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

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STATISTICAL ANALYSIS PLAN

CLI 00183

Protocol Title:	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 Number:	CLI 00183 Version 2.0
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CONFIDENTIAL

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ABBREVIATIONS

Abbreviations list pertains to the SAP only.

AABB	Association for the Advancement of Blood & Biotherapies
AE	Adverse Event
ATP	Adenosine 5'-Triphosphate
BEST	Biomedical Excellence for Safer Transfusion
BMI	Body Mass Index
CI	Confidence Interval
CD62P	P-selectin expression on the surface of platelets
^{51}Chromium, ^{51}Cr	An isotope of chromium with a radioactive half-life of 27.7 days
Control	Fresh autologous platelets
CSR	Clinical Study Report
DPI	Days post infusion of autologous radiolabeled platelets
eCRF	Electronic Case Report Form
FDA	US Food and Drug Administration
^{111}Indium, ^{111}In	An isotope of indium with a radioactive half-life of 2.80 days
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.
ITT	Intention-to-Treat
LED	Light-emitting Diode
LV	Large Volume (one storage container)
MedDRA	Medical Dictionary for Regulatory Activities
LDH	Lactate Dehydrogenase
MPV	Mean Platelet Volume
mITT	Modified Intention-to-Treat
PC	Platelet Component
PPS	Per-Protocol Set
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
TEAE	Treatment-Emergent Adverse Event
Test	Autologous INTERCEPT platelet
WB	Whole Blood
WBC	White Blood Cell

1. INTRODUCTION

This Statistical Analysis Plan (SAP) details the statistical methods that will be implemented to analyze data collected from clinical 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]. It applies to the study protocol (version 2.0) and provides detailed instructions as to how each analysis will be performed.

Results obtained from the analyses specified in the final approved version of the SAP will become the basis of the final report for this study. Any deviations from the final approved version of the SAP must be substantiated by sound statistical reasoning and documented in the clinical study report (CSR).

2. OBJECTIVES AND STUDY DESIGN

2.1 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.

2.2 General Study Design

This is a randomized, multi-center, controlled, *in vivo* study which has 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 for non-inferiority testing.

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 apheresis collection will be processed using the INTERCEPT Blood System for Platelets with the LED Illuminator. Platelets components containing 4.0 to 5.2 × 10^{11} platelets in 300 to 390 mL of plasma will be processed using the INTERCEPT Large Volume (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 platelets components will be stored for 5 days, from day of collection, in 100% plasma.

Samples for in vitro platelet function 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 **TABLE 1** for details).
At the end of storage, an aliquot of Test platelets will be aseptically removed and randomly labeled with ^{51}Chromium (^{51}Cr) or ^{111}Indium (^{111}In) using the Variant 1 methodology. At the same time, the same healthy subjects will return to the site, and a whole blood (WB) sample will be drawn to prepare fresh Control platelets using the alternating radiolabel following the Variant 1 of BEST 4.2.1 methodology.

After radiolabeling, the autologous Control and Test platelet samples will be simultaneously infused into the subject. Blood samples will be drawn for radioactivity measurements immediately before infusion and 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) to infer the recovery and survival endpoints.

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

2.3 Sample Size Planning

A sufficient number of subjects will be enrolled to provide a minimum of 24 evaluable subjects with both paired (referred to Test and Control) recovery and paired survival data. 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.

3. ANALYSIS SETS

The analysis sets will be defined as follows:

Consented Set:
All consented subjects.

Intention-to-Treat (ITT) Set:
All subjects who initiated an apheresis collection per the "Platelet Apheresis Collection" electronic case report form (eCRF).

Modified Intention-to-Treat (mITT) Set
All subjects who have any recovery and survival data 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 survival less than Control recovery and survival and otherwise complied with the protocol without any protocol deviation potentially affecting the primary efficacy outcome.

For the criterion of "Test recovery and survival less than Control recovery and survival" in the PPS, both calculated recovery and survival values as well as the individual fitted recovery curve will be assessed to determine whether each subject is eligible for inclusion in the PPS.

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

4. SUBJECT DISPOSITION AND DEVIATION

Data will be summarized by site and treatment (Test vs. Control) as applicable. The number and percentage of subjects who completed the study and the number and percentage of subjects who were withdrawn will be presented. Subjects who discontinued prematurely will be summarized by the primary reason for withdrawal as specified in the eCRFs. Any deviations during the study will be documented in the eCRF and identified and detailed in the listings prior to database lock. Subjects with deviations may be excluded from the PPS depending on the nature of the violation. The rationale for exclusion from the PPS will be described in the CSR.

5. STUDY ENDPOINTS

5.1 Efficacy Endpoints

5.1.1 Primary Efficacy Endpoints

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

Recovery and survival of INTERCEPT (Test) components will be evaluated. Platelets will be prepared for radiolabeling using the Variant 1 of BEST 4.2.1 method. See Appendix B: Calculations and Data Handling Rules of Recovery and Survival for information and derivation details for recovery and survival.

5.1.2 Secondary Efficacy Endpoints

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

Product parameters at the end of INTERCEPT treatment:

- Platelet dose ($\geq 3.0\times10^{11}$ platelets)
- Platelet yield retention ($\geq 80\%$)

Test Product parameters at Day 5:

- $pH_{22°C}$ (≥ 6.2)

5.1.3 Additional Efficacy Endpoints

Additional analyses will include the *in vitro* function parameters of the INTERCEPT platelet component after INTERCEPT treatment and at the end of storage. Appendix A: Derivation Details contains derivation details for these endpoints.

Product Parameters at Input (Day 0/1), post-INTERCEPT (Day 1/2), and at end of storage (Day 5) (Prior to radiolabeling preparation process) (**TABLE 1**):

- Platelet count, platelet dose, component volume, and mean platelet volume (MPV).

- Biochemical assessments: pH, pO_2, pCO_2, bicarbonate (HCO_3^-), supernatant glucose, supernatant lactate, ATP, supernatant LDH, total LDH, LDH as a % of total LDH, and baseline adjusted LDH as a % of total LDH. Normalized pO_2 consumption, normalized pCO_2 production, normalized HCO_3^- production, normalized glucose consumption, normalized lactate production, normalized ATP production, and normalized supernatant LDH values will be calculated per platelet count.
- Functional assessments: CD62P (% P-selectin expression).

TABLE 1 *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 ($\times 10^3$ cells/µL)	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.

Radioactivity Measurements:

Radiolabel spontaneously dissociates (elution) from platelets both *in vivo* and *in vitro*. Therefore, elution will be calculated to correct sample radioactivity for recovery and survival calculations. Radiolabeling efficiency will serve as a quality-control metric for the radiolabeling process.

In vivo elution at 2-hour post-infusion (for ^{51}Cr and ^{111}In)

- *In vivo* elution will be calculated by dividing the supernatant dose by the combined doses of supernatant and pellet from gamma counter at 2 hours ±15 min post-infusion. Formula and details are given in Appendix A: Derivation Details.

In vitro elution

- *In vitro* elution will be calculated by using the "Elution of Radioactivity from Labeled Platelets in Whole Blood" from the BEST elution assessment method. Formula and details are given in Appendix A: Derivation Details.

Radiolabeling efficiency (for Test and Control)

- The activity of supernatant and the labeled pellet will be determined in a dose calibrator. The radiolabeling efficiency will be calculated by dividing the activity of the pellet by the combined activities of the pellet and supernatant. Formula and details are given in Appendix A: Derivation Details.

Platelet Yield Retention and Volume Recovery

- Platelet yield retention will be determined at post-INTERCEPT (Day 1/2) and at end of storage (Day 5) compared to the Input dose by dividing the post-INTERCEPT or end of storage dose by the Input dose. The platelet yield retention post INTERCEPT treatment will be determined and assessed if meets ≥ 80% criteria.
- Volume recovery will be determined at post-INTERCEPT (Day 1/2) compared to the Input volume by dividing the post-INTERCEPT by the Input volume.

Formulas and details are given in Appendix A: Derivation Details.

5.1.4 Methods and Timing of Efficacy Endpoints

5.1.4.1 Radioactivity Measurements and Recovery and Survival Estimation

Samples will be obtained from the radiolabeled fresh 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. Blood samples will be drawn immediately before infusion and for radioactivity measurements at 2 hours ± 15 min post-infusion (0 DPI), and 6 more samples will be drawn at 1 (±4 hours), 2, 3, 5±1, 7/8, and 11±1 DPI. The radioactivity of the samples will be determined by using 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. Radioactivity of the standards, elution measurements, pre-infusion, and post-infusion blood samples will be corrected for radioactive decay if the counts for each subject are taken at different times. Duplicates of the subject's WB samples for radioactivity will be measured for radioactivity while singletons of all other samples will be evaluated.

The post-infusion recovery and survival data from 5-day Test 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 DPI and compared with its paired Control descriptively. Post-infusion blood samples will be corrected for plasma associated radioisotope and the spontaneous *in vitro* dissociation (elution) of radioisotopes using the BEST elution assessment method. Moreover, the corrected count samples will be adjusted for cell-bound proportion and baseline counts. Then, the fully adjusted timed-sample counts for times greater than 20 hours are used for kinetic curve fitting and estimation of recovery and survival. A non-linear curve fitting model, with the multiple-hit algorithm, will be used to estimate platelet recovery and lifespan based on the processed (fully adjusted) ^{51}Cr and ^{111}In counts, respectively. Additional models might be explored as suggested by the data. Appendix B: Calculations and Data Handling Rules of Recovery and Survival contains more detailed information about the quantitative formulas and calculations of recovery and survival.

5.1.4.2 *In Vitro* Platelet Assessments

Samples for analysis of pH need to be analyzed within 30 minutes of preparation. The remaining indices listed in **TABLE 1** should be tested or prepared for testing following site standard operating procedures (SOP). 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. Specific assays and equipment for the assessment of the *in vitro* parameters will be detailed in the clinical study report.

5.2 Safety Endpoints

Safety will be assessed through monitoring of adverse events (AEs), blood donor physical examinations (including vital signs), and laboratory tests. Safety endpoints are listed below:

- AEs
- Vital signs
- Hematological profile
- Serum chemistry profile

6. STATISTICAL ANALYSIS AND CONSIDERATIONS

6.1 General Analysis Consideration

Unless stated otherwise, data will be summarized and tabulated for Test and Control. In addition, a summary will be presented by site (and with both sites combined).

For categorical measurements, summaries will be presented using counts and proportions. For continuous measurements, summaries will be presented using descriptive statistics including the mean, standard deviation (SD), median, minimum, maximum, and confidence interval (CI) for the mean. Individual data points will be presented in data listings. Due to the nature of CRF, if the reported value for a continuous variable is below the limit of quantitation, the limit of quantitation number will be used in generating descriptive statistics in tables and will be reported with the "<" in listings.

Data is read directly as input and used for calculation; rounding will occur at the final step of calculation for each summarized variable.

For the following assessment criteria, the comparison will be made after rounding the calculated value to the same precision as the threshold value:

- For platelet post-infusion recovery, Test ≥ 0.66 × Control (e.g., a ratio of 0.6551 for Test/Control will be rounded to 0.66 and considered as meeting this criterion)
- For platelet survival, Test ≥ 0.58 × Control (e.g., a ratio of 0.5792 for Test/Control will be rounded to 0.58 and considered as meeting this criterion)
- Platelet dose ≥ 3.0×10^{11} cells/unit (e.g., 2.951×10^{11} will be rounded to 3.0×10^{11})
- Platelet yield retention ≥ 80% (e.g., 79.51% will be rounded to 80%)
- pH ≥ 6.2 (e.g., 6.151 will be rounded to 6.2)

All statistical analyses will be performed using SAS® version 9.4 (or higher) or R version 4.5.1 (or higher).

6.1.1 Subject Disposition

Subject disposition will be summarized by site (and with both sites combined). Numbers of subjects terminating the study early and their reasons for early termination will be summarized.

6.1.2 Demographics, Baseline, and Clinical Measurements

The following demographic and clinical variables collected for the study will be summarized by site (and with both sites combined):

- Demographic variables: Age (at enrollment), Sex at Birth, Ethnicity, Race
- Baseline characteristics (Vital Signs at Screening): Weight, Height, BMI
- Clinical measurements: Blood Type, Rh Factor

6.1.3 Substance Use

Substance use (including nicotine use or cannabis use) will be summarized descriptively.

6.2 Efficacy Analyses

6.2.1 Analysis of Primary Endpoints

The primary endpoints are platelet post-infusion recovery and survival at end of storage. Subjects who do not have both paired recovery and paired survival data may be replaced to ensure a sufficient number of evaluable subjects is reached for the primary analysis. Reasons for absence of primary endpoint measurement(s) for any subject will be listed in the CSR.

The post-infusion recovery and survival data will be calculated based on the corrected radioactive counts observed from the six post-infusion sampling time points at 1 (within ±4 hours from time of infusion), 2, 3, 5±1, 7/8 and 11±1 DPI. The following acceptance criteria for recovery and survival will be used to demonstrate non-inferiority of Test (INTERCEPT with LED Illuminator treated and stored) platelets against Control (fresh) platelets:

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

The primary efficacy endpoints will be analyzed on PPS and mITT set (for sensitivity analysis). The two-sided 95% CIs will be constructed based on the following formula:

$$95\%\ CI = \bar{d} \pm t_{0.025}\frac{s_d}{\sqrt{n}},$$

where

$\bar{d}$ = mean treatment difference:

Test – 0.66×Control for post-infusion recovery or Test – 0.58×Control for survival,

s_d = the sample standard deviation of the treatment differences,

$n =$ number of subjects in PPS or mITT set,

$t_{0.025}$ = the *t*-score in the Student's *t*-distribution with the degrees of freedom of n-1.

If non-inferiority cannot be declared for the primary endpoints, 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 (PC) greater than or equal to 66% of platelet recovery from its paired fresh Control PC). Observed recovery will be plotted with a fitted non-linear curve from the multi-hit model for visualization.

6.2.2 Analysis of Secondary Endpoints

Data will be summarized descriptively. The proportions of

(1) Test PCs with platelet dose ≥ 3.0×10^{11} cells/unit (end of INTERCEPT treatment),
(2) Test PCs with platelet yield retention ≥ 80% (end of INTERCEPT treatment), and
(3) Test PCs with pH at 22°C ≥ 6.2 (end of storage)

will be summarized, with the corresponding lower bound of a one-sided 95% Clopper-Pearson CI for the proportion provided. Correlations between the primary and secondary efficacy endpoints may be explored using correlation plots or linear regression analysis.

6.2.3 Analysis of Additional Efficacy Endpoints

Descriptive summary, including mean, SD, median, minimum, maximum, and 95% CI of mean will be provided for *in vitro* function parameters of the stored INTERCEPT PCs. In addition, the radiolabeling efficiency will be summarized descriptively. Correlations between the post-infusion recovery or survival and additional efficacy endpoints on Day 5 may be explored using correlation plots.

6.3 Exploratory Analysis

Mixed models will be used to assess whether there is a site or radiolabeling sequence effect in recovery or survival.

6.4 Subgroup Analysis

Primary endpoints will be explored by the site and radiolabeling sequence. Additional subgroups may be explored as suggested by the data.

6.5 Safety Analyses

Treatment-emergent AEs (TEAEs; defined as AEs with onset at or after the initiation of apheresis platelet collection) and serious adverse events (SAEs) will be summarized descriptively. Other safety data including vital signs, hematological profile, and serum chemistry profile will be provided in the data listings. The observed results and change from pre-infusion for vital signs, and hematology and chemistry parameters will be summarized descriptively by DPI.

Vital signs are collected at screening, and prior to apheresis collection (Day 0), on the day of infusion (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. All verbatim AE terms detailed in the CRF will be mapped to their preferred terms and system organ class (SOC) using MedDRA.

Blood samples for hematology and chemistry panels will be obtained from each subject at screening, prior to apheresis platelet collection (Day 0), prior to platelet infusion, and at the end of the study period (11±1 DPI). Clinically significant laboratory findings will be recorded as AEs. The subject laboratory assessments are identified as follows.

Hematology: Hematocrit, Hemoglobin, Red Blood Cell (RBC) Count, Platelet Count, and White Blood Cell (WBC) Count (with differential).

Serum Chemistry: Blood Urea Nitrogen, Calcium, Carbon Dioxide, Chloride, Creatinine, Glucose, Potassium, and Sodium.

7. HANDLING MISSING DATA

Missing data will be noted as such and not be imputed.

8. CHANGES TO PROTOCOL SPECIFIED ANALYSES

The following changes to the data analysis pre-specified in the protocol are made after the study started.

1) For duplicated data at a specific visit, only the one with a later collection or assessment date will be analyzed and summarized. This detailed information was not stated in the protocol.

2) In the process of calculating the recovery and survival, the elution of radioactivity from labeled platelets in whole blood and the fully adjusted count at time "t" post-infusion will be derived using the average of the results from the duplicate blood samples. This detailed information was not stated in the protocol.

3) For any data collected on unscheduled visits, it will not be reported in the Tables where only values from scheduled visits are included. However, it will be reported in the Listings.

4) For the inclusion criterion "Test recovery and survival less than Control recovery and survival" for PPS, individual fitted recovery curve will also be evaluated with the calculated recovery and survival results. This detailed information was not stated in the protocol.

9. REFERENCES

1. FDA Draft Guidance for Industry, Draft Guidance for Industry For Platelet Testing and Evaluation of Platelet Substitute Products. 1999
2. Gahmberg CG, Jokinen M, Andersson LC. Expression of the major sialoglycoprotein (glycophorin) on erythroid cells in human bone marrow. Blood. 1978;52:379-387.
3. Reid ME. MNS blood group system: a review. Immunohematology. 2009;25(3):95-101.
4. The Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Platelet radiolabeling procedure. Transfusion 2006;46 Suppl:59S-66S.

10. APPENDICES

Appendix A: Derivation Details

ATP Normalized for Platelet Count

ATP will be measured and reported in µmol/dL.

Normalized ATP will be calculated using the following formula:

$$\text{ATP (nmol/}10^8\text{ platelets)} = \frac{\text{ATP (µmol/dL)}}{\text{Platelet Count (} \times 10^3\text{ platelets/µL)}} \times 10^3 .$$

Body Mass Index (BMI)

$$\text{BMI} = \frac{\text{Weight (kg)}}{[\text{Height (cm)}/100]^2}$$

The weight and height at Screening will be used for the BMI calculation.

Component Volume and Platelet Dose

The component volume and platelet dose will be calculated using the following formulas:

$$\text{Component Volume (mL)} = \frac{\text{Component Gross Weight (g)} - \text{Tare Weight (g)}}{1.03\text{ g/mL}}$$

$$\text{Platelet Dose } (\times 10^{11}\text{platelets/unit}) = \frac{\text{Component Volume (mL)} \times \text{Platelet Count } (\times 10^3\text{ platelets/µL})}{10^5} ,$$

where Component Gross Weight = Final Input Gross weight or Pre-sampling gross weight at the post-INTERCEPT and End of storage; Tare Weight = Empty container weight

Unless otherwise noted, the gross weight (pre-sampling weight if available) and platelet count collected at each timepoint will be used to derive the timepoint specific volume and dose. The tare weights (Wt) will be set to the following values:

Input (Day 0):

- Tare Wt = 39g

Post-INTERCEPT (Day 1/2):

- Tare Wt = 40g

Post-INTERCEPT Storage Volume (Day 1/2) and End of Storage (Day 5):

- Tare Wt = 40g

End of storage volume will be also corrected for sampling taken during the storage process including the sampling for the post-INTERCEPT sample and bacterial culture sample. The corrected volume will be used to calculate the platelet dose at end of storage. The corrected weight will be used to calculate the corrected volume. Sample weights will be calculated by the following formulas:

$$\text{End of storage volume Corrected (mL)} = \frac{\text{Pre-Sampling Component Gross Weight (g)} - \text{Tare Weight (g)}}{1.03\ \text{g/mL}} + \frac{\text{Post INTERCEPT sample weight}}{1.03\ \text{g/mL}} + \frac{\text{BacT sample weight}}{1.03\ \text{g/mL}}$$

- Post-INTERCEPT sample weight (g) =
  Storage Container Pre-Sampling Gross Weight (g) – Storage Container Post-Sampling Gross Weight after Sampling for Post-INTERCEPT Processing (g)
- BacT sample weight (g) =
  Gross weight of platelet unit pre-sampling (g) – Gross weight of platelet unit post-sampling (g)

CPM Adjusted for Decay

CPM will be adjusted when radioactivity samples for Recovery and Survival calculations—specifically standards, elution measurements, and pre-infusion and post-infusion blood samples—are not analyzed by the gamma counter on the same day. The earliest gamma counter run time will be identified, and the duration between this earliest run time and run time of the specific CPM sample (if it does not coincide with the earliest run time run time) will be calculated to adjust for decay.

For Chromium:

CPM Adjusted for Decay = CPM × exp (0.0250 × t)

where t = Duration in days = CPM sample date/time start on gamma counter – earliest date/time start on gamma counter.

For Indium:

CPM Adjusted for Decay = CPM × exp (0.24714 × t)

where t = Duration in days = CPM sample date/time start on gamma counter – earliest date/time start on gamma counter.

Platelet Yield Retention and Volume Recovery

Volume recovery will be determined at post-INTERCEPT (Day 1/2), and platelet yield retention will be determined at post-INTERCEPT (Day 1/2) and end of storage (Day 5), and will be calculated as follows:

- Platelet Yield Retention at Post-INTERCEPT (%) = Post-INTERCEPT Platelet Dose / Input Platelet Dose × 100
- Platelet Yield Retention at End of Storage (%) = End of Storage Platelet Dose / Input Platelet Dose × 100
- Volume Recovery at Post-INTERCEPT (%) = Post-INTERCEPT Volume / Input Volume × 100

Platelet yield retention at end of storage will be calculated based on gross weights that are corrected for sampling.

Glucose and Lactate Conversion of Units

If glucose or lactate is measured in mg/dL, then it will be converted to mmol/L using the following formula:

$$\text{Glucose (mmol/L)} = \text{Glucose (mg/dL)} \div 18$$
$$\text{Lactate (mmol/L)} = \text{Lactate (mg/dL)} \div 9$$

Glucose and Lactate Normalized for Platelet Count

Glucose and lactate will be measured in mmol/L (or converted to mmol/L).

On all collection days, glucose and lactate will be normalized to platelet count by converting from mmol/L to mmol/10^{12} platelets using the following formula:

$$\text{Glucose or Lactate (mmol/}10^{12}\text{ platelets)} = \frac{\text{Glucose or Lactate (mmol/L)}}{\text{Platelet Count (} \times 10^3 \text{ platelets/}\mu\text{L)}} \times 10^3 .$$

HCO_3^- Normalized for Platelet Count

HCO_3-will be measured in mmol/L.

On collection days, HCO_3- will be normalized to platelet count by converting from mmol/L to mmol/10^{12} platelets using the following formula:

$$HCO_3^- \text{ (mmol/}10^{12}\text{ platelets)} = \frac{HCO_3^- \text{ (mmol/L)}}{\text{Platelet Count (} \times 10^3 \text{ platelets/}\mu\text{L)}} \times 10^3$$

In Vivo Elution at 2-hour Post-Infusion

In vivo elution will be determined using the blood draw for radioactivity at 2 hours ± 15 min. The *in vivo* elution will be calculated using the following formula:

$$In\ Vivo\ \text{Elution}\ (\%)\ =\ \frac{\text{2hr plasma CMP/g}}{(\text{2hr plasma CPM/g} + \text{2hr Packed Cells CPM/g})} \times 100$$

In Vitro Elution

In vitro elution will be determined using the "Elution of Radioactivity from Labeled Platelets in Whole Blood" from the BEST method where 10 µL of the mixed injectate will be mixed into a 5 or 7 mL EDTA tube of blood, incubated for 2 hours at 37ºC in a wet incubator, spun to separate the plasma and packed cells and counted on the gamma counter. The *in vitro* elution will be calculated using the following formula:

$$In\ Vitro\ \text{Elution}\ (\%)\ =\ \frac{\text{Elution Plasma CMP/g}}{(\text{Elution Plasma CPM/g} + \text{Elution Packed Cells CPM/g})} \times 100$$

MPV

Depending on the hematology analyzer, MPV will be measured in fL or ×femto m^3. These units are interchangeable. MPV will be summarized in fL in the tables.

pH Temperature Conversion

Any pH measurement taken at a temperature outside of the range of 19°C to 25°C will be corrected to $pH_{22°C}$, calculated according to the CLSI (formerly NCCLS) Patient Temperature Correction, as follows:

$$pH_{22°C} = pH_{X°C} + (X - 22) \times 0.015$$

where X is the degrees of temperature in Celsius.

Platelet Count Units

Depending on the hematology analyzer, platelet counts will be measured in $\times 10^3$ platelets/µL or $\times 10^6$ platelets/mL. Platelet counts will be summarized in $\times 10^3$ platelets/µL in the tables.

Platelet Dose

Platelet dose will be derived using the following formulas across all timepoints:

$$\text{Dose}\ (\times 10^{11}\ \text{platelets/unit}) = \frac{\text{Volume (mL)}\ \times\ \text{Platelet Count}\ (\times 10^3\ \text{platelets/µL})}{10^5}$$

The volumes calculated from the corrected gross weight are used.

pO_2 and pCO_2 Normalized for Platelet Count

pO_2 and pCO_2 will be measured in mmHg.

On all collection days, pO_2 and pCO_2 will be normalized to platelet count by converting from mmHg to µmol/hrs/10^{12} platelets by taking advantage of the ideal gas law where:

$$pO_2 \text{ and } pCO_2 \text{ (atm)} = \frac{\text{Moles (mol) x Gas Contstant } ([\text{L} \cdot \text{atm}]/[\text{mol} \cdot \text{K}]) \text{ x Temperature (K)}}{\text{Volume (L)}}$$

By reorganizing terms, pO_2 and pCO_2 values will be initially converted into molarity values (mol/L) prior to normalization. Thus:

$$\text{Molarity (mol/L)} = \frac{\text{Moles (mol)}}{\text{Volume (L)}} = \frac{pO_2 \text{ and } pCO_2\text{(atm)}}{\text{Gas Contstant } ([\text{L} \cdot \text{atm}]/[\text{mol} \cdot \text{K}]) \times \text{Temperature (K)}}$$

By using the following list of constants and conversions:

Gas Constant ([L·atm]/[mol·k]) = 0.08206 ([L·atm]/[mol·k])

Temperature (K) = 310.15

Moles (µmol) = Micromoles (mol) × 10^6

pO_2 and pCO_2 (atm) = pO_2 and pCO_2 (mmHg) ÷ 760,

the molarity can be re-written in terms of µmol/L, and furthermore, the pO_2 and pCO_2 values can also be expressed in mmHg units as collected in the study:

$$\text{Molarity (mol/L)} = \frac{[pO_2 \text{ and } pCO_2\text{(mmHg)}] \div 760}{\text{Gas Contstant } ([\text{L} \cdot \text{atm}]/[\text{mol} \cdot \text{K}]) \times \text{Temperature (K)}} \times 10^6$$

$$= \frac{[pO_2 \text{ and } pCO_2\text{(mmHg)}] \div 760}{0.08206 \times 310.15} \times 10^6$$

Once the molarity is calculated, the final portion of the normalization will be carried out as follows:

$$pO_2 \text{ and } pCO_2 \text{ (µmol/hrs/}10^{12}\text{ platelets)} = \frac{\text{Molarity (µmol/L)}}{\text{Time (Hrs)} \times \text{Platelet Count } (\times 10^3 \text{ platelets/µ}L)} \times 10^3,$$

where Time (hrs) will be set to 120 hrs (or 5 days) for Day 5 normalizations.

Radiolabeling Efficiency

Radiolabeling efficiency will be calculated using the dosimeter readings from the platelet pellet and the supernatant from the radiolabeling process for both the Test and the Control using the following equation:

$$\text{Radiolabeling Efficiency (\%)} = \frac{\text{Pellet Dose}}{(\text{Pellet Dose} + \text{Supernatant Dose})} \times 100$$

Supernatant LDH Normalized for Platelet Count

Supernatant LDH activity will be measured in U/L.

Supernatant LDH will be normalized to platelet count by converting from U/L to U/10^{12} platelets using the following formula:

$$\text{Supernatant LDH (U/}10^{12}\text{ platelets)} = \frac{\text{Supernatant LDH (U/L)}}{\text{Platelet Count (} \times 10^3 \text{ platelets/}\mu\text{L)}} \times 10^3.$$

Total LDH Dilution Correction

Total LDH from the database will be corrected first for sample dilution by the following formula:

Total LDH Dilution (U/L, Dilution Correction) = Total LDH Triton Lysate × 8

LDH as a % of Total LDH and Baseline Adjusted LDH as a % of Total LDH

On all collection days, the LDH as a % of Total LDH (%) and baseline adjusted LDH as a % of Total LDH will be calculated using the following formulas:

$$\text{LDH as a \% of Total LDH (\%)} = \text{Supernatant LDH (U/L)} \div \text{Total LDH (U/L)} \times 10^2$$

$$\text{Day } \mathbf{X} \text{ Baseline Adjusted LDH as a \% of Total LDH (\%)} = \frac{\text{Day } \mathbf{X} \text{ Supernatant LDH (U/L)} - \text{Day } \mathbf{0} \text{ Supernatant LDH (U/L)}}{\text{Day } \mathbf{X} \text{ Total LDH (U/L)}} \times 10^2$$

X indicates the specific time point for assessment: 1/2 for post-INTERCEPT, or 5 for end of storage. If baseline adjusted LDH is negative, it will be converted to 0.

Appendix B: Calculations and Data Handling Rules of Recovery and Survival

Objective: To describe calculations for post-infusion platelet recovery and survival at end of storage (Day 5) for radioisotopic labeling and infusion of stored platelets with ^{111}In Oxine (^{111}Indium) and $Na_2{}^{51}Cr\,O_4$ (^{51}Chromium).

Data Sources: Raw Data is collected at American Red Cross Research Laboratory and Bloodworks Northwest Research Institute. Fresh platelets and stored platelets will be radiolabeled using ^{51}Cr (Chromium) or ^{111}In (Indium). 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 (±4 hours from infusion time), 2, 3, 5±1, 7/8, and 11±1 days post-infusion (DPI). Duplicates of the subject's whole blood (WB) will be measured for radioactivity while singletons of all other samples will be evaluated. The following data information will be included in the calculation:

- Radiolabeled platelet infusion date/time
- Weight of syringe to be used for infusion including pre-infusion weight of filled syringe and post-infusion weight
- Weight of syringe in standard flasks (total of three flasks) including pre-infusion weight of filled syringe and post-infusion weight
- Mixed standard counted for weight and counts per minute (CPM) including pre-infusion values for empty tube and post-infusion values for filled tube
- Sample weights and CPMs with corresponding collection date/time for the radioactivity of the samples, determined by use of a gamma counter
- Individual sex and weight, height, and hematocrit values recorded at Screening
- Solution volumes and densities of standard flasks

Methods: The post-infusion recovery and survival of INTERCEPT components will be calculated based on the corrected radioactive counts observed from samples at 1 (±4 hours from infusion time), 2, 3, 5±1, 7/8, and 11 (±1) days post infusion. The post-infusion blood samples will be corrected for plasma associated radioisotope and the spontaneous *in vitro* and *vivo* dissociation (elution) of radiolabels using the Biomedical Excellence for Safer Transfusion (BEST) assessment method as described in the Variant 1 of BEST version 4.2.1 method. A linear regression fitting model and a validated non-linear curve fitting model, the multiple-hit algorithm (from the COST program), will be used to estimate platelet recovery and lifespan based on the processed (fully adjusted) ^{51}Cr and ^{111}In counts, respectively.

Steps for calculations:

Step 1: Calculate decay adjusted CPM that was not measured at the earliest Gamma counter run time and determined corrected CPM/g ($CPM_{corrected}$) of whole blood for each duplicate sample taken and measured for radioactivity.

Because collection of gamma counter data can happen at different time points for mixed standard, elution of radioactivity from labeled platelets in whole blood, whole blood samples, and packed cells and plasma, the earliest

gamma counter time will be identified and any CPM that was measured later than that will be adjusted to the decay adjusted CPM, which will be used to determine the corrected CPM.

Adjusted CPM_StdCnt1Cr = exp(0.025*difft_m) * CPM_StdCnt1Cr
Adjusted CPM_StdCnt2Cr = exp(0.025*difft_m) * CPM_StdCnt2Cr
Adjusted CPM_StdCnt3Cr = exp(0.025*difft_m) * CPM_StdCnt3Cr
Adjusted CPM_StdCnt1In = exp(0.24714*difft_m) * CPM_StdCnt1In
Adjusted CPM_StdCnt2In = exp(0. 24714*difft_m) * CPM_StdCnt2In
Adjusted CPM_StdCnt3In = exp(0. 24714*difft_m) * CPM_StdCnt3In

Where difft_m is the day difference (with decimal points accurate to minute) between the gamma counter run time for mixed standard counted and the earliest gamma counter run time.

Adjusted CPM_EluRBCCrA = exp(0.025*difft_b) * CPM_EluRBCCrA
Adjusted CPM_EluRBCCrB = exp(0.025*difft_b) * CPM_EluRBCCrB
Adjusted CPM_EluPLSCrA = exp(0.025*difft_b) * CPM_EluPLSCrA
Adjusted CPM_EluPLSCrB = exp(0.025*difft_b) * CPM_EluPLSCrB
Adjusted CPM_EluRBCInA = exp(0.24714*difft_b) * CPM_EluRBCInA
Adjusted CPM_EluRBCInB = exp(0. 24714*difft_b) * CPM_EluRBCInB
Adjusted CPM_EluPLSInA = exp(0. 24714*difft_b) * CPM_EluPLSInA
Adjusted CPM_EluPLSInB = exp(0. 24714*difft_b) * CPM_EluPLSInB

Where difft_b is the day difference (with decimal points accurate to minute) between the gamma counter run time for elution of radioactivity from labeled platelets in whole blood and the earliest gamma counter run time.

Adjusted CPM_PreInfCra = exp(0.025*difft_pi) * CPM_PreInfCra
Adjusted CPM_PreInfCrb = exp(0.025*difft_pi) * CPM_PreInfCrb
Adjusted CPM_PreInfIna = exp(0.24714*difft_pi) * CPM_PreInfIna
Adjusted CPM_PreInfInb = exp(0.24714*difft_pi) * CPM_PreInfInb

Where difft_pi is the day difference (with decimal points accurate to minute) between the gamma counter run time for whole blood samples at pre-infusion and the earliest gamma counter run time.

Adjusted CPM_CrWBa = exp(0.025*difft) * CPM_CrWBa
Adjusted CPM_CrWBb = exp(0.025*difft) * CPM_CrWBb
Adjusted CPM_InWBa = exp(0.24714*difft) * CPM_InWBa
Adjusted CPM_InWBb = exp(0.24714*difft) * CPM_InWBb
Adjusted CPM_CrRBC = exp(0.025*diff2t) * CPM_CrRBC
Adjusted CPM_CrPLS = exp(0.025*diff2t) * CPM_CrPLS
Adjusted CPM_InRBC = exp(0.24714*diff2t) * CPM_InRBC
Adjusted CPM_InPLS = exp(0.24714*diff2t) * CPM_InPLS

Where difft is the day difference (with decimal points accurate to minute) between the gamma counter run time for the whole blood sample from each sampling day's collection post infusion and the earliest gamma counter run time; diff2t is the day difference (with decimal points accurate to minute) between the gamma counter run time for the packed cells and plasma from each sampling day's collection post infusion and the earliest gamma counter run time.

The CPM variables used in the following steps refer to the CPM values that have been adjusted for the decay.

Formula for $CPM_{corrected}$:

$CPM_{corrected}$ (CPM/g) = CPM / WB Weight

Actual Derivation with respect to ^{51}Cr and ^{111}In:

Post-infusion $CPM_{corrected}$ at Time t:
c_CPM_WBa = CPM_CrWBa / Wt_WBa;
c_CPM_WBb = CPM_CrWBb / Wt_WBb;
i_CPM_WBa = CPM_InWBa / Wt_WBa;
i_CPM_WBb = CPM_InWBb / Wt_WBb;

Time t is the elapsed time in hours from the time of infusion. If there is only one WB sample collected, either sample A or B, that CPM and WB weight will be used as the values for both sample A and B for following calculations.

WB weight (Wt_WB) is calculated by "post-weight, filled tube" – "pre-weight, empty tube".

The average from two WB samples will be used to determine pre-infusion $CPM_{corrected}$:
c_Pa = CPM_PreInfCra / (Wt_PreInf_WBaPos - Wt_PreInf_WBaPre) ;
i_Pa = CPM_PreInfIna / (Wt_PreInf_WBaPos - Wt_PreInf_WBaPre) ;
c_Pb = CPM_PreInfCrb / (Wt_PreInf_WBbPos - Wt_PreInf_WBbPre) ;
i_Pb = CPM_PreInfInb / (Wt_PreInf_WBbPos - Wt_PreInf_WBbPre) ;
c_P = (c_Pa + c_Pb) / 2;
i_P = (i_Pa + i_Pb) / 2;
if c_P < 0 then c_P = 0;
if i_P < 0 then i_P = 0;

Step 2: Determine Elution of Radioactivity from Labeled Platelets in Whole Blood.

The activity found in the cells and supernatant plasma will be used to evaluate the *in vitro* cell-bound activity (elution) of the labeled platelets for each of the two radiolabels.

Formula:

Elution = (Elution CPM_{Plasma}) / [(Elution $CPM_{Packed\ Cells}$) + (Elution CPM_{Plasma})]

Actual Derivation with respect to ^{51}Cr and ^{111}In:

$$c\_EA = \frac{CPM\_EluPLSCrA/abs(Wt\_EluPLSPosA - Wt\_EluPLSPreA)}{CPM\_EluPLSCrA/abs(Wt\_EluPLSPosA - Wt\_EluPLSPreA) + CPM\_EluRBCCrA/abs(Wt\_EluRBCPosA - Wt\_EluRBCPreA)};$$

$$c\_EB = \frac{CPM\_EluPLSCrB/abs(Wt\_EluPLSPosB - Wt\_EluPLSPreB)}{CPM\_EluPLSCrB/abs(Wt\_EluPLSPosB - Wt\_EluPLSPreB) + CPM\_EluRBCCrB/abs(Wt\_EluRBCPosB - Wt\_EluRBCPreB)};$$

$$c\_E = \frac{c\_EA + c\_EB}{2};$$

$$i\_EA = \frac{CPM\_EluPLSInA/abs(Wt\_EluPLSPosA - Wt\_EluPLSPreA)}{CPM\_EluPLSInA/abs(Wt\_EluPLSPosA - Wt\_EluPLSPreA) + CPM\_EluRBCInA/abs(Wt\_EluRBCPosA - Wt\_EluRBCPreA)};$$

$$i\_EB = \frac{CPM\_EluPLSInB/abs(Wt\_EluPLSPosB - Wt\_EluPLSPreB)}{CPM\_EluPLSInB/abs(Wt\_EluPLSPosB - Wt\_EluPLSPreB) + CPM\_EluRBCInB/abs(Wt\_EluRBCPosB - Wt\_EluRBCPreB)};$$

$$i\_E = \frac{i\_EA + i\_EB}{2};$$

Note: Averaged activity found in the packed cells and plasma (elution) from two duplicate samples will be used.

Step 3: Determine Corrected Standard.

All three standards are used and averaged to determine corrected Standard adjusted for injectate elution. The gamma counters correct automatically for background activity and decay during counting as well as for "cross-up" and "cross-down". Corrected CPM values reported by the gamma counter ($CPM_{corrected}$), after adjusting for decay as described in Step 1, determine CPM/g for standards.

Formula:

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

Corrected standard: $STD_{corrected}$ = STD * (1 – Elution)

Actual Derivation with respect to ^{51}Cr and ^{111}In:

c_S = {[CPM_StdCnt1Cr*StdSolnVol*Density / abs(Wt_SyrStd1Pos - Wt_SyrStd1Pre)] / Wt_StdCnt1 + [CPM_StdCnt2Cr* StdSolnVol*Density / abs(Wt_SyrStd2Pos - Wt_SyrStd2Pre)] / Wt_StdCnt2 + [CPM_StdCnt3Cr* StdSolnVol*Density / abs(Wt_SyrStd3Pos - Wt_SyrStd3Pre)] / Wt_StdCnt3} / 3 * (1- c_E);

i_S = {[CPM_StdCnt1In*StdSolnVol*Density / abs(Wt_SyrStd1Pos - Wt_SyrStd1Pre)] / Wt_StdCnt1 + [CPM_StdCnt2In*StdSolnVol*Density / abs(Wt_SyrStd2Pos - Wt_SyrStd2Pre)] / Wt_StdCnt2 + [CPM_StdCnt3In*StdSolnVol*Density / abs(Wt_SyrStd3Pos - Wt_SyrStd3Pre)] / Wt_StdCnt3} / 3 * (1- i_E)

Note: The density 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. StdSolnVol is 100. The average from three mixed standards will be used for the derivation of the corrected standard.

Step 4: Determine cell-bound proportion ($CBF_{Time\ t}$) for timed count adjustment, using timed split sample to calculate the proportion of the activity in each timed sample that can be associated with the cellular fraction.

Formula:

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

Actual Derivation with respect to ^{51}Cr and ^{111}In:

if CPM_CrRBC~=0 then c_$CBF_{Time\ t}$ = [CPM_CrRBC / abs(Wt_RBCPos - Wt_RBCPre)] /
[CPM_CrRBC/abs(Wt_RBCPos - Wt_RBCPre) + CPM_CrPLS/Wt_PLS)];
if CPM_CrRBC=0 then c_$CBF_{Time\ t}$ = 0;
if CPM_InRBC~=0 then i_$CBF_{Time\ t}$ = [CPM_InRBC / abs(Wt_RBCPos - Wt_RBCPre)] /
[CPM_InRBC/abs(Wt_RBCPos - Wt_RBCPre) + CPM_InPLS/Wt_PLS];
if CPM_InRBC=0 then i_$CBF_{Time\ t}$ = 0;

Step 5: Determine adjusted CPM of Whole Blood by Pre-infusion corrected CPM and Cell Bound Fraction.

Formula:
Adjusted $CPM_{corrected}$ at Time t = ($CPM_{corrected}$ at Time t – Pre-infusion $CPM_{corrected}$) × $CBF_{Time\ t}$.

Actual Derivation with respect to ^{51}Cr and ^{111}In:

c_CPM_WBaT = (c_CPM_WBa - c_P) * c_$CBF_{Time\ t}$;
c_CPM_WBbT = (c_CPM_WBb - c_P) * c_$CBF_{Time\ t}$;
i_CPM_WBaT = (i_CPM_WBa - i_P) * i_$CBF_{Time\ t}$;
i_CPM_WBbT = (i_CPM_WBb - i_P) * i_$CBF_{Time\ t}$;

Step 6: Calculate Fully Adjusted Count $_{Time\ t}$ at Time t post infusion with adjustment for cell-bound proportion (from Step 5) and non-platelet residual activity baseline.

Formula:

Adjusted SAMPLE $_{Time\ t}$ (derived from Step 5) with further adjustment for non-platelet residual activity baseline

If last time point is on Day 10,

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

If last time point is on Day 11,

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

If last time point is on Day 12,

Fully adjusted count = adjusted SAMPLE $_{\text{Time t}}$ – [adjusted SAMPLE $_{\text{Day 12}}$ * (1.24 – 0.24 * (Time t/Time of Day 12 sample)]

The adjusted sample at the last day is from average of the results from the dual blood samples. Any negative fully adjusted counts will be converted to 0 due to the inherent nature of the definition.

Actual Derivation with respect to ^{51}Cr and ^{111}In:

```
if 4<=Time_NominalL<=10 then do;
    c_CPM_WBaTr = c_CPM_WBaT - 0.5 * (c_CPM_WBaTL + c_CPM_WBbTL) * (1.20-0.20*Time/TimeL);
    c_CPM_WBbTr = c_CPM_WBbT - 0.5 * (c_CPM_WBaTL + c_CPM_WBbTL) * (1.20-0.20*Time/TimeL);
    i_CPM_WBaTr = i_CPM_WBaT - 0.5 * (i_CPM_WBaTL + i_CPM_WBbTL) * (1.20-0.20*Time/TimeL);
    i_CPM_WBbTr = i_CPM_WBbT - 0.5 * (i_CPM_WBaTL + i_CPM_WBbTL) * (1.20-0.20*Time/TimeL);
    end;
else if Time_NominalL=11 then do;
    c_CPM_WBaTr = c_CPM_WBaT - 0.5 * (c_CPM_WBaTL + c_CPM_WBbTL) * (1.22-0.22*Time/TimeL);
    c_CPM_WBbTr = c_CPM_WBbT - 0.5 * (c_CPM_WBaTL + c_CPM_WBbTL) * (1.22-0.22*Time/TimeL);
    i_CPM_WBaTr = i_CPM_WBaT - 0.5 * (i_CPM_WBaTL + i_CPM_WBbTL) * (1.22-0.22*Time/TimeL);
    i_CPM_WBbTr = i_CPM_WBbT - 0.5 * (i_CPM_WBaTL + i_CPM_WBbTL) * (1.22-0.22*Time/TimeL);
    end;
else do;
    c_CPM_WBaTr = c_CPM_WBaT - 0.5 * (c_CPM_WBaTL + c_CPM_WBbTL) * (1.24-0.24*Time/TimeL);
    c_CPM_WBbTr = c_CPM_WBbT - 0.5 * (c_CPM_WBaTL + c_CPM_WBbTL) * (1.24-0.24*Time/TimeL);
    i_CPM_WBaTr = i_CPM_WBaT - 0.5 * (i_CPM_WBaTL + i_CPM_WBbTL) * (1.24-0.24*Time/TimeL);
    i_CPM_WBbTr = i_CPM_WBbT - 0.5 * (i_CPM_WBaTL + i_CPM_WBbTL) * (1.24-0.24*Time/TimeL);
    end;

c_CPM = max((c_CPM_WBaTr + c_CPM_WBbTr) / 2, 0);
i_CPM = max((i_CPM_WBaTr + i_CPM_WBbTr) / 2, 0);
```

Note: Averaged fully adjusted Count $_{\text{Time t}}$ for each Time t post infusion from two samples will be used.

Step 7: Determination of expected Count $_{\text{Time 0}}$ at Time 0 following infusion.

Formula:

Count $_{\text{Time 0}}$ = STD$_{\text{corrected}}$ * Infuscate mass (g) / Blood mass (g)

Where

Blood mass (g) = specific gravity (g/mL) * BV (blood volume in mL)

and

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

and

specific gravity = (1541+Hct) / 1500

Actual Derivation with respect to ^{51}Cr and ^{111}In:

c_CPM0 = c_S * abs(Wt_SyrInfPre - Wt_SyrInfPos) / BM;
i_CPM0 = i_S * abs(Wt_SyrInfPre - Wt_SyrInfPos) / BM;

Where

if sex = "M" then BM = (0.3669*(height (in) * 0.0254)**3 + 0.03219*(weight (lb) * 0.453592) + 0.6041) *1000 * (1541+Hct)/1500;
if sex = "F" then BM = (0.3561*(height (in) * 0.0254)**3 + 0.03308*(weight (lb) * 0.453592) + 0.1833) *1000 * (1541+Hct)/1500;

Note: Hct refers to hematocrit lab result at screening if available.
Weight is collected in pounds (lb) and height is collected in inches (in) from raw datasets at most sites. If weight is collected in kilograms (kg) or height is collected in centimeters (cm), they will be converted to values in pounds and inches to be used in the BM formula as follows:
Weight (lb) = weight (kg) * 2.205;
Height (in) = height (cm) / 2.54.

Step 8: Determine observed recovery (%) for each fully adjusted timed sample.

Formula:

Recovery $_{\text{Time t}}$ = Fully Adjusted Count $_{\text{Time t}}$ / Count $_{\text{Time 0}}$

Actual Derivation with respect to ^{51}Cr and ^{111}In:

c_REC = c_CPM / c_CPM0 * 100%;

i_REC = i_CPM / i_CPM0 * 100%;

Step 9: Estimate platelet recovery and lifespan based on the processed ^{51}Cr and ^{111}In counts by using a validated non-linear curve fitting routine, the multiple hit model, with the x-values (time after infusion in hours) and y-values (observed recovery (%) of fully adjusted timed sample count from Step 8) for timed sample from 1 DPI to 7 (or 8) DPI.

The non-linear multiple-hit algorithm was developed by Murphy and Francies (Murphy and Francies 1971 and 1973). This method assumes that the platelets are repeatedly subjected to hits that cause some damage in the platelets until their final destruction. By fitting the multiple hit curve with the ordinary least squares approach, we can estimate the number of hits the platelets get before being destroyed, and the rate which is the reciprocal of the mean waiting time between hits. Then the estimated recovery from this model is the intercept of the fitted curve on the

vertical y-axis and the mean survival time is calculated by the number of hits multiplying mean waiting time between hits.

- 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.

Sample Results

Data for Subject 001-105 are used.

subjid	Time_Nominal	Time	c_CPM0	c_CPM	c_REC(%)	i_CPM0	i_CPM	i_REC(%)	c_E	i_E
001-105	1	22.83333	227.9890	139.1890	61.0508	205.3476	137.0338	66.7326	0.0733	0.1325
001-105	2	45.5000	227.9890	118.5677	52.0059	205.3476	122.7726	59.7877	0.0733	0.1325
001-105	3	73.83333	227.9890	91.9704	40.3398	205.3476	108.1287	52.6564	0.0733	0.1325
001-105	4	93.33333	227.9890	74.3048	32.5914	205.3476	92.5090	45.0499	0.0733	0.1325
001-105	7	167.6167	227.9890	22.3752	9.8142	205.3476	41.9942	20.4503	0.0733	0.1325
001-105	11	263.1167	227.9890	0	0	205.3476	0	0	0.0733	0.1325

Unit for CPM variables is counts/g; Elution is a ratio.

Results of Recovery and Survival

Primary Analysis (as stated in the protocol) - Using Non-linear Curve Fitting Routine, the Multiple Hit Model results for subject 001-105:

Recovery_Cr_MultiHit	Survival_Cr_MultiHit	Recovery_In_MultiHit	Survival_In_MultiHit
70.4366	172.6731	74.8049	233.1309

REFERENCES

Murphy, E.A., Francis, M.E. and Bulling, D.R., 1973. Estimation of blood platelet survival—V. A method for the analysis of population data. *Journal of Chronic Diseases*, *26*(12), pp.797-815.

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Appendix C: Rounding Table of In Vitro Platelet Function Assays Results

Parameter	Unit of Measure	Listings	Precision as Reported for Mean, SD, Min, and Max
pH at 22°C	Not applicable	0.000	0.0
Platelet Count	$\times 10^3$ cells/µL	0	0
Platelet Dose	$\times 10^{11}$ platelets	0.0	0.0
Platelet Yield Retention	%	0	0
Mean Platelet Volume (MPV)	fL	0.0	0.0
Component Volume	mL	0	0
Volume Recovery	%	0	0
Supernatant Glucose	mmol/L	0.0	0.0
Normalized Supernatant Glucose	mmol/10^{12} platelets	0.0	0.0
Supernatant Lactate	mmol/L	0.00	0.0
Normalized Supernatant Lactate	mmol/10^{12} platelets	0.0	0.0
pO_2 at 37°C	mmHg	0.0	0.0
Normalized pO_2 at 37°C	µmol/hrs/10^{12} platelets	0.0	0.0
pCO_2 at 37°C	mmHg	0.0	0.0
Normalized pCO_2 at 37°C	µmol/hrs/10^{12} platelets	0.0	0.0
HCO_3^- at 37°C	mmol/L	0.0	0.0
Normalized HCO_3^- at 37°C	mmol/10^{12} platelets	0.0	0.0
Total ATP	µmol/dL	0.00	0.0
Normalized ATP	nmol/10^8 platelets	0.00	0.0
Total LDH	U/L	0	0
Supernatant LDH	U/L	0	0
Normalized Supernatant LDH	U/10^{12} platelets	0	0
LDH as a % of Total LDH	%	0.0	0.0
Baseline Adjusted LDH as a % of Total LDH	%	0.0	0.0
CD62P (% P-selectin expression)	%	0.0	0.0