Abbreviated Title: SOLID

Protocol #: 19-CC-0005

Version Date: October 7, 2019

Protocol Title: *Short or Long Infusion Duration for Platelets: The SOLID Platelet Study*

NIH Principal Investigator: Willy A. Flegel, MD
Department of Transfusion Medicine
Clinical Center
Building 10, Rm 1C711
9000 Rockville Pike
Bethesda, MD 20892

Subjects of Study: *Number*: Up to 30
Sex: Either
Age Range: 18 – 100 years old

PRECIS

Platelet transfusion can be a life-saving procedure in preventing or treating serious bleeding in patients who have low and/or dysfunctional platelets. Treatment of blood cancer and other blood diseases, as well as bone marrow transplantation, is not possible without platelet transfusion support. Unfortunately, 15-25% of chronically transfused patients' platelet counts will stop responding to these transfusions, putting them at risk for serious bleeding complications. The development of HLA antibodies is responsible for 4-8% of this platelet transfusion refractoriness. The presence of HLA antibodies is a clinical complication that is generally managed by the selection of products that are negative for the antigens for which the patient has antibodies. Often, for patients with chronic and ongoing need, this selection is facilitated by targeted recruitment of donors with known HLA types (i.e., types that lack antigens cognate to the patient's known antibodies and are thus predicted to be compatible). However, for very broadly HLA-alloimmunized patients, compatible products may be exceedingly scarce or completely unavailable, precluding the ability to consistently provide products the patient will likely increment from. This research protocol is designed to evaluate the efficacy of a 4-HOUR continuous infusion of single donor, apheresis platelets in overcoming both alloimmune-mediated and non-alloimmune-mediated platelet refractoriness. We hypothesize that when we transfuse patients over a long duration, who have platelet refractoriness, the platelet counts will increase to higher numbers for an extended period of time in the peri-transfusion period when compared to shorter transfusion intervals.

Table of Contents

List of Abbreviations

AUC – Area Under the Curve

CCI – Corrected Count Increment

CPT – Continuous Platelet Transfusion

PTR – Platelet Transfusion Refractoriness

IBS – Intercept Blood System

DTM – Department of Transfusion Medicine at the NIH Clinical Center

LSS – Laboratory Services Section of DTM

SDP – Single Dose Platelet

1. OBJECTIVE

1.1. Primary Objective:

To evaluate efficacy of continuous platelet transfusions in overcoming platelet transfusion refractoriness, including HLA-alloimmune-mediated refractoriness, in patients with severe thrombocytopenia.

1.2. Secondary Objective:

To characterize the efficacy of continuous platelet infusion on bleeding outcomes, as measured during the peri-transfusion period by daily hemostatic assessments using the World Health Organization (WHO) bleeding scale (see section 6.3.4).

2. INTRODUCTION, BACKGROUND, AND SCIENTIFIC JUSTIFICATION

Rationale:

Platelet transfusion can be a life-saving procedure in preventing or treating serious complications from hemorrhage in patients who have disorders manifesting as thrombocytopenia and/or platelet dysfunction. Allogeneic platelet support is indispensable to the treatment of hematological and non-hematological diseases. Prophylactic platelet transfusion to prevent bleeding -- rather than waiting to treat active bleeding -- is now the standard practice for the treatment of profound thrombocytopenia (with platelet counts below 10 x 10^9 cells/L) and is recommended by various clinical practice guidelines [1-6]. Higher "triggers" are recommended when invasive procedures are anticipated. In 2011, an estimated 2.1 million platelet products were transfused in the USA [7]. However, depending on the patient, and disease characteristics, 15-25% of patients chronically transfused with leukocyte-reduced blood components will ultimately develop platelet refractoriness, making this a major worldwide health problem [8-10].

Platelet refractoriness is defined by an inadequate post-transfusion platelet count increment and is calculated based on pre- and post-transfusion platelet counts, the number of transfused platelets, and the patient's body surface area. The most commonly used definition to establish platelet refractoriness is a post-transfusion corrected count increment (CCI) less than 7500/uL 10-60 min following the transfusion (CCI_{1hr}), and less than 5000/uL at the 18-24 hrs time points [11], where CCI is calculated as follows: CCI = (absolute count increment per µL x body surface area in m^2)/number of platelet transfused (x 10^{11}). The CCI is also commonly reported as a "unit-less" multiple of 1000 (i.e., a failed CCI_{1hr} would be <7.5).

Platelet refractoriness is associated with bleeding complications, reduced overall survival and longer hospital stays and higher hospital costs [12-14]. The National Institutes of Health Clinical Center is a major research referral center for hematologic disorders, including severe aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), congenital thrombocytopenia, and a multitude of premalignant and malignant hematological disorders. Patients with these disorders are characterized by pancytopenia, a hypocellular bone marrow, and suppression of hematopoiesis, resulting in hypoproliferative thrombocytopenia. Further, one treatment modality that many of the Clinical Center's research protocols utilize in the treatment of hematologic and immunologic conditions is hematopoietic progenitor cell transplantation (HPCT), which relies on the recovery of the hematopoiesis from engrafting donor progenitor cells. However, until patients recover hematopoiesis they remain pancytopenic. Thrombocytopenia leads to easy bruisability and purpura, petechiae of the skin and mucous membranes, epistaxis, irregular and heavy menses, and gum bleeding. Bleeding can be brisk in the presence of accompanying physical lesions, as in gastritis and fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage, which has a high degree of mortality. The

primary modality to prevent life-threatening hemorrhage is platelet transfusion [5]. However, patients who develop platelet refractoriness do not increment appropriately to platelet transfusions. Any intervention that augments hemostasis in these patients pending the recovery of hematopoiesis and endogenous platelet count would potentially be lifesaving.

HPCT is not possible without adequate platelet support. While most platelet refractoriness is associated with a range of multifactorial non-immune-mediated clinical factors such as fever, sepsis, splenomegaly and certain medications, a significant subset of refractoriness is due to antibodies directed against foreign HLA antigens that developed as a result of exposure to foreign leukocytes through pregnancy or prior transfusion [8,15,16]. Management of platelet refractoriness at the Clinical Center focuses on providing platelets of HLA types compatible with the recipient's antibody profile (i.e., platelets that do not contain the HLA antigens to which the patient has developed antibodies). Providing HLA matched platelets is primarily accomplished via targeted donor recruitment or less frequently purchasing products procured from blood collection agency searches at both the regional and national level. However, for very broadly HLA-alloimmunized patients, compatible products may be exceedingly scarce or completely unavailable, precluding the platelet transfusion support necessary to overcome the patient's refractoriness.

While clinical studies have described the phenomenon of platelet alloimmunization in detail, a full elucidation of its pathophysiology remains elusive [16]. The majority of clinically relevant HLA antibodies have been shown to be immunoglobulins G and M (IgG and IgM) that are directed against HLA class I epitopes, namely, HLA-A and B antigens. When patients generate IgG and IgM to allogeneic platelets, the destruction of platelets occurs by one of the known antibody dependent mechanisms, namely eradication via the Fc receptor-expressing phagocytes [17] residing in the reticuloendothelial system (RES) and via complement activation [18]. However, attempts to reduce the production of HLA antibodies using immunosuppressive or immunomodulatory agents (e. g. IVIG, cyclosporine, corticosteroids) typically do not achieve a sustained resolution of platelet refractoriness [19-22]. Our experience with a pilot trial treating HLA alloimmune-mediated platelet refractoriness with eculizumab, a monoclonal antibody that inhibits complement-mediated destruction by binding C5, shows that while preliminarily results indicate some promise in a subset of patients, refractoriness persists in others [23]. Thus, alternative approaches to manage patients who are heavily HLA alloimmunized are in great demand. It is also clear that alloimmunization to HLA antigens does not explain all platelet refractoriness and even for immune refractoriness, HLA alloimmunization may be a marker for other conditions (platelet-specific antibodies (HPA), non-HLA antibodies to antigens on the platelet surface).

Recently, slow, continuous infusion of platelet products (continuous platelet transfusion, CPT) has garnered renewed interest as one potential way to manage platelet transfusion refractoriness (PTR) [24-26]. In a case series of three patients with PTR, Narvios *et al* reported successful post-transfusion increments after administration of platelet concentrates infused over six hours, with one patient, who also had hematuria prior to the infusion, experiencing clinical hemostasis 24-hours post-infusion [24]. However, details regarding the nature of the refractoriness (alloimmune vs non-alloimmune) and the CCI were not reported. In a larger, retrospective analysis of 21 patients with PTR, Tzadok *et al* evaluated the impact of 24-hour CPT -- consisting of consecutive transfusions of single-donor apheresis platelets (SDP) half-doses (i.e., comprising 1.5×10^{11} platelets each) infused over 4-6 hours each -- on the CCI obtained after 12 and 24-hours post-transfusion. The authors found CPT resulted in a statistically significant higher mean CCI at 24 hours (1.16 vs. 0.37, $p<0.05$) but not at 12 hours (2.45 vs. 0.36, $p=0.058$) [25]. The HLA/HPA alloimmune status of the patients was not reported. Most recently, employing a similar 24-hour CPT method as Tzadok *et al*, Cid *et al* reported a range of measures (including HLA alloimmune status, bleeding outcomes and median platelet counts before and after CPT) in their retrospective analysis of CPT in 14 patients (6 alloimmune-mediated,

8 non-alloimmune-mediated). The difference in increments prior to, and after, CPT was not formally statistically assessed. However, nine bleeding episodes (WHO grade≥2) were present prior to 19 CPT infusions and all nine resolved during the 24-hour infusions, notably without any complicating adverse transfusion reactions [26]. Similar other anecdotal successes with using 24-hour CPT or massive platelet transfusion to manage bleeding risk and/or alloimmunization have also been reported [9].

The theoretical underpinnings of how CPT might be effective in augmenting hemostasis and minimizing bleeding events, despite PTR -- including alloimmune-mediated PTR, were the subject of recent speculation [26]. Briefly, it has been observed that transfusion of HLA-incompatible platelets, while not appreciably improving hemostasis as measured by 10- and 60-minute post-transfusion forearm bleeding times, nevertheless results in increases in deposition of fibrin on the subendothelial surface and in circulating thrombin-antithrombin complexes, suggesting a procoagulant effect [27]. The volar bleeding times were performed with positive capillary pressure. Thus, it is not known to what extent, if any, this apparent procoagulant effect may mitigate the overall bleeding diathesis that results from severe thrombocytopenia, and, in particular, in tissues with physiologic or low shear rates. Importantly, Tzadok *et al* suggests a proportion of incompatible platelets infused within a 24-hour CPT may escape immune-mediated clearance mechanisms [25]. An alternative hypothesis that derives some support from animal studies is that massive platelet transfusion may be able to directly reduce the level of alloimmunization by *in vivo* alloadsorption [9], thus increasing the likelihood of adequate CCI on each sequential platelet transfusion from donors sharing a given HLA type.

In general, a fixed 7.1×10^{9} platelets/L/day is necessary to maintain the vascular endothelial integrity [28], suggesting that the average 70 kg adult with a total blood volume of ~5L would require ~0.36×10^{11} platelets/day. A 24-hour CPT, consisting of five to six half-dose SDPs (1.5×10^{11} platelets each) in 24 hours, would provide, at minimum, 7.5×10^{11} platelets/L/day. In PTR, and in particular in alloimmune-mediated PTR, the minimum number of transfused platelets needed per unit time to maintain vascular endothelial integrity, mitigate bleeding risk, manage hemorrhage or obtain adequate CCIs is unknown. The kinetics of platelet survival in patients with PTR, and the increment in circulating platelet count during transfusion, is similarly unknown.

Taken altogether, these data support slow, continuous transfusion as a potential transfusion approach that may minimize the number and/or duration of adverse hemorrhagic events and that may result in greater platelet increments per unit time than the conventional infusion approach. Furthermore, there is no evidence in the published literature or any reported relevant experience of physicians who treat thrombocytopenic patients to suggest that CPT would be deleterious. Therefore there is equipoise in this trial design.

This study only utilizes Cerus Intercept Blood System (IBS) single, apheresis donor platelets (SDP) to compare two treatment approaches: 4-HOUR continuous infusion ("Long Transfusion") versus <60-minute infusion ("Short Transfusion"). The IBS is an FDA-approved pathogen reduction technology that reduces the risk of transfusion-transmitted infections by inactivating a broad range of pathogens such as viruses, bacteria and parasites that may be present in donated blood. This technology is the standard of care processing for all platelet products collected by the Department of Transfusion Medicine (DTM), implemented January 14th, 2016. The 4-HOUR infusion duration was selected as the maximum infusion time since it is undesirable for components that contain red cells to remain at room temperature longer than 4 hours, and since it is generally recommended for components to complete transfusion within 4 hours by the FDA-recognized AABB *Circular of Information* (https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/G

uidances/Blood/UCM364587.pdf, accessed 11/07/2017). Sixty-minutes was selected for the maximum duration for the "Short Transfusion" based on retrospective data on platelet transfusion durations and to proscriptively not exceed the 300 mL/hour ("or as tolerated") rate recommended by the AABB Technical Manual (18th edition). As each transfusion will use a half-SDP, and since each full SDP is no greater than 390 mL, the maximum volume in each product issued within this protocol will not exceed 195 mL; thus, ensuring that the slowest infusion rate allowed within this protocol for the "Short Transfusion" does not exceed 195 mL/hour (that is, is not prescriptively forced to exceed the 300 mL/hour recommended standard rate).

3. STUDY DESIGN

3.1. Indication:

Platelet transfusion refractoriness (PTR)

3.2. Subject Population:

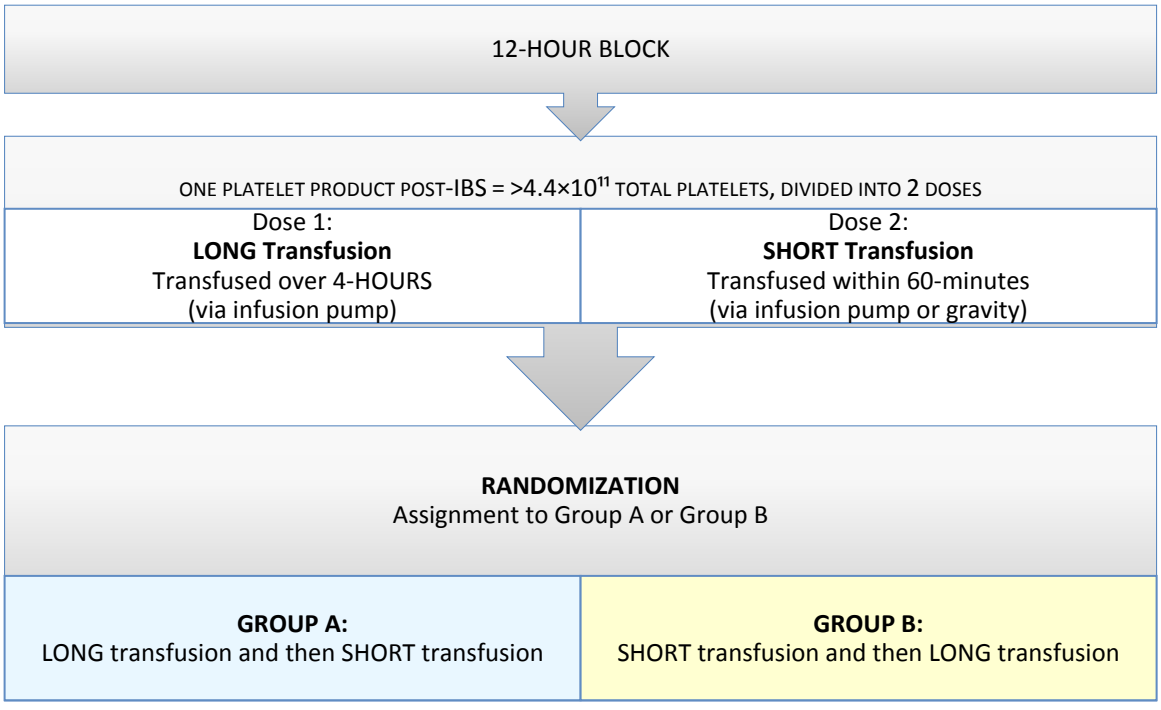
Subjects/Patients with thrombocytopenia (due to congenital causes, bone marrow failure, hematologic malignancies, and treatment-related), already enrolled on a primary protocol at the NIH CC, who require platelet transfusions, as well as demonstrate PTR diagnosed by the following:

Lack of an adequate post-transfusion platelet count increment, defined by CCI <5000/ul at 10-60 minutes after each of at least 2 consecutive platelet transfusions.

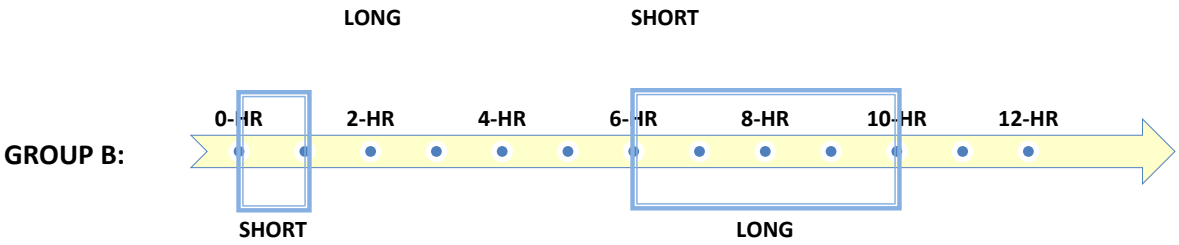
If, in addition to meeting the above criteria for PTR, anti-HLA class 1 type A and/or type B antibodies are detected, the PTR will be classified as HLA-alloimmune-mediated. If HPA antibodies are detected in the setting of PTR, the PTR will be classified as HPA alloimmune-mediated.

If neither HLA nor HPA antibodies are detected, the PTR will be classified as non -alloimmune-mediated. Both alloimmune and non-alloimmunte types of PTR are eligible for this study.

3.3. Randomized Block Schema:



GROUP A:

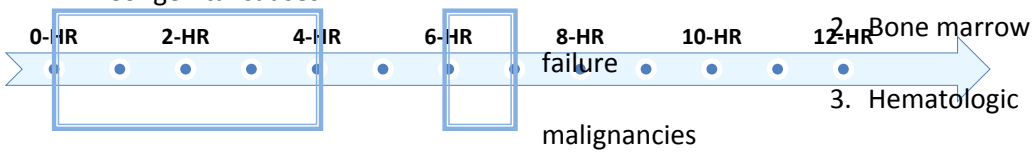


● ALL PATIENTS WILL HAVE PLATELET COUNTS OBTAINED VIA COMPLETE BLOOD COUNTS (CBC), DRAWN AT THE ABOVE SCHEDULED INTERVALS, DURING EACH 12-HOUR BLOCK.

4. ELIGIBILITY ASSESSMENT

4.1. Inclusion Criteria

- Ability to comprehend the investigational nature of the study and provide informed consent
- Thrombocytopenia
    a) Causes of thrombocytopenia may be due to:
        1. Congenital causes



        2. Bone marrow failure
        3. Hematologic malignancies
        4. Treatment related
    b) Thrombocytopenia is generally defined as one of the following:
        1. <10K/uL without bleeding
        2. <20K/uL for "complicated prophylaxis" in patient's determined to be at increased risk of bleeding or other complications
        3. <50K/uL with evidence of active bleeding, such as intracranial hemorrhage, GI bleeding, pulmonary hemorrhage, uncontrolled epistaxis, hematuria.

    The treating provider may change the platelet transfusion threshold based on the clinical circumstance, patient population, and/or concurrent primary protocol considerations – similar to the PLADO study[29].
- Diagnosed with PTR, characterized by the following:
    a) Lack of adequate post-transfusion platelet count increment, defined by, CCI <5000/ul at 10-60 min after each of at least 2 consecutive platelet transfusions
    b) Presence of anti-HLA class 1 type A and/or type B antibody, in the setting of PTR, as defined above, constitutes the HLA alloimmune-mediated subtype of PTR. Presence of one or more HPA antibodies in the setting of PTR, as defined above, constitutes the HPA alloimmune-mediated subtype of PTR. Failure to detect HLA or HPA antibodies will be categorized as "non-alloimmune-mediated PTR."

4.2. Exclusion Criteria

- Less than 18-years-old
- Lack of ability to obtain informed consent

- Pregnant female
- Presence of ITP/autoimmune thrombocytopenia
- Immune platelet refractoriness responsive to treatment with IVIg or eculizumab, or other immunosuppressive therapy within the 3 preceding months. This is based on the wide variation in the duration therapeutic antibodies, with the upper limit frequently cited as 3 months.

5. RESPONSE CRITERIA

Statistically significant difference in the adjusted platelet increment area under the curve (AUC) obtained between 0-HOURS and 6-HOURS after the start of the platelet transfusion (Long Transfusion or Short Transfusion); this is determined at the interim and final analysis. The interim analysis will include a test of the null hypothesis of no difference in efficacy, using a threshold of significance of 0.01, with a threshold of 0.04 used for the final analysis. See *Study Analysis*, Section 8.2 for further details.

6. TREATMENT PLAN

6.1. Pre-Study Evaluation (screening)

- Conducted as part of the subject's primary research protocol:
  - Clinical history and body surface area (BSA)
  - CBC or CBC with differential
- Conducted as part of this protocol's screening:
  - PT/INR and aPTT
  - HLA typing and HLA class I A, B, C antibody screen for HLA alloantibodies
  - HPA antibody screen ("Platelet antibody screen")

6.2. Randomized Block Design

6.2.1. Randomization

- Upon IRB approval, the randomization schema for the first block for each subject will be specified using the "sealed envelope" web application at https://www.sealedenvelope.com/simple-randomiser/v1/lists, using the following input specifications:
  1) Treatment Groups
     - Group A: Long Transfusion followed by Short Transfusion in the first block
     - Group B: Short Transfusion followed by Long Transfusion in the first block
  2) Block sizes – 4, 6, or 8
  3) List Length – 30

  This web application has been used for randomization in various published clinical trial studies over the last decade (https://scholar.google.com/scholar?q="Sealed+Envelope+Ltd.").
- The randomization list will be held by the TSL Operations Coordinator, who will reveal the first block assignment (Group A versus Group B) for each subject at the time of that subject's enrollment

- Only one block per day will be permitted for a subject. Up to two further subsequent blocks (one per day) may be administered for a subject, thus bringing the total maximum number of blocks per subject to three. Subsequent blocks will alternate the sequence of the Long and Short Transfusions.

6.2.2. Platelet Component Preparation for Block Administration

- This study will only utilize platelet components collected in the NIH Clinical Center, Department of Transfusion Medicine
- Only IBS platelet components that are $>4.4\times10^{11}$ platelets, as measured from a sample obtained from the storage container post-transfer from the compound adsorption device (CAD) container, will be used; this number was selected in order to closely approximate the number of platelets transfused during routine issue of a single dose of platelets
- 2 ml will be withdrawn into a sample pouch or tubing segment, heat sealed, detached, and then emptied into a labeled EDTA tube to be submitted to CPS for platelet count determination
- Each component will be split by dividing the tubing at the point of union, into two, roughly equal, "doses", following established TSL procedures (TSL-SOP-3941)
- Each dose will have its weight documented in order to calculate the number of platelets in each dose
- Each block consists of two doses from one platelet product, one for a Long Transfusion duration and one for a Short Transfusion duration:
- Both doses will be gently swirled for ~20 revolutions, every 30-minutes, to thoroughly mix the product; this will done by the nursing staff
- Both doses will be weighed to ascertain volume
- One dose within each block will be transfused over a LONG duration, over 4-HOURS from the time the back is spiked and one dose within each block will be transfused over a SHORT duration, within 60-minutes from the time the bag is spiked; both transfusions may occur via an infusion pump or by gravity.

6.3. Treatment Monitoring

6.3.1. CBC Collection

All CBC blood draws will be collected in a 2 ml EDTA tube, after wasting the first 1ml of blood drawn. The sample should be obtained via the patient's peripheral access device, but a peripheral venipuncture or draw from the patient's central access device may be done if access the patient's peripheral line is not feasible. The initial 1 ml sample discard, prior to filling the 2ml EDTA tube, is to prevent a false reading by drawing the sample from the same line through which the patient is receiving the transfusion.

Patients will have platelet counts obtained by CBCs collected at the following scheduled intervals:

1) Pre-transfusion (0-HOUR) CBC: obtained 15-minutes prior to transfusion start time
2) 2-HOUR CBC: Obtained +/- 15-minutes of the 2-HOUR mark after the infusion start time

3) 4-HOUR CBC: Obtained +/- 15-minutes of the 4-HOUR mark after the infusion start time
4) 6-HOUR CBC: Obtained +/- 15-minutes of the 6-HOUR mark after the infusion start time. Also serves as the PRE (0-HOUR) CBC for the subsequent, transfusion; the subsequent transfusion will also have scheduled CBCs drawn at q2hr intervals (i.e. CBC at 8-HOUR, 10-HOUR, 12-HOUR), collected +/- 15-minutes of each time mark)

CBCs unable to be obtained as indicated will result in an unsuccessful, or "failed," block. Failed blocks will be precluded from further analysis. All attempts will be made to repeat the block as soon as clinically possible. Blood draws for other clinical purposes should be avoided, but will not result in a failed block unless it/they preclude the aforementioned scheduled CBCs needed for this protocol's treatment monitoring.

From the CBC, platelet value will be used to calculate the platelet AUC using the trapezoid rule and adjusted for the absolute number of platelets transfused, on a rolling basis for each transfusion.

6.3.2. Documentation and Results Review

Testing for HLA-alloimmune-mediated PTR and HPA-alloimmune-mediated PTR will be performed upon enrollment, if not previously completed prior to enrollment. Weight and platelet count of the platelet component will be documented concurrently in the DTM Transfusion Services Laboratory. Treatment monitoring, including transfusion duration, CBC collection times, platelet counts, etc., will be occur concurrently via *The SOLID Platelet Study Nursing Worksheets* (Appendices B and C), the electronic medical record system, Clinical Research Information System (CRIS), and the already operational "DTM Platelet Transfusion Summary" and "Patient Platelet Count Summary" SQL reports.

Orders and results will be tracked through CRIS. Should CRIS not be available, the NIH form 2803-1 will be completed and accompany the specimen; it will be filed in the patient's medical record.

To evaluate the frequency and extent of hemorrhagic complications, bleeding will be assessed by the modified World Health Organization bleeding scale (Appendix A). This will be done by the Principal Investigator (PI), the Lead Associate Investigator (LAI), or Associate Investigator (AI) using chart reviews for bleeding events one day prior to the Transfusion Blocks, the day(s) of the Transfusion Blocks, and one day following the Transfusion Blocks. This approach is deemed reasonable in the absence of a standardized or consensus method to evaluate bleeding in platelet transfusion clinical trials [31].

Long-term follow-up observations will not be necessary after the study's completion.

7. BIOSTATISTICAL CONSIDERATIONS

This is a two arm, randomized block study.

7.1. Sample Size and Study Design

We will determine the sample size using the Two-Stage Design. At the first stage, 12 subjects will be accrued. An interim analysis will be performed to compare the platelet count AUC values, adjusted for the absolute number of platelets transfused (adjusted platelet AUC), between the within-block treatment pairs. If the difference is significant at the 0.01 one-sided level favoring

the slow infusion technique, then accrual will cease and the null hypothesis rejected. At the interim analysis we will also perform a futility analysis. If the conditional power for rejecting the null hypothesis at the end of the study is less than 0.3, then accrual will cease. Otherwise, an additional 12 subjects will be accrued, bringing the total number of subjects to n=24. To account for dropouts, up to 30 subjects will be enrolled. Anticipated dropouts are due to inability to tolerate blood draws.

This design (n=24) has power >80% using the sign test on the matched pairs from the first block for each patient under the alternative hypothesis that the probability that a matched pair of adjusted platelet AUC values favors the test regimen is 0.80, assuming no tied observations [32]. The actual analysis will use the Wilcoxon matched pair signed rank test. A mixed model analysis for multiple blocks per patient will be performed as a secondary analysis.

8. ENDPOINTS

8.1. Primary Endpoint

To evaluate the efficacy of Long Transfusion in overcoming PTR in patients with severe thrombocytopenia.

Efficacy will be measured in terms of the adjusted platelet increment AUC, obtained between 0-HOURS and 6-HOURS after start of the platelet transfusion. The AUC (i.e., AUC above the pre-transfusion CBC's platelet count, minus the AUC below the pre-transfusion CBC's platelet count, which can be a net increment or net decrement) determined by the 0-HOUR, 2-HOUR, 4-HOUR, and 6-HOUR CBC platelet concentrations, will be estimated by the trapezoid rule and used as the primary outcome measure.

Adjustment to the measured AUC will be done for the number of platelets transfused during the 2 transfusion periods (Long and Short Transfusion durations) in 1 block: The weights of the 2 bags will be checked to estimate the platelet product volumes; the volumes will be multiplied by the post-illumination CBC (obtained after removal of the platelet product from the compound adsorption device).

The outcomes (AUC) will be compared between the 2 transfusion periods within the first block for the primary analysis. The null hypothesis for efficacy will be that the adjusted platelet increment AUC does not differ between the 2 transfusion periods (Long and Short infusion durations).

8.2. Study Analysis

All subjects who receive one 12-HOUR block of Short and Long infusion duration of platelets will be included in the primary analysis. Every effort will be made to evaluate the subjects before transfusion as well as at 2-HOURS, 4-HOURS and 6-HOURS after starting each platelet dose infusion. Adverse events will be tabulated by severity and attribution to treatment and disease (transfusion reactions will be classified using the CDC NHSN *Hemovigilance Module Surveillance Protocol*, which may be accessed at https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf).

Final statistical analysis will be performed once all 24 subjects have completed the end of study assessments and all data have been archived for analysis. An interim analysis will be conducted after 12 subjects have completed at least one 12-HOUR block of transfusions. The planned

analyses will include descriptive statistics on the proportions of platelet response and 2-HOUR to 6-HOUR platelet increment AUC values, adjusted for number of platelets given. Hypothesis testing will utilize Wilcoxon matched pair signed rank test for the primary analysis. Mixed model analysis will be employed for multiple blocks per patient and reported descriptively.

The interim analysis will include a test of the null hypothesis of no difference in efficacy using a threshold of significance of 0.01, with a one-sided threshold of 0.04 used for the final analysis. The interim analysis will also include a futility analysis based on the conditional power for rejecting the null hypothesis at the final analysis given the interim results. The futility analysis reduces the type I error of the design.

Secondary analysis based on mixed effect regression, and logistic regression analysis will also be employed if deemed appropriate.

8.3. Stopping Rules

At the interim analysis we will perform a futility analysis. If the conditional power for rejecting the null hypothesis at the end of the study is less than 0.2, then accrual will cease.

8.4. Off-Study Criteria (and Subject Replacement)

Patients will be taken off study for the following:

- Subject has completed three successful 12-HOUR blocks
- Subject no longer requires frequent platelet transfusions, as defined as 5-days of platelet transfusion independence
- Withdrawal per subject choice
    - Subjects have the opportunity to withdraw from the study; if subjects withdraw, they will be replaced, to reach a total of 24 patients
- Withdrawal by physician decision
    - Subject will be withdrawn from the study if he/she cannot tolerate blood draws, or if subject no longer requires platelet transfusions
- Completion of the study

9. SAMPLE COLLECTION, STORAGE AND TRACKING PLAN

Plasma samples can be stored in the NIH DTM HLA laboratory with an aliquot of each sample separated and set aside for the purpose of this study. Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

9.1. Sample Storage

Research aliquots will be coded by sample number and be stored in a secure freezer in the NIH/CC/DTM Laboratory Service Section. Access will be restricted to protocol research staff only (i.e., the PI and AIs). All research aliquots will be discarded after testing is performed.

9.2. Intended Use

Aliquots of stored archival plasma from prior clinical use will be extracted and prepared for testing against allogenic platelets for platelet crossmatch method validation.

9.3. End of Study Procedures

Samples will be stored until they are no longer of use for the purposes within the context of this study, or until the subject withdraws consent – at which time they will be destroyed.

9.4. Loss or Destruction of Samples

Should it become known that a major breech in the plan for tracking and storage of samples has occurred, the IRB will be notified by the PI.

9.5. Future Use

Other investigators may want to study stored samples. If so, the NIH study team may send samples to them along with the coded label. Although the study team will not share information that can directly identify subjects, they may share information regarding health history, as well as general demographic information such as gender, age, and ethnicity. Future studies may need health information (such as smoking history or present health status) that is not already known about the subjects; if so, the NIH study team will contact subjects for this information.

10. NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

10.1. Definitions

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

10.2. OHSRP Office of Compliance and Training / IRB Reporting

For all human subjects research in which an NIH IRB is the Reviewing IRB, NIH Principal Investigators (PIs) and, as applicable, non-NIH Site PIs/Lead Investigators (further referred to as non-NIH investigators), are required to ensure that all reportable events, as defined in NIH *Policy – 801*, are reported to the OHSRP office of Compliance and Training within the time frames as specified in the policy**.**

Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here.

IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

10.3. Clinical Director (CD) Reporting

Problems expeditiously reported to the OHSRP/IRB in iRIS will also be reported to CD. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

10.4. NIH Required Data and Safety Monitoring Plan

The clinical research team will meet weekly when patients are being actively treated on the trial to discuss each patient. All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **10.2** will be submitted within the appropriate timelines.

The principal investigator will review adverse event data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

10.5. Adverse Transfusion Events Management

All adverse transfusion events will be investigated by the study PI. If it is determined that an adverse transfusion event is, indeed, related to the study treatment, each adverse transfusion reaction will be classified according to the Centers for Disease Control (CDC) April 2018*National Healthcare Safety Network (NHSN) Biovigilance Component Hemovigilance Module Surveillance Protocol*, v2.5.2 (https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf). This reference guide includes reaction-specific case definitions, as well as severity, and imputability criteria, further outlined below:

- **Defined Adverse Transfusion Reactions**:
    - Transfusion-associated circulatory overload (TACO)
    - Transfusion-related acute lung injury (TRALI)
    - Transfusion-associated dyspnea (TAD)
    - Allergic reaction (where severity is severe, life threatening, or death)
    - Hypotensive transfusion reaction
    - Febrile non-hemolytic transfusion reaction (FNHTR)
    - Acute hemolytic transfusion reaction (AHTR)
    - Delayed hemolytic transfusion reaction (DHTR)
    - Delayed serologic transfusion reaction (DSTR)
    - Transfusion-associated graft vs. host disease (TAGVHD)
    - Post-transfusion purpura (PTP)
    - Transfusion-transmitted infection (TTI)
    - Other or Unknown

- **Severity:**
    - *Non-severe*: Medical intervention (e.g. symptomatic treatment) is required but lack of such would not result in permanent damage or impairment of a bodily function.
    - *Severe:* Inpatient hospitalization or prolongation of hospitalization is directly attributable to the adverse reaction, persistent or significant disability or incapacity of the patient occurs as a result of the reaction, or a medical or surgical intervention is necessary to preclude permanent damage or impairment of a body function.
    - *Life-threatening:* Major intervention required following the transfusion (e.g. vasopressors, intubation, transfer to intensive care) to prevent death.
    - *Death:* The recipient died as a result of the adverse transfusion reaction. Death should be used if death is possibly, probably or definitely related to transfusion. If the patient died of a cause other than the transfusion, the severity of the reaction should be graded as appropriate given the clinical circumstances related to the reaction.
- **Imputability:**
    - *Definite:* Conclusive evidence exists that the adverse reaction can be attributed to the transfusion
    - *Probable:* Evidence is clearly in favor of attributing the adverse reaction to the transfusion.
    - *Possible:* Evidence is indeterminate for attributing the adverse reaction to the transfusion or an alternate cause.
    - *Doubtful:* Evidence is clearly in favor of a cause other than the transfusion, but transfusion cannot be excluded.

- *Ruled Out:* There is conclusive evidence beyond reasonable doubt of a cause other than the transfusion.

10.6. Data Management

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. Laboratory values from referring home physicians will be entered into the system. The PI, AI(s), research nurses, and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable. Data will be extracted from CRIS and will be imported electronically from CRIS into an in-house database.

Data will be located in a password protected folder on the Department of Transfusion Medicine (DTM) secure G drive. Access will be restricted to PI and AI(s).

- **End of Study Procedures:**
  - Data and samples will be stored until they are of no longer of scientific value or until the subject withdraws consent at which time it will be destroyed
- **Loss or destruction of data:**
  - Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified
  - Data will not be sent outside NIH without IRB notification and an executed MTA or CTA.

- **Research use of study data:**
  - The IRB will be notified and the protocol and consent amended if samples and data collected under this protocol will be used for research not described in the current protocol or if the study records are transferred to another party.
- **Publication policy:**
  - Given the research mandate of the NIH, subject data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research (OHSR).

10.7. Data Sharing

Data and samples may be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained (if applicable: or under waiver of informed consent as described below). Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with a Federal Wide Assurance (FWA) or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be

reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

11. HUMAN SUBJECTS PROTECTIONS

The investigators will protect the rights and welfare of human research subjects set forth in 45 C.F.R. Part 46 and 21 C.F.R Part 50, *Protection of Human Subjects.*

Rationale for Subject Selection:

Thrombocytopenia leads to petechiae of the skin and mucous membranes, epistaxis, menses that last more than a month, and gum bleeding. The most feared complication of thrombocytopenia is intracranial hemorrhage, which has a high degree of mortality. The only modality to prevent life threatening hemorrhage is platelet transfusion. However, patients who develop platelet refractoriness do not respond to platelet transfusions. A subset of this refractoriness is due to HLA antibodies. Data support the observation that continuous platelet transfusion (CPT) may overcome transfusion refractoriness and result in an adequate number of platelets in circulation to support hemostasis. Therefore, we hypothesize that when we transfuse patients who have platelet refractoriness with "Long Transfusion", the platelet counts will increase to higher numbers after these transfusions than intermittent "Short Transfusion" platelet infusions, thus decreasing the risk of bleeding complications or ameliorating, if not completely resolving, bleeding present prior to enrollment.

Recruitment Effort:

Strategies for subject recruitment will include announcement to the medical team during rounds. DTM consultations are routinely performed for HLA alloimmunized patients thus there is a keen awareness of potential candidates for study. If a subject is deemed suitable by the primary medical team for this protocol, the patient will be approached by an Investigator for a full explanation of the study. The study will be listed on the clinicaltrials.gov, and Clinical Center research studies.

11.1. Competition between Branch Protocols

This protocol currently does not conflict or compete with any other Branch protocols. This protocol is targeted to the same patient population as NHLBI protocol 15-H-0015, which has completed enrollment and is thus closed.

11.2. Reimbursement

- Reimbursement for protocol participation, travel, food, and lodging will be consistent with NHLBI policy.
- Payment for participation: $0

11.3. Participation of Children

Pediatric subjects are excluded, due to the additional confounding that may be introduced when additional processing (i.e., volume reduction) is required of the platelet product due to the patient's size.

11.4. Participation of Pregnant Females

Pregnant women are excluded, because the hypothesis should first be explored in patients without incurring any risk for a pregnant woman or her fetus.

11.5. Participation of Subjects Unable to Provide Informed Consent

Subjects incapable of providing informed consent are excluded, because the hypothesis should first be explored to assess whether their prospective participation in the study outweighs any potential risk, as per human subjects research protections outlined in NIH SOP-14E, *Research Involving Adults Who Are or May Be Unable to Consent*.

11.6. Anticipated Benefit, Risks and Discomforts

The research risk of the protocol is no greater than minimal risk with the prospect of direct benefit, due to the potential for prolonging platelet survival with a 4-HOUR infusion.

11.6.1. Transfusion-Associated Circulatory Overload (TACO)

Volume overload as a result of transfusion occurs in 1-8% of patients who are transfused, or one in ~10,000 transfused components, and is generally under-recognized and lacks international consensus criteria. However, the CDC NHSN criteria (https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf; *Hemovigilance Module Surveillance Protocol* v2.4) define the reaction as 3 or more of the following within 6 hours of cessation of transfusion:

- Acute respiratory distress (dyspnea, orthopnea, cough)
- Elevated brain natriuretic peptide (BNP)
- Elevated central venous pressure (CVP)
- Evidence of left heart failure
- Evidence of positive fluid balance
- Radiographic evidence of pulmonary edema

A recent review published by an international panel recommends transfusing patients at risk of TACO slowly over up to 4 hours, using divided units[33]. Since this protocol is an extended version of the recommended approach, the risk of TACO is mitigated to some degree and is not thought to be substantially increased. TACO is treated by stopping the transfusion, administering oxygen as needed, and diuretic administration.

11.6.2. Risks Common to all Blood Products

- Uncommon (1-5%) chance)
  - Mild reactions resulting in itching, rash, fever, headaches.
- Rare (<1% chance)
  - Respiratory distress (shortness of breath) or lung injury
  - Exposure to blood borne micro-organisms (bacteria and parasites) that could result in an infection*
  - Possible effects on the immune system, which may decrease the body's ability to fight infection
  - Exposure to blood borne viruses such as hepatitis B (an inflammatory disease affecting the liver)*
  - Shock
- Extremely rare (one in a million or less)
  - Exposure to blood borne viruses such as hepatitis C (an inflammatory disease affecting the liver)and Human Immunodeficiency Virus (HIV, the virus that causes AIDS)*
  - Death

*The above incidence refers to non-pathogen reduced platelets. At the NIH CC, all platelet products collected by DTM are pathogen-reduced via the Intercept Blood System; only these products will be used in this study. Pathogen-reduced platelets using the Intercept Blood System for Platelets (IBS) has FDA marketing approval for pathogen reduction based on demonstrated effectiveness against a range of viruses, protozoa, and Gram-positive, Gram-negative, anaerobic and spirochete bacteria. Thus, actual rates are likely much lower.

11.6.3. Risks Related to Blood Tests

Some subjects may experience localized bruising at the site of venipuncture. Some subjects may experience a vasovagal response. In order to minimize the potential for fall injuries after a blood draw, subjects will be closely monitored for dizziness or lightheadedness before they are allowed to stand.

11.7. Risks in Relation to Anticipated Benefit

The investigational nature and objectives of this study, the procedures and treatments involved and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the patient or the patient's surrogate, and a signed informed consent document will be obtained.

There is a possibility that a platelet AUC may show a sustained increase via "Long Transfusion", though it is not known at this time. Thus, this study may offer a benefit from participation by reducing the risk of bleeding from refractory thrombocytopenia.

This study is likely to yield generalizable knowledge about the role of slow transfusion in ameliorating platelet transfusion refractoriness.

11.8. Classification of Risk for the Study as a Whole

The research risk of the protocol is no greater than minimal risk with the prospect of direct benefit, due to the potential for prolonging platelet survival with a 4-HOUR infusion.

The risks of this study are reasonable in relation to the indirect benefits that will be provided. For adult research subjects, the level of risk is greater than minimal risk, as defined in 45 CFR 46.102.

11.9. Informed Consent, Consent Documents and Process

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study. Study investigators designated as able to obtain consent (asterisked investigators), will obtain informed consent.

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing.

<u>Informed Consent for Non-English Speaking Subjects</u>

We anticipate the enrollment of non-English speaking research participants into our study. The IRB approved full consent document will be translated into the subject's native language in accordance with the Clinical MAS Policy M77-2.
If there is an unexpected enrollment of a research participant for which there is no translated IRB approved consent document, the PI and/or those authorized to obtain informed consent will use the short form oral consent process as described in MAS Policy M77-2, 45 CFR 46.117 (b) (2) and 21 CFR 50.27 (b) (a). The English version of the IRB approved consent document will be used as the summary of the text to be provided.

We prospectively request the IRB approve the use of the short form consent process in up to five of enrollees who are non-English speaking. We will notify the IRB at the time of continuing review of the frequency of the use of the short form process (number per language). Should we reach the threshold of five of participants, we will notify the IRB of the need for an additional use of the short form and that we will have the consent document translated into the given inherent language.

11.10. Conflict of Interest

The PI assures that each AI listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation will be provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial members of the research team reported a potential conflict of interest.

12. ALTERNATIVES TO PARTICIPATION

Subjects receive standard care platelets on this study and do not forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

13. TECHNOLOGY TRANSFER

No technology transfer agreement is in place for this protocol.

14. REFERENCES

1. Blajchman MA, Slichter SJ, Heddle NM, Murphy MF. New Strategies for the Optimal Use of Platelet Transfusions. *Hematology* 2008; **2008**(1): 198-204.
2. Meyer E, Delaney M, Lin Y, et al. A reporting guideline for clinical platelet transfusion studies from the BEST Collaborative. *Transfusion* 2012; **53**(6): 1328-34.
3. Slichter SJ. Evidence-Based Platelet Transfusion Guidelines. *Hematology* 2007; **2007**(1): 172-8.
4. Slichter SJ. Platelet Transfusion Therapy. *Hematology/Oncology Clinics of North America* 2007; **21**(4): 697-729.
5. Kaufman RM, Djulbegovic B, Gernsheimer T, et al. Platelet transfusion: a clinical practice guideline from the AABB. *Ann Intern Med* 2015; **162**(3): 205-13.
6. Hochsmann B, Moicean A, Risitano A, Ljungman P, Schrezenmeier H. Supportive care in severe and very severe aplastic anemia. *Bone Marrow Transplant* 2013; **48**(2): 168-73.
7. The 2011 National Blood Collection and Utilization Survey Report. http://www.hhs.gov/ash/bloodsafety/2011-nbcus.pdf (accessed March 25, 2017.

8. Legler TJ, Fischer I, Dittmann J, et al. Frequency and causes of refractoriness in multiply transfused patients. *Ann Hematol* 1997; **74**(4): 185-9.
9. Hod E, Schwartz J. Platelet transfusion refractoriness. *Br J Haematol* 2008; **142**(3): 348-60.
10. Slichter SJ, Davis K, Enright H, et al. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. *Blood* 2005; **105**(10): 4106-14.
11. Novotny VMJ. Prevention and Management of Platelet Transfusion Refractoriness. *Vox Sanguinis* 1999; **76**(1): 1-13.
12. Rebulla P. Refractoriness to platelet transfusion. *Current Opinion in Hematology* 2002; **9**(6): 516-20.
13. Meehan KR, Matias CO, Rathore SS, et al. Platelet transfusions: utilization and associated costs in a tertiary care hospital. *Am J Hematol* 2000; **64**(4): 251-6.
14. Kerkhoffs JL, Eikenboom JC, van de Watering LM, van Wordragen-Vlaswinkel RJ, Wijermans PW, Brand A. The clinical impact of platelet refractoriness: correlation with bleeding and survival. *Transfusion* 2008; **48**(9): 1959-65.
15. Rebulla P. A mini-review on platelet refractoriness. *Haematologica* 2005; **90**(2): 247-53.
16. Pavenski K, Freedman J, Semple JW. HLA alloimmunization against platelet transfusions: pathophysiology, significance, prevention and management. *Tissue Antigens* 2012; **79**(4): 237-45.
17. Handin RI, Stossel TP. Phagocytosis of antibody-coated platelets by human granulocytes. *N Engl J Med* 1974; **290**(18): 989-93.
18. Fontaine MJ, Kuo J, Chen G, et al. Complement (C1q) fixing solid-phase screening for HLA antibodies increases the availability of compatible platelet components for refractory patients. *Transfusion* 2011; **51**(12): 2611-8.
19. Blanchette VS. Intravenous Gamma-Globulin (Sandoglobulin®) Therapy in the Management of Four Patients with Immune Thrombocytopenic Purpura. *Vox Sanguinis* 1985; **49**: 32-41.
20. Christie DJ, Howe RB, Lennon SS, Sauro SC. Treatment of refractoriness to platelet transfusion by protein A column therapy. *Transfusion* 1993; **33**(3): 234-42.
21. Schiffer CA, Keller C, Dutcher JP, Aisner J, Hogge D, Wiernik PH. Potential HLA-matched platelet donor availability for alloimmunized patients. *Transfusion* 1983; **23**(4): 286-9.
22. Zeigler ZR, Shadduck RK, Rosenfeld CS, et al. Intravenous gamma globulin decreases platelet-associated IgG and improves transfusion responses in platelet refractory states. *American Journal of Hematology* 1991; **38**(1): 15-23.
23. Vo PT, Purev E, West KA, et al. Complement Inhibition Using Eculizumab Overcomes Platelet Transfusion Refractoriness in Allo-Immunized Patients Receiving HLA Mismatched Platelets. Am Soc Hematology; 2016.
24. Narvios A, Reddy V, Martinez F, Lichtiger B. Slow infusion of platelets: a possible alternative in the management of refractory thrombocytopenic patients. *Am J Hematol* 2005; **79**(1): 80.
25. Tzadok S, Gurevich A, Inbal A, Bar-Natan M, Wolach O, Raanani P. Continuous Platelet Transfusion Increases Platelet Increment in Refractory Hemato-Oncological Patients–a Single Center Experience. *Blood* 2014; **124**(21): 2888-.

26. Cid J, Guijarro F, Carbasse G, Lozano M. 24-h continuous infusion of platelets for patients with platelet transfusion refractoriness. *Br J Haematol* 2017.
27. Mazzara R, Escolar G, Garrido M, et al. Procoagulant effect of incompatible platelet transfusions in alloimmunized refractory patients. *Vox Sang* 1996; **71**(2): 84-9.
28. Slichter SJ. Relationship between platelet count and bleeding risk in thrombocytopenic patients. *Transfus Med Rev* 2004; **18**(3): 153-67.
29. Slichter SJ, Kaufman RM, Assmann SF, et al. Dose of prophylactic platelet transfusions and prevention of hemorrhage. *N Engl J Med* 2010; **362**(7): 600-13.
30. Tambur AR, Herrera ND, Haarberg KM, et al. Assessing Antibody Strength: Comparison of MFI, C1q, and Titer Information. *Am J Transplant* 2015; **15**(9): 2421-30.
31. Estcourt LJ, Heddle N, Kaufman R, et al. The challenges of measuring bleeding outcomes in clinical trials of platelet transfusions. *Transfusion* 2013; **53**(7): 1531-43.
32. Nietert PJ, Dooley MJ. The power of the sign test given uncertainty in the proportion of tied observations. *Contemporary clinical trials* 2011; **32**(1): 147-50.
33. Delaney M, Wendel S, Bercovitz RS, et al. Transfusion reactions: prevention, diagnosis, and treatment. *Lancet* 2016; **388**(10061): 2825-36.
34. INTERCEPT Blood System for Platelets SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED). December 18, 2014. https://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/UCM431243.pdf.
35. INTERCEPT® Blood System for Platelets Manufacturer insert. 2016. https://intercept-usa.com/images/resources/Package_Inserts/INTERCEPT_Blood_System_SV_Platelets_Package_Insert_v3.1.pdf.

APPENDIX A: World Health Organization Bleeding Scale

	Grade 1	*Grade 2*	*Grade 3*	*Grade 4*
Oral	• Oropharyngeal bleeding – total duration of all episodes in previous 24 hours ≤ 30 minutes* • Petechiae of oral mucosa • Epistaxis – total duration of all episodes in previous 24 hours ≤ 30 minutes*	• Oropharyngeal bleeding – total duration of all episodes in previous 24 hours > 30 minutes* • Epistaxis – total duration of all episodes in previous 24 hours • 30 minutes*	Any bleeding requiring RBC transfusion over routine transfusion needs†	Any bleeding associated with severe hemodynamic instability (hypotension; >50mm/Hg fall or >50% decrease in either systolic or diastolic blood pressure, with associated tachycardia (heart rate increase of ≥ 20% for 20 minutes) and requiring RBC transfusion over routine transfusion needs • Fatal bleeding from any source • Retinal bleeding with visual impairment (Visual impairment is defined as a field deficit, and patients with suspected visual impairment require an ophthalmologic consult for documentation) • CNS symptoms with non-traumatic bloody lumbar puncture • CNS bleeding on imaging study with or without dysfunction
Skin, soft tissue, musculoskeletal	• Petechiae of skin • Purpura ≤ 1 inch diameter • One or more spontaneous • hematomas in the soft tissue or muscle > 1 inch	• Purpura > 1 inch diameter • Spontaneous hematoma in deeper tissues • Joint bleeding (confirmed by aspiration, imaging study or other accepted technique)	Any bleeding requiring RBC transfusion over routine transfusion needs†	
Gastrointestinal	• Positive stool occult blood test‡	• Melanotic stool • Hematochezia – visible red blood mixed in stool, not requiring a transfusion • Hematemesis – grossly visible blood in emesis or in nasogastric drainage tube (not related or secondary to swallowed blood)	Any bleeding requiring RBC transfusion over routine transfusion needs†	
Genitourinary	• Any biochemical or microscopic Hb/RBCs without red urine‡ • Abnormal vaginal bleeding (Unexpected bleeding out of normal cycle *or* bleeding heavier than normal *or* breakthrough bleeding (patient on hormonal therapy to prevent bleeding)) with spotting	• Gross/visible hematuria without need for transfusion • Abnormal vaginal bleeding (Unexpected bleeding out of normal cycle *or* bleeding heavier than normal *or* breakthrough bleeding (patient on hormonal therapy to prevent bleeding)) more than spotting	Any bleeding requiring RBC transfusion over routine transfusion needs†	
Pulmonary		- Hemoptysis – visible blood - Blood in broncho-pulmonary lavage, or blood tinged sputum (excluding those with nose or oropharyngeal bleeding)	Any bleeding requiring RBC transfusion over routine transfusion needs†	
Body cavity		- Visible blood in body cavity fluid (e.g. red cells apparent in fluid aspirate) short of criteria for Grade 3 or 4	Grossly bloody body cavity fluids and organ dysfunction with symptoms, and/or need to intervene (e.g. to aspirate), and/or need for transfusion	
Central nervous system		- Retinal bleeding without visual impairment - Lumbar puncture with blood (>5 RBC/mL in CSF on microscopic analysis and non-traumatic tap), no symptoms and no visible red color	Lumbar puncture with visible red color in absence of symptoms, and non-traumatic tap	
Invasive sites		- Bleeding at invasive sites (venipuncture sites, intravenous lines or catheter exit sites): active oozing at site for a cumulative total of >1 hour in the previous 24 hours	Any bleeding requiring RBC transfusion over routine transfusion needs†	
Hemodynamic instability			Any bleeding associated with moderate hemodynamic instability (hypotension; >30mmHg fall or >30% decrease in either systolic or diastolic blood pressure) and requiring RBC transfusion over routine transfusion needs†	

RBC indicates red blood cell; Hb, hemoglobin; CSF, cerebrospinal fluid; Hg, mercury; and CNS, central nervous system †Red cell transfusion must be specifically related to treatment of bleeding within 24 hours of onset of bleeding

** Count actual bleeding (i.e. "running out" or need for basin, Kleenex, towel, etc.) not minor bleeding ‡Not assessed in PLADO*

APPENDIX B: Protocol Worksheet

The SOLID Platelet Study, Protocol #19-CC-0005

LONG Transfusion followed by SHORT Transfusion

GROUP	BLOCK	Date *(mm-dd-yy)*	Randomly Assigned ID

PATIENT CRIS LABEL

INSTRUCTIONS:

- *All CBCs should be collected in a 2 mL EDTA tube;* ***please WASTE the first 1 mL at each sample collection***
- *CBC collection times may occur (+/-) 15 minutes of the target draw time*
- *Documenting 'EXPECTED TIME' is optional; it is provided as a reference tool for planning the CBC collection and transfusion START / STOP Schedule*
- *Please call Dr. Flegel (DTM, Protocol PI) with any questions, concerns, or if you run in to any problems, at 301.496.5000.*

	EXPECTED TIME *(Optional)*	ACTUAL TIME	PLATELETS (10^9/L)	HEMOGLOBIN (g/DL)	TO BE COMPLETED BY DTM
1. PRE (0-HOUR) *i.* ***START Long Transfusion AFTER CBC collection*** *ii.* ***Continue transfusing over 4-HOURS*** *iii.* ***Gently Swirl Bag for 20 revolutions every 30-min***					
A. **CBC COLLECTION**					
B. **LONG TRANSFUSION START**					
2. 2-HOUR (1b) + 2-HOURS					
A. **CBC COLLECTION**					
3. 4-HOUR (1b) + 4-HOURS - STOP Long Transfusion BEFORE CBC Collection					
A. **LONG TRANSFUSION STOP**					
B. **CBC COLLECTION**					
4. 6-HOUR (1b) + 6-HOURS *i.* ***START Short Transfusion AFTER CBC Collection*** *ii.* ***Also serves as the PRE (0-HOUR) CBC for the Short Transfusion Transfuse for no more than 60-minutes*** *iii.* ***Gently Swirl Bag for 20 revolutions every 30-minutes***					
A. **CBC COLLECTION**					
B. **SHORT TRANSFUSION START**					
5. 7-HOUR (4b) + 1-HOUR - STOP Short Transfusion					
A. **SHORT TRANSFUSION STOP**					
6. 8-HOUR (4b) + 2-HOURS					
A. **CBC COLLECTION**					
7. 10-HOUR (4b) + 4-HOURS					
A. **CBC COLLECTION**					
8. 12-HOUR (4b) + 6-HOURS					
A. **CBC COLLECTION**					

Print Name of RN(s) Completing form	Signature	Date
1)		
2)		

APPENDIX C: Protocol Worksheets

The SOLID Platelet Study, Protocol # 19-CC-0005

SHORT Transfusion followed by LONG Transfusion

GROUP	BLOCK	Date *(mm-dd-yy)*	Randomly Assigned ID

PATIENT CRIS LABEL

INSTRUCTIONS:

- *All CBCs should be collected in a 2 mL EDTA tube;* ***please WASTE the first 1 mL at each sample collection***
- *CBC collection times may occur (+/-) 15 minutes of the target draw time*
- *Documenting 'EXPECTED TIME' is optional; it is provided as a reference tool for planning the CBC collection and transfusion START / STOP Schedule*
- *Please call Dr. Flegel (DTM, Protocol PI) with any questions, concerns, or if you run in to any problems, at 301.496.5000.*

	EXPECTED TIME *(Optional)*	ACTUAL TIME	PLATELETS (10^9/L)	HEMOGLOBIN (g/DL)	TO BE COMPLETED BY DTM
1. PRE (0-HOUR) *i.* ***START Short Transfusion AFTER CBC collection*** *ii.* ***Transfuse for no more than 60-minutes*** *iii.* ***Gently swirl bag for 20 revolutions every 30-minutes***					
A. **CBC COLLECTION**					
B. **SHORT TRANSFUSION START**					
2. 1-HOUR *(1b) + 1-HOUR - STOP Short Transfusion*					
A. **SHORT TRANSFUSION STOP**					
3. 2-HOUR *(1b) + 2-HOURS*					
A. **CBC COLLECTION**					
4. 4-HOUR *(1b) + 4-HOURS*					
B. **CBC COLLECTION**					
5. 6-HOUR *(1b) + 6-HOURS* *i.* ***START Long Transfusion AFTER CBC Collection*** *ii.* ***Also serves as the PRE (0-HOUR) CBC for the Long Transfusion*** *iii.* ***Continue transfusing over 4-HOURS*** *iv.* ***Gently swirl bag for 20 revolutions every 30-minutes***					
A. CBC COLLECTION					
B. LONG TRANSFUSION START					
6. 8-HOUR (4b) + 2-HOURS					
A. CBC COLLECTION					
7. 10-HOUR (4b) + 4-HOURS					
A. LONG TRANSFUSION STOP					
B. CBC COLLECTION					
8. 12-HOUR (4b) + 6-HOURS					
A. CBC COLLECTION					

Print Name of RN(s) Completing form	Signature	Date
1)		
2)		