterile transfer pipette, transfer the resuspended platelets to the other tube containing the platelet pellet. Resuspend the second platelet button. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting the next step

5.6 Platelet Labeling with $Na_2{}^{51}CrO_4$

5.6.1 Add approximately 50 to 100 μCi $Na_2{}^{51}CrO_4$ to the washed platelet suspension. Incubate at 20 to 24°C for 20 minutes to achieve labeling

5.6.2 At the end of incubation, add 2.5 mL of ACD-A/saline and 0.5 mL of the reserved PPP (from Step 5.4) directly to the incubation tube containing the platelets in ACD-A/saline and $Na_2{}^{51}CrO_4$.

5.6.3 Centrifuge the tube at 1200-1250 ×g at 20 to 24°C for 10 minutes with brake off

5.6.4 Remove the supernatant and retain in a separate test tube. Determine the activity of this supernatant and the labeled pellet in a dose calibrator. Calculate and note the efficiency of labeling by dividing the activity of the pellet by the combined activities of the pellet and supernatant

5.6.5 Gently resuspend the platelet-pellet in 2 mL of PPP reserved previously (from Step 5.4). (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure). After complete resuspension, add an additional 4 mL of PPP.

6.0 Injectate and Standard Preparation

6.1 Label five 100 mL volumetric flasks and five 12 × 75 mm polystyrene tubes with the isotope used, subject name and/or unit identification, date prepared and In STD, Cr STD, and STD I, II or III

NOTE: The In STD and the Cr STD are necessary only if calculation of "cross-p" and "cross-down" corrections requires them

6.2 Add 1.8 mL 0.9% saline to each 12 × 75 mm standard capped tube. Weigh each tube and record the weights. Take care that the saline does not touch the cap.

6.3 Add 10 mL of 0.2% SDS-saline solution (1-part saturated (20%) SDS + 99-parts 0.9% saline) to each flask.

6.4 Mix each single labeled platelet aliquots (from Steps 4.6.5 and 5.6.5). Draw 0.3mL of each suspension into its own 1cc syringe through a 16-gauge needle.

6.5 Weigh the filled syringe, needle, and needle cover accurately. Record the weight

6.6 Carefully add 0.2 mL of each injectate directly into each flask, "Cr STD" or "In STD," as appropriate. Add 0.9% saline to the flask to bring to volume. Mix carefully. Weigh the syringe, needle, and needle cover accurately and determine the mass of labeled injectate placed in each standards flask

6.7 Draw the appropriate radioactive dose of ^{51}Cr-labeled platelets into a labeled syringe. Weigh the syringe, and record the radioactive dose

NOTE: The activity needed to be injected is dependent on a number of variables, such as the gamma counter's crystal size. In general, 10-20 μCi are required to be injected for accurate determinations. The injectates of the fresh and stored platelets should have approximately the same activities

6.8 Remove ^{111}In-labeled platelets from the centrifuge tube until the dose intended to be injected is remaining. Measure and record the radioactive dose. Record the weight of the tube

6.9 Carefully verify subject identification on the chromium syringe and indium tube. Inject the ^{51}Cr-labeled platelets into the centrifuge tube containing the ^{111}In-labeled platelets

6.10 Mix the labeled injectate in the tube. Prepare final mixed injectate for infusion by using an 18-gauge spinal needle and a syringe labeled with subject information to aspirate all but at least 1 mL of labeled solution in conical tube. (This remaining amount will be used for preparation of standards and determination of cell bound activity.)

6.11 Remove the spinal needle and replace with a sterile syringe cap. Record the weight of the final injectate syringe and cap

6.12 Draw approximately 0.8 mL of the remaining labeled solution from the tube containing the ^{51}Cr- and ^{111}In-labeled platelets into a 1cc syringe through a 16-gauge needle to be used for standard preparation

6.13 Record the weight of the filled syringe, needle, and needle cover

6.14 Carefully add 0.2 mL of the injectate directly into the flask labeled “STD I.” Add 0.9% saline to the flask to bring to volume. Mix carefully

6.15 Reweigh the 1 cc syringe, needle and needle cover and record this weight and the difference between the two weights (this being the net weight of the mixed injectate added to the standards flask)

6.16 Repeat Steps 6.14 and 6.15 for the remaining two flasks

6.17 Pipet 0.2 mL of each standard into the appropriate pre-weighed capped tube containing 1.8 mL saline, one sample tube per standard. Alternatively, if this process results in standard counts less than 10-fold higher than background, pipet 2.0 mL of the standard from the 100ml diluted volumetric flask into an appropriate pre-weighed capped tube for direct counting

6.18 Weigh the capped tubes and record the weight. Take care that the sample does not touch the cap

7.0 Infusion Procedure

7.1 Allow recipient to sit in the donor bed to stabilize. Record pre-infusion vital signs

7.2 Verify that recipient's identifiers match the information on the infusion syringe

7.3 Perform venipuncture with a 19–21-gauge butterfly infusion needle. Draw one **(**or 2 if tube for elution was not drawn earlier**)** 10 mL EDTA (lavender-top) tube for the pre-infusion sample

7.4 Flush the line slowly with 10 mL of sterile saline to ensure patency of the vein. Inject labeled platelet suspension slowly. Record exact time (starting from time 1/2 of injectate has been infused)

7.5 Flush butterfly line and needle with 10 mL of sterile 0.9% sodium chloride

7.6 Remove butterfly needle and apply pressure to injection site. Apply bandage when bleeding has stopped

7.7 Remove needle, cap syringe with syringe cap, and weigh syringe with syringe cap. Record weight on the data sheet. Record the net weight of injectate

7.8 Dispose of used supplies in the receptacle labeled for radioactive waste

7.9 Measure and record post-infusion vital signs

8.0 Sampling Procedure and Sample Preparation

8.1 One 5-10 mL EDTA (lavender-top) tube sample is to be drawn by direct venipuncture at the post-infusion times:

8.1.1 Pre-infusion (from Step 7.3)

8.1.2 Collect blood draws at 2 hours ±15 minutes after infusion. (Collect from the contralateral arm to that used for infusion.)

8.1.3 Sample on days 1, 2, 3, 5±1, 7/8. And one final sample on day 11±1. Sample on day 1 should be taken ±4 hours from the time of infusion. (The last sample is called the "baseline" sample and is used to correct for inadvertent labeling of other blood components.)

NOTE: As the points at the beginning of the curve are most important in determining the parameters of the curve, daily sampling on Days 1, 2, and 3 afford more accurate projections of recovery and survival parameters and should be taken if at all possible. A total of at least 5 data points from samplings on Days 1-7 should be obtained.

Delaying the final sample from Day 10 to Day 12 results in lower counts and, potentially, counts that are inadequate to achieve desired accuracy. Obtaining the final sample on Day 10, if possible, is desirable.

8.2 Sample Processing:

NOTE: Processing should be completed as soon as possible after sampling to prevent post-sampling elution from skewing the proportion of activity found in the cellular fraction.

8.2.1 Prior to the infusion, label and weigh capped 12 × 75 mm polystyrene tubes: one for each sampling time (including pre-sample) for whole blood, packed cells, and plasma. Tubes for daily samples may be labeled and weighed on the day of use or beforehand

8.2.2 Just prior to aliquoting sample, thoroughly mix each timed sample from the subject manually or for a maximum of two minutes on a rocker

8.2.3 Pipet approximately 2.0 mL of the mixed whole blood into the appropriate pre-weighed tube using a positive displacement pipettor and replace cap. This sample is prepared in duplicate and counted.

8.2.4 Weigh the capped tube and record the weight. Take care that the sample does not touch the cap

8.2.5 Pipet approximately 2.0 mL of blood into the appropriate tube labeled for packed cells

8.2.6 Centrifuge this second set of tubes at 2000 ×g for 15 minutes at room temperature

8.2.7 Pipet the plasma from the packed cell tubes to the appropriate tube labeled for plasma

8.2.8 Count the "splits" (packed cell and plasma tubes, including the injectate). (When possible, count the “splits” on the day of sampling.)

NOTE: The size of sample volumes may be increased if desired

9.0 Elution of Radioactivity from Labeled Platelets in Whole Blood

9.1 Add 10 µL of the mixed injectate into a 5 to 7 mL EDTA tube of blood collected from the subject as soon as possible after the infusion of the labeled cells procedure. (See Step 4.1.2 or 5.1.2.)

Note: This timing is anticipating infusion of the radiolabeled platelets at the earliest possible time. If delay in infusion is anticipated, the elution steps described in this section should be begun so that the separation of the supernatant after the next step occurs approximately coincidently with the infusion

9.2 Mix and incubate the tube for two hours at 37ºC in a wet incubator (water bath) or a dry incubator in a cylinder of water

9.3 Pipet approximately 2 mL into a 12 × 75 mm capped tube. This sample is prepared in duplicate and counted. Spin at 2000 ×g for 15 minutes

9.4 Transfer the supernatant plasma to a capped 12 × 75 mm capped tube

9.5 Count the splits (this may be done when the samples are counted)

9.6 The activity found in the cells and plasma will be used to evaluate the *in vivo* cell-bound activity of the labeled platelets for each of the two radiolabels

Elution = $(CPM_{supernate}/g)/[(CPM_{cells}/g) + (CPM_{supernate}/g)]$

10.0 Counting the Samples

10.1 Ensure required quality control and preventive maintenance procedures are performed according to the operating instructions. Load racks into counter and start counting. Count whole blood samples once all samples from the subject have been collected and processed. If any sample is run on a separate day (for example the Day 11±1 sample), the counts must be corrected for decay. (One can also count standards and splits, daily.)

10.2 Load the gamma counter in a standard order, such as: blank/background, standards, elutions, pre-infusion, and post-infusion timed specimens in order collected

10.3 Select appropriate counting program. Set count time to achieve a 2% accuracy, including background.

10.4 When the tubes have finished counting, label the instrument printout. Verify that the background has not drifted significantly. Verify that all samples counted for expected times. Place the gamma counter printouts in the appropriate file

10.5 Transfer all data to appropriate spreadsheets for calculations. Verify accurate transcription

11.0 Calculations

11.1 Determine corrected CPM/g for each sample

Most gamma counters correct automatically for background activity and decay during counting as well as for "cross-up" and "cross-down," that is, counting of one isotope's activity in the other's channels. Samples and standards must all be corrected for these parameters as well as corrected to the same time for calculations to be valid. Using these corrected CPM values reported by the gamma counter ($CPM_{corrected}$), determine CPM/g for standards and timed subject samples as follows:

11.1.1 Standards: STD = $CPM_{corrected}$ * dilution factor/mass counted, where dilution factor = mass of standard/mass of injectate added

NOTE: Since the diluent for the standard is water, the specific gravity of the standard may be assumed to be 1.00, and the final volume of the standard in mL may be taken as its mass in g.

Dilution of standards must be calibrated to ensure that measurements at baseline are > 10-fold higher than background

11.1.2 Timed sample activity: SAMPLE = $CPM_{corrected}$ /mass counted

11.2 Determine cell-bound proportion for timed count adjustment

Using timed split samples (Step 8.2.8), determine the proportion of the activity in each timed sample that can be associated with the cellular fraction:

Cell Bound Fraction ($CBF_{Time\ t}$) = (Activity of cells)/ (Activity of cells + Activity of plasma) = ($CPM_{cell\ bound}$)/ [($CPM_{cell\ bound}$) + (CPM_{plasma})]

11.3 Standard adjustment for injectate elution:

11.3.1 Determine the mean CPM of the three mixed standards (Steps 6.0 and 11.1.1)

11.3.2 Multiply the mean CPM of the standard samples by the elution correction (Step 9.6)

$STD_{corrected}$ = STD * (1-Elution)

11.4 Adjustment for cell-bound proportion and baseline

The following two adjustments should be made to the timed sample CPM/g counts. These adjustments result in "fully adjusted counts" used for curve fitting

11.4.1 Adjustment of timed sample for cell-bound proportion (Steps 11.1.2, 11.2):

$SAMPLE_{Time\ t}$ = SAMPLE (CPM/g) × ($CBF_{Time\ t}$)

11.4.2 Adjust for non-platelet residual activity baseline, according to the day on which the "baseline" sample was taken. (This results in fully adjusted timed sample counts.)

11.4.2.1 Day 10

Fully adjusted count = $SAMPLE_{Time\ t}$ – [$SAMPLE_{Day\ 10}$ × (1.20–0.20 (Time t/Time of Day 10 sample)]

11.4.2.2 Or Day 11

Fully adjusted count = $SAMPLE_{Time\ t}$ – [$SAMPLE_{Day\ 11}$ × (1.22–0.22 (Time t/Time of Day 11 sample)]

11.4.2.3 Or Day 12

Fully adjusted count = $SAMPLE_{Time\ t}$ – [$SAMPLE_{Day\ 12}$ × (1.24–0.24 (Time t/Time of Day 12 sample)]

Note: "Time t" is the elapsed time in hours from the time of infusion (Step 7.4) to the time of sampling. "Time of Day 10 sample" is the elapsed time in hours from time of infusion (Step 7.4) to the time the sample was obtained on Day 10 following infusion

These calculations are based on the published experience of reference 2.3 and the expectation that ^{51}Cr persistence is the primary source of persistent activity. Persistent activity attributable to ^{111}In is less of a concern because of its shorter half-life, its higher elution from any contaminating red cells that were labeled and the lack of oxine to facilitate labeling after elution from platelets

11.5 Determination of expected $time_0$ count following infusion

11.5.1 Calculate blood volume:

Male: BV (mL) = ([0.3669*{height (m)} ^{3}] + 0.03219*weight (kg) + 0.6041) *1000

Female: BV (mL) = ([0.3561*{height (m)}^{3}] + [0.03308*weight (kg)] + 0.1833) *1000

11.5.2 Convert the blood volume calculated in Step 11.5.1 to blood mass by multiplying the volume by the specific gravity of whole blood. The specific gravity may be taken as 1.05 g/mL, or, if the subject's hematocrit is known, it may be estimated as (1541 + Hct)/1500 (Subject hematocrit is expressed as a percentage rather than a decimal; expected SG usually between 1.053 and 1.060 g/mL.).

11.5.3 Determine expected $time_0$ (t=0) count (Steps 11.3.2, 7.0, 11.5.2)

$Time_0$ Count = $STD_{corrected}$ * Infusate mass (g) / Blood mass

11.6 Determine recovery for each timed sample (optional)

Take each fully adjusted sample count (Step 11.4.2) and divide by the expected $time_0$ count (Step 11.5.3) to calculate the recovery at each sampling time:

Recovery $_{Time\ t}$ = Fully Adjusted Count $_{Time\ t}$ / $Time_0$ Count

NOTE: The recovery result that is reported from the study is determined using the time0 extrapolation of the curve fitting program

11.7 Kinetic curve fitting

The fully adjusted timed-sample counts (Step 11.4.2) for times greater than 20 hours are used for kinetic curve fitting and estimation of recovery and survival. That is, the 2-hour time sample is not used for kinetic curve fitting. These parameters may be estimated using a validated non-linear curve fitting routine found in various statistical and pharmacokinetic computer program packages. The use of COST software, one of these software packages, is described below. Optionally, the recovery of timed samples calculated in Step 11.6 may be used.

Recovery is determined as the extrapolated y-axis intercept (t=0) of the survival curve, expressed as the blood cell-bound activity in proportion to the expected activity as projected from the blood volume-based dilution

The fully adjusted count data (CPM/g) for each timed sample point are entered into the COST program as y values, the time after infusion (in hours) as x

values, and the expected time$_0$ count. (Optionally, the recovery of each timed sample from 12.6 may be entered as y values.)

11.7.1 Activate the COST program

11.7.2 Select the appropriate group in COST into which the subject's data will be entered

11.7.3 Enter the number of data points to be entered

11.7.4 Enter the x- (time in hours) and y-values (fully adjusted timed sample count, Step 11.4.2) for each timed sample. Double check entry accuracy. (Optionally, enter the y value as recoveries from Step 11.6)

11.7.5 Enter the time$_0$ count from Step 11.5.3 into counts/milliliter field. (If the individual timed recoveries from Step 11.6 are used as y values, enter 100 into the counts/milliliter field in COST to set the 100% recovery point)

11.7.6 Perform data analyses using the multiple hit model

11.7.7 If outliers are found, determine potential source of error and flag for review by medical director. Remove point from curve only if clearly a spurious result

11.7.8 Save and print results. Append to unit records

12.0 Completion of Records

12.1 Complete, review and file all forms, data, and other paperwork.