ORIGINAL ARTICLE

A randomized controlled clinical trial evaluating the performance and safety of platelets treated with MIRASOL pathogen reduction technology

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BACKGROUND: Pathogen reduction of platelets (PRT-PLTs) using riboflavin and ultraviolet light treatment has undergone Phase 1 and 2 studies examining efficacy and safety. This randomized controlled clinical trial (RCT) assessed the efficacy and safety of PRT-PLTs using the 1-hour corrected count increment (CCI_{1hour}) as the primary outcome.

STUDY DESIGN AND METHODS: A noninferiority RCT was performed where patients with chemotherapy-induced thrombocytopenia (six centers) were randomly allocated to receive PRT-PLTs (Mirasol PRT, Caridian-BCT Biotechnologies) or reference platelet (PLT) products. The treatment period was 28 days followed by a 28-day follow-up (safety) period. The primary outcome was the CCI_{1hour} determined using up to the first eight on-protocol PLT transfusions given during the treatment period.

RESULTS: A total of 118 patients were randomly assigned (60 to PRT-PLTs; 58 to reference). Four patients per group did not require PLT transfusions leaving 110 patients in the analysis (56 PRT-PLTs; 54 reference). A total of 541 on-protocol PLT transfusions were given (303 PRT-PLTs; 238 reference). The least square mean CCI was 11,725 (standard error [SE], 1.140) for PRT-PLTs and 16,939 (SE, 1.149) for the reference group (difference, −5214; 95% confidence interval, −7542 to −2887; $p < 0.0001$ for a test of the null hypothesis of no difference between the two groups).

CONCLUSION: The study failed to show noninferiority of PRT-PLTs based on predefined CCI criteria. PLT and red blood cell utilization in the two groups was not significantly different suggesting that the slightly lower CCIs (PRT-PLTs) did not increase blood product utilization. Safety data showed similar findings in the two groups. Further studies are required to determine if the lower CCI observed with PRT-PLTs translates into an increased risk of bleeding.

Over the past two decades significant progress has been made to prevent transmission of viruses and bacteria through blood transfusion including improved donor screening at the time of donation, introduction of nucleic acid testing for virus detection, screening for bacteria, and the diversion pouch used at the time of donation to reduce bacterial contamination.[1,2] In spite of these improvements, notable risks still remain for transmitting some blood-borne pathogens. Viral transmission can still occur during the window period when tests are unable to detect low pathogen load, because some tests lack optimal sensitivity, or due to the fact that practical and effective donor screening methods for certain known pathogens may not be available. Transfusion-associated sepsis due to bacteria in the blood product also occurs as bacterial testing is not performed universally, and current detection systems are only partially effective at identifying contaminated products. However, the greatest concern driving the development of new technologies to prevent pathogen transmission is the risk of blood supply contamination by new pathogens, or new strains of known pathogens, for which no tests currently exist.[1]

For more than a decade, research has focused on the development of safe and effective methods of pathogen

reduction in the anticipation that these methods would be effective in preventing transmission of known pathogens and provide protection against emerging or mutant strains or viruses and bacteria.

Methods of pathogen reduction for red blood cells (RBCs), platelets (PLTs), and plasma are currently in development with some of these methods already in clinical use in Europe.[3] Several of these technologies use photochemical agents, which can be activated by ultraviolet (UV) light resulting in chemical modifications to DNA and RNA that prevent their replication.[4-12] This renders the pathogens present in the blood product incapable of replication during storage and also incapable of causing infectious complications in the patient after transfusion. One pathogen reduction process for PLTs (Mirasol pathogen reduction technology [PRT]; CaridianBCT, Lakewood, CO), utilizes exposure to UV light in the presence of riboflavin to introduce irreparable lesions to nucleic acids thereby inhibiting pathogens and white blood cell (WBC) replication.[13] Riboflavin is a nontoxic and nonmutagenic compound; hence, it does not have to be removed at the end of the process.[14] This technology has been shown to substantially reduce the active pathogen load[15-21] in PLT products, and effectively inactivate residual WBCs that may be present in blood components; hence, there is strong evidence that this technology prevents transfusion-associated graft-versus-host disease.[22-25] Laboratory studies have also documented acceptable metabolic and functional characteristics as measured by a battery of in vitro PLT function tests.[26,27] Hence, with Phase 1 and 2 studies suggesting that this technology appears safe and effective for reducing pathogen transmission, a larger clinical study was warranted.

We report on a randomized controlled trial (RCT) conducted to determine if pathogen-reduced PLTs (PRT-PLTs) are as effective as standard untreated PLT products when transfused to patients with chemotherapy-induced thrombocytopenia with respect to the corrected count increment 1 hour posttransfusion (CCI_{1hour}). The study was also designed to provide safety information of PRT-PLTs by documenting all adverse events.

MATERIALS AND METHODS

Study design

This was a multicenter, open-label, parallel-group noninferiority RCT conducted in France by the Etablissement Francais du Sang and university hospitals (n = 6; see Acknowledgments), which compared PRT-PLTs and standard (reference) PLT products when transfused to thrombocytopenic hematology and/or oncology patients. The study was approved by the central research ethics committees for the participating centers, and was registered at http://www.clinicaltrials.gov (NCT00263809) and at the AFSSAPS official trial site.

Study population

There was a two-stage process for assessing patient eligibility. In Phase 1, patients were deemed eligible for further assessment if they met the following inclusion criteria: age 16 years or older; thrombocytopenia due to chemotherapy, malignant hemopathy, allogeneic or autologous hematopoietic stem cell transplantation, or diagnosis of a solid tumor with expectation to receive at least two PLT transfusions; and being treated as an inpatient. Eligible patients were excluded if one or more of the following criteria were satisfied: pregnancy, lactation, splenomegaly, and history or diagnosis of an autoimmune disease affecting hemostasis. Patients meeting the Phase 1 eligibility criteria were approached for informed consent. The rationale and objectives of the study were explained to patients by the site investigator or coinvestigator. Informed consent was required from all participants in accordance with the Declaration of Helsinki. Consenting patients underwent a Phase 2 screening process to confirm eligibility. Patients were excluded if any of the following criteria were present: positive serum or urine pregnancy test within 72 hours of randomization; history of hypersensitivity to riboflavin or metabolites; history of refractoriness to PLT transfusion (two successive $CCI_{1hour} < 5000$); presence of HLA antibodies, positive lymphocytotoxicity test, or previously documented alloimmunization to PLTs (as per individual site testing protocols); active bleeding requiring one or more RBC transfusions; acute or chronic disseminated intravascular coagulation; history or a diagnosis of immune/idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, or hemolytic uremic syndrome; history of solid organ transplant; evidence of veno-occlusive disease; temperature of more than 39.5°C and/or signs of infection; enrollment in a pathogen reduction clinical trial within the previous 6 months; exposure to any other investigational product within 30 days of randomization; taking study-prohibited medications within 14 days of randomization (see Supporting Appendix S1, available as supporting information in the online version of this paper); evidence of chronic alcohol misuse;[28] and any other medical condition that could compromise participation.

Patients meeting the Phase 2 eligibility criteria were randomly assigned to receive reference PLTs or PRT-PLTs. The random treatment allocation scheme involved stratification by center and blocking and was computer generated by the coordinating center (MedPass International, Paris, France). Patient allocation was performed at each site using opaque envelopes containing the treatment assignment. Due to the slight yellow color of PRT-PLTs the study could not be conducted in a double-blind manner; however, those individuals assessing PLT counts and performing patient assessments were blinded to the patient's treatment allocation.

The following data were collected at the initial randomization visit: height, weight, vital signs, concomitant treatments, and laboratory test results (D-dimer, albumin, alkaline phosphatase, alanine aminotransferase [ALT], blood urea nitrogen, lactate dehydrogenase [LDH], potassium, total protein, fibrinogen, creatinine, urea, bilirubin, complete blood count, and lymphocytotoxicity testing). Blood samples were also collected for detection of photoproducts and neoantigen formation (results reported in a separate article).[28]

Interventions

Reference and PRT-PLT products were collected by apheresis (Trima Version 5.0, CaridianBCT) or prepared from buffy coats using pools from six whole blood collections using the OptiPress (Fenwal, Inc., Round Lake, IL) device with a top-and-bottom separation process and conventional PLT pooling methods. All PLTs were leukoreduced in accordance with French requirements (residual WBC content below 10^6/product in >97% of production). Product requirements included: volume of 170 to 360 mL, concentration of 1180×10^9 to 2100×10^9 PLTs/L plasma, and minimum-maximum PLT yield of 3.0×10^{11} and 5.1×10^{11} PLTs, respectively. All products were suspended in plasma and stored at 22°C with agitation for a maximum of 5 days. Products that failed requirements (see Supporting Appendix S2, available as supporting information in the online version of this paper) were not used in the study.

The PRT-PLTs were prepared using MIRASOL PRT. After the rest period (2 hr postcollection of apheresis PLTs and 1 hr postpreparation of buffy coat PLTs), the PLTs were transferred into an illumination/storage bag and riboflavin solution was added (500 µmol/L, 35 ± 5 mL). The bag was sealed using the MIRASOL PRT Welder. The product was placed in the illuminator and exposed to light at 6.24 J/mL[26] and then labeled "Exclusively for Clinical Investigation."

The recommended transfusion trigger was 10×10^9/L when clinical risk factors were absent; 20×10^9/L when there was fever, hypertension, evidence of Grade 2 mucositis, lesions with bleeding potential and/or a rapid decrease in PLT count occurred within 72 hours; and 50×10^9/L if antithrombotics were administered, if there was evidence of fibrinolysis or coagulopathy, or invasive surgery was required.[29] Patients could withdraw from the study at any time or could be withdrawn at their physician's discretion based on clinical or laboratory findings that suggested that participation may not be in the patient's best interest.

The treatment period started at the time of randomization (Day 0) and continued for a maximum of 28 days. The following reasons accounted for patient termination before Day 28: no need for additional on-protocol PLT transfusions, withdrawal due to an adverse event, withdrawal of consent, lost to follow-up, transfer to another hospital service (e.g., intensive care unit), or death. After the treatment period, a safety follow-up period began with Day 1 being the day after the last on-protocol transfusion in the treatment period continuing for 28 days (range, 23-42 days considered acceptable), day of withdrawal (adverse event/withdrawal of consent), lost to follow-up, or death, whichever occurred first. A transfusion was defined as off-protocol if 1) the product did not meet the prespecified criteria (defined above), 2) a patient randomized to PRT-PLTs received a non–PRT-PLT product, or 3) a PLT transfusion was given outside of the 28-day treatment period.

Product information collected with each on-protocol transfusion included weight (g), PLT count, ABO group, collection and/or manufacturing method, whether the product was gamma irradiated, transfused volume, and date and time of transfusion. Patient information for each on-protocol PLT transfusion was collected before and 24 hours posttransfusion and included weight, vital signs, evidence of bleeding, concomitant treatments, creatinine, urea, bilirubin, and complete blood count. Similar documentation occurred at 1 hour posttransfusion with the exception of creatinine, urea, and bilirubin. At the end of the safety follow-up period the same assessment was performed as the pretransfusion assessment. Bleeding assessments for on-protocol PLT transfusion were performed by hospital staff (physicians or nursing staff) who were appropriately trained to score according to WHO bleeding assessment criteria.[30] This included a physical examination for signs and symptoms of bleeding and a review of the patient's chart for documentation of bleeding. A bleeding assessment was also performed at the last study follow-up visit.

Study outcomes

The primary efficacy outcome was the CCI_{1hour} measured 30 to 90 minutes posttransfusion for each of a maximum of eight on-protocol PLT transfusions per patient occurring within the 28-day treatment period. The patient's pretransfusion PLT count for this calculation had to be measured within 12 hours of the transfusion. Transfusions where the 1-hour measurement was taken 30 to 90 minutes posttransfusion were considered time compliant. Measurements taken within 0 to 120 minutes posttransfusion were also analyzed as an extended time period. Transfusions with measurements taken after 120 minutes were not included in these analyses. CCI was calculated using the formula

$$CCI = \frac{Post - pre\ count\ (\times 10^9/L)}{Platelet\ dose\ transfused\ (\times 10^{11})} \times BSA^*$$

$$*BSA\,(Body\ Surface\ Area)$$
$$= 0.0202457 \times Height_m^{0.725} \times Weight_{kg}^{0.425}.$$

Secondary outcomes included CCI_{24hour} (specimens collected 18-26 hr posttransfusion were considered time compliant and 15-30 hr posttransfusion defined the extended time period), interval between transfusions, number of PLT and RBC transfusions per subject during the treatment period, number of PLTs transfused normalized by body surface area and for the number of days in the treatment period, evidence of refractoriness (two consecutive transfusions with a $CCI_{1hour} < 5000$), frequency of transfusion associated infections, and bleeding (WHO Grades 1-4).

Safety outcomes were captured during the treatment and follow-up periods including adverse events, serious adverse events (SAEs), bleeding status on days of PLT transfusion, transfusion-associated infections, and death. Adverse events were categorized as mild, moderate, or severe. The causal relationship was classified as unrelated, unlikely, possible, probable, or highly probable (see Supporting Appendix S3, available as supporting information in the online version of this paper). Adverse events were coded according to the Common Toxicity Criteria Scale (CTCAE Version 3.0/MedDRA Version 6.0, MedDRA MSSO, Chantilly, VA). All SAEs were reported to the coordinating center within 24 hours of the event being identified and to other relevant authorities. Alloimmunization to neoantigens was also assessed with results reported in a separate publication.[28]

Sample size

It was estimated that the mean CCI_{1hour} in the reference group would be 14,700 (standard deviation [SD], 5200; based on the results of the TRAP study).[31] With a Type 1 error of 2.5% and power of 80%, it was determined that 50 patients would be required per group to claim noninferiority of PRT-PLTs compared to standard practice with a noninferiority margin of 20% (CCI difference of 2940). This sample size was increased to 118 to accommodate some loss to follow-up. If the lower limit of a two-sided 95% confidence interval (CI) for the difference (PRT-PLTs—reference) in mean CCI_{1hour} is above –2940, noninferiority would be demonstrated.

Data Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) was composed of two transfusion medicine experts, one biostatistician, and one physician, all independent of the study sponsor. The DSMB monitored unblinded safety and performance data, made recommendations related to protocol changes and continuing/stopping the study, and reviewed all SAEs providing their final adjudication. An interim analysis was planned a priori and performed by an independent group after 54 randomized patients completed follow-up; however, formal stopping rules were not specified a priori.

Statistical analysis

Descriptive analyses were conducted for the demographic and clinical variables. Continuous variables were summarized by their means and SDs and categorical variables by frequencies and percentages. The frequency of on-and off-protocol transfusions was tabulated.

The primary and secondary outcomes (CCI_{1hour} and CCI_{24hour}, respectively) were analyzed using a mixed-effects analysis of covariance model with a random patient effect to accommodate the association in the responses within patients over multiple transfusions and controlling for pretransfusion PLT count and treatment group.[32] For each treatment group, least square (LS) means and standard errors (SE) were reported based on fits using computer software (PROC MIXED, SAS 9.1.3, SAS Institute, Inc., Cary, NC) and compared between treatment arms. By recognizing that responses to serial transfusions may not be independent within patients, this approach recognizes all sources of variability and ensures valid inferences. Analysis included up to the first eight time-compliant on-protocol PLT transfusions received during the treatment period for all randomized patients who received at least one transfusion. A secondary analysis also included transfusions where posttransfusion measurements occurred within the extended time period.

Interactions between treatment group and pretransfusion PLT count were tested to examine whether there was evidence that the effect of PRT-PLTs varied for different pretransfusion PLT counts. Similar tests were carried out for interactions between response and site to test for the poolability of data across sites.

A mixed longitudinal logistic regression model[33] was also fit to assess the effect of PRT-PLTs versus reference PLT products on achieving a 7500 CCI at 1 hour and 4500 CCI at 24 hours posttransfusion.[34] Pretransfusion PLT count and a random patient effect were included in this model with the latter accounting for an association in the responses over time. Frailty models were fit to estimate the distribution of times between transfusions while accounting for the within-patient dependence in the gap times.[35] All p values for secondary outcome comparisons were two-tailed tests. Adverse event data were summarized in tabular form and analyzed descriptively.

The primary and secondary analyses were repeated in a post hoc subgroup analysis of 95 patients. This subgroup was obtained by excluding 15 patients with incomplete data (eight receiving reference PLTs and seven receiving PRT-PLTs) after discussion with the DSMB.

RESULTS

Six centers enrolled 118 patients into the study between December 2005 and September 2007: 60 patients received PRT-PLTs and 58 received reference PLTs. Four patients in each treatment group did not receive PLT transfusions leaving 110 patients that could be included in the intention-to-treat analysis. There were 10 of 110 patients who withdrew from the study before Day 28 in the treatment period (six in the PRT-PLT arm; four in the reference arm); hence, the proportion of patients completing the treatment period in the PRT-PLT group was 91.1% (51/56) and 98.1% (53/54) in the reference group. Data from these 10 patients were included in the analyses up until the time of their withdrawal. The proportion of patients completing the safety follow-up period was 73.2% (41/56) for PRT-PLTs and 81.5% (44/54) for the reference arm (median durations both study periods being 45 and 44 days, respectively). Patient flow through the study is summarized in Fig. 1.
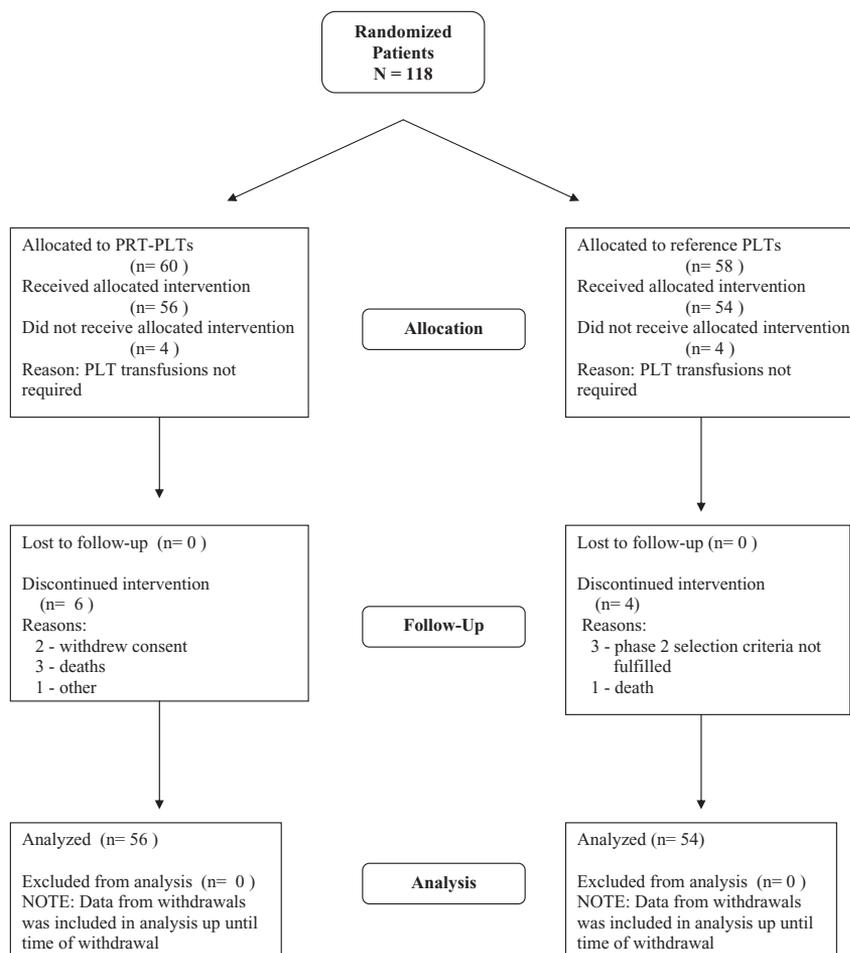
Baseline demographics for the study patients were similar between the two groups and are summarized in Table 1. Other baseline characteristics were documented (data not shown) and showed a similar distribution in both groups (physical findings, vital signs, complete blood count, fibrinogen, albumin, alkaline phosphatase, ALT, creatinine, urea, direct and total bilirubin, blood urea nitrogen, LDH, potassium, and total protein).

There were a total of 678 PLT transfusions given to patients during the study period: 368 PRT-PLT transfusions (303 on-protocol; 65 off-protocol) and 310 reference group transfusions (238 on-protocol; 72 off-protocol). The frequency of off-protocol PLT transfusions was 17.7% for PRT-PLTs and 23.2% in the reference group. Criteria for off-protocol transfusions were prespecified in the protocol; however, the data collection process did not capture the reason.

The prespecified primary outcome analysis for the CCI_{1hour} was based on a maximum of eight PLT transfusions per patient occurring in the 28-day treatment period: 258 for PRT-PLTs and 209 for the reference group (total 467). The test for homogeneity of treatment effects between sites for the CCI_{1hour} was not significant (p = 0.1728), indicating that data from all sites could be pooled to estimate the treatment effect. The LS mean CCI_{1hour} in the PRT-PLT group was 11,725 (SE, 1140) and in the reference group 16,939 (SE, 1149), a difference of −5214 (95% CI, −7542 to −2887; p < 0.0001). The CI for the difference includes the prespecified upper limit of the zone of noninferiority (set at 20% of the mean CCI anticipated in the reference group, which was 2940); hence, noninferiority could not be claimed since to do so would have required the lower limit of this CI to be above −2940. The CCI_{1hour} was also calculated for the extended time period, adjusted for pretransfusion PLT count (continuous variable) and site (Table 2). The CCI_{1hour} data for time-compliant and extended time period transfusions are illustrated in Fig. 2 using box plots.

Secondary outcomes

The CCI_{24hour} was analyzed according to the time-compliant and extended time periods and adjusted for pretransfusion PLT count as a continuous variable and site. The test for homogeneity of the effect of treatment between sites for the

Fig. 1. CONSORT flow diagram showing the flow of patients through the study from the time of randomization to analysis.

TABLE 1. Baseline characteristics for the patients in the PRT-PLTs and reference group

Demographic characteristic	Treatment arm	
	PRT-PLTs (n = 56)	Reference (n = 54)
Median age, years (range)	58 (20-73)	53 (20-74)
Sex (male/female)	32/24	34/20
Median height, m (range)	1.7 (1.5-1.86)	1.7 (1.51-1.93)
Median weight, kg (range)	71.5 (46.3-121.0)	73.6 (45.0-110.3)
ABO blood group, number (%)		
A	30 (53.6)	32 (59.3)
B	5 (8.9)	6 (11.1)
O	21 (37.5)	16 (29.6)
AB	0 (0.0)	0 (0.0)
Diagnosis, number (%)		
Acute lymphocytic leukemia	2 (3.6)	2 (3.7)
Acute myelogenous leukemia	26 (46.4)	27 (50.0)
Multiple myeloma	4 (7.1)	2 (3.7)
Non-Hodgkin's lymphoma	19 (33.9)	18 (33.3)
Hodgkin's lymphoma	1 (1.8)	3 (5.6)
Other*	4 (7.1)	2 (3.7)
Mean (SD duration of thrombocytopenia, days†	16.3 (7.2)	14.8 (7.0)
Median (range) baseline test results		
Hemoglobin (g/dL)	9.5 (8-14)	9.5 (7-15)
PLT count ($\times 10^9$/L)	42.5 (8-479)	43.0 (6-206)
WBC count ($\times 10^9$/L)	1.05 (0-14)	1.30 (0-51)

* Other includes severe idiopathic medullary aplasia (1), biphenotypic acute leukemia (1), chronic lymphocytic leukemia (1), myelodysplasia-refractive anemia with excess blasts (2), and mediastinal teratocarcinoma (1).
† During treatment period.

TABLE 2. Summary CCI values by treatment group based on the first eight on-protocol transfusions (primary outcome) and all on protocol PLT transfusions within the treatment period*

Outcome	PRT-PLTs		Reference		PRT-PLTs minus Reference		
CCI—continuous outcome	Number	LS mean (SE)	Number	LS mean (SE)	Difference	95% CI	p value
Analysis based on the first eight on-protocol transfusions within the 28-day treatment period							
CCI_{1hour}							
Time compliant	195	11,725 (1,140)	164	16,939 (1,149)	−5214	(−7542 to −2887)	<0.0001
Extended time	216	11,766 (1,072)	174	17,170 (1,057)	−5404	(−7721 to −3088)	<0.0001
CCI_{24hour}							
Time compliant	175	6,676 (883)	160	9,886 (915)	−3210	(−5160 to −1260)	0.0014
Extended time	209	6,998 (811)	179	10,385 (811)	−3387	(−5232 to −1542)	0.0004
Analysis based on all on-protocol transfusions within the 28-day treatment period							
CCI_{1hour}	273	11,005 (962)	220	16,614 (977)	−5609	(−7791 to −3427)	<0.0001
CCI_{24hour}	267	7,162 (831)	211	10,070 (839)	−2907	(−4802 to −1013)	0.0027
CCI—dichotomous outcome	Number	Number (%)	Number	Number (%)	OR	95% CI	p value
Analysis based on the first eight on-protocol transfusions within the 28-day treatment period							
Time compliant							
$CCI_{1hour} > 7500$	195	139 (71.3)	164	138 (84.1)	0.284	(0.105 to 0.767)	0.0130
$CCI_{24hour} > 4500$	175	103 (58.9)	160	109 (68.1)	0.481	(0.211 to 1.098)	0.0822
Extended time							
$CCI_{1hour} > 7500$	216	151 (69.9)	174	147 (84.5)	0.233	(0.081 to 0.667)	0.0067
$CCI_{24hour} > 4500$	209	118 (56.5)	179	120 (67.0)	0.423	(0.189 to 0.945)	0.0360

* Results for the first eight on-protocol transfusions are also presented using CCI as a dichotomous outcome.

CCI_{24hour} was not significant (p = 0.1336) allowing for data to be pooled. The LS mean for time-compliant CCI_{24hour} was 6676 (SE, 883) for the PRT-PLTs and 9886 (SE, 915) in the reference group (difference, −3210; 95% CI, −5160 to −1260). The CCI_{24hour} results are summarized in Table 2 and Fig. 2. Table 2 also contains the results of the mixed logistic regression models and reports the odds ratios (ORs) for achieving the desired CCI increment (7500 and 4500 for CCI_{1hour} and CCI_{24hour}, respectively). The odds of achieving a successful response is significantly lower in the PRT-PLTs arm for the CCI_{1hour} among time-compliant transfusions (OR, 0.284; 95% CI, 0.105 to 0.767; p = 0.0130) but not significantly lower for the CCI_{24hour} among time-compliant transfusions (OR, 0.481; 95% CI, 0.211 to 1.098; p = 0.0822). Similar results were found when considering transfusions within the extended time period although the 24-hour CCI result becomes significant in this analysis.

A meaningful interval between transfusions was difficult to calculate as patients in both treatment groups had off-protocol transfusions within the treatment period.
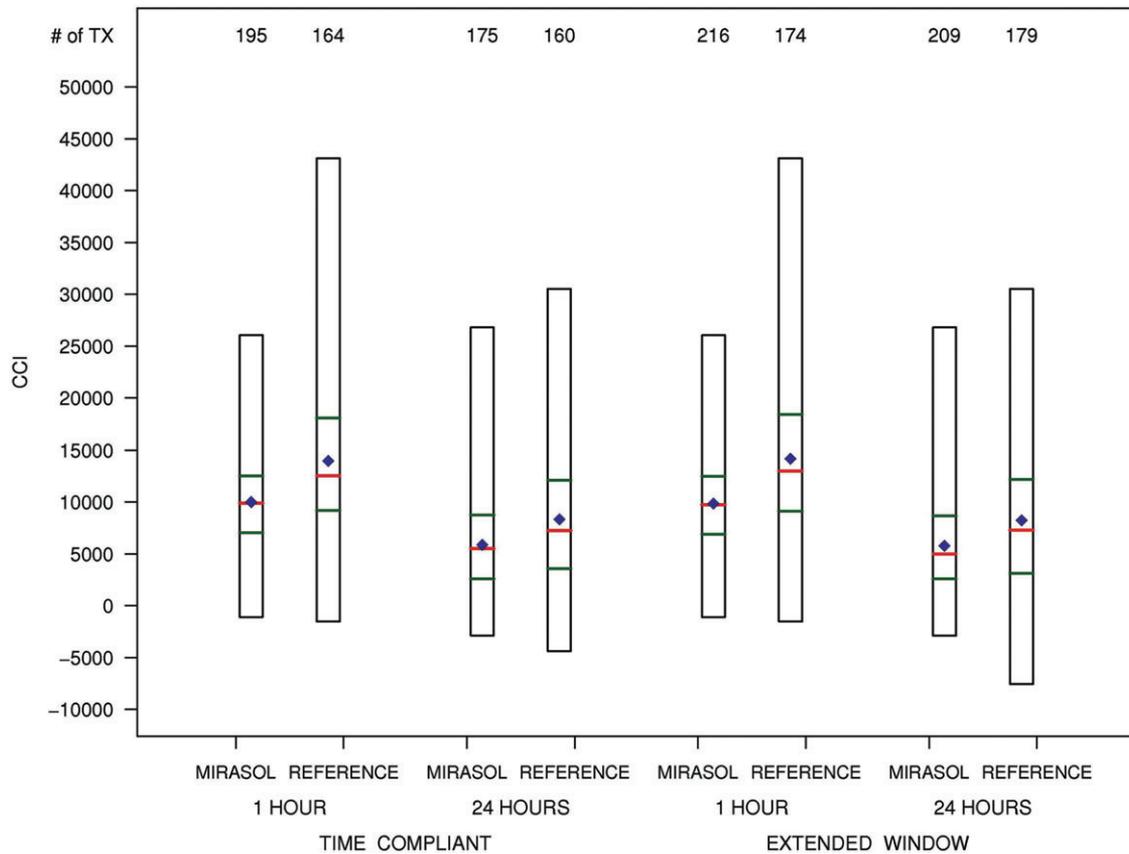
Fig. 2. Box plots of 1- and 24-hour CCIs for transfusions in the time-compliant and extended time periods by treatment group. The lines within the boxes represent the Q_{75} (upper line), median (middle line) and Q_{25} (lower line). The diamond indicates the raw means (the mean of the raw CCI values). The height of the rectangular box indicates the minimum and maximum values.

When both on- and off-protocol transfusions within the 28-day treatment period are included in this analysis the mean number of days between transfusions was 2.16 (SD, 1.69) for PRT-PLTs and 2.30 (SD, 1.48) for the reference arm (p = 0.2903). The mean number of PLT transfusions per patient-day during the treatment period (includes on- and off-protocol transfusions) was not significantly different: PRT-PLTs 0.24 (SD, 0.16) and reference group 0.20 (SD, 0.19; p = 0.2046). Summaries of secondary outcomes are given in Table 3. None of the differences observed were significant.

RBC requirements were similar in the two groups. In the PRT-PLT group 183 RBC units were transfused in the treatment and follow-up periods: 155 were given in the treatment period with a mean (SD) per patient of 2.8 (1.7). In the reference group 142 of 160 RBC units were given in the treatment group with a mean per patient of 2.6 (2.4; p = 0.7257).

Alloimmunization and refractoriness

Two patients in the PRT-PLT group (3.6%) became alloimmunized and four patients in the reference group (7.45%) developed HLA antibodies (p = 0.4336; Fisher's exact). Only 5 of 110 patients (4.5%) became refractory during the study: three (5.4%) in the PRT-PLT group and two (3.7%) in the reference group (p = 1.0000; Fisher's exact).

Infections

There were a total of 88 infectious adverse events reported in 58 study patients. In the PRT-PLT group 45 infections were reported in 28 patients (1.61 infections/patient). Six infections were categorized as severe adverse events: cytomegalovirus (CMV; 1), *Klebsiella* (2), *Escherichia* urinary tract (1), infection (1), and sepsis (1). The one patient who developed CMV infection had positive CMV serology before stem cell transplantation and transfusion. In the reference group there were 43 infections in 30 patients (1.42 infections/patient): nine of these were categorized as severe adverse events: bacterial infection (1), bacterial sepsis (1), bronchopulmonary aspergillosis (1), *Clostridium* colitis (1), *Clostridium difficile* colitis (1), herpes virus infection (1), urinary tract infection enterococcal (1), sepsis (1), and septic shock (1), but none were considered transfusion related. There were no significant

TABLE 3. Summary of the characteristics of the PLT transfusions and the secondary outcomes related to PLT transfusion

| | PLT transfusions | | | | | |
| | On-protocol, limited to first eight transfusions within the 28-day treatment period | | | On protocol, within the 28-day treatment period | | |
Outcome/characteristic	PRT-PLTs	Reference	p value	PRP-PLTs	Reference	p value
Total number of PLT transfusions	258	209		303	238	
Number of apheresis PLT transfusions (%)	180 (69.8)	149 (71.3)		224 (73.9)	178 (74.8)	
Number of buffy coat PLT transfusions (%)	78 (30.2)	60 (28.7)		79 (26.1)	60 (25.2)	
Median number of PLT transfusions/patient (range)	4.0 (1-8)	3.0 (1-8)		4.5 (1-21)	3.0 (1-19)	
Mean (SD) PLT dose transfused ($\times 10^{11}$)	5.37 (2.14)	5.38 (2.10)	0.9615	5.23 (2.09)	5.22 (2.02)	0.9867
Mean age of product at transfusion (days)	2.8 (1.1)	2.6 (1.1)	0.0891	2.7 (1.1)	2.6 (1.1)	0.2210
Total number of ABO-mismatched transfusions (%)	41 (15.9)	37 (17.7)		50 (16.5)	40 (16.8)	
Major mismatch	39 (15.1)	32 (15.3)		46 (15.2)	35 (14.7)	
Minor mismatch	2 (0.8)	5 (2.4)		4 (1.3)	5 (2.1)	
Number (%) of transfusions CCI_{1hour} compliant	195 (81.9)	164 (85.4)		222 (81.3)	185 (84.1)	
Number (%) of transfusions CCI_{24hour} compliant	175 (75.8)	160 (83.3)		196 (73.4)	173 (82.0)	
Mean (SD) interval between PLT transfusions*	2.32 (1.79)	2.72 (1.44)	0.0107	2.16 (1.69)	2.30 (1.48)	0.2903

* The mean (SD) interval between PLT transfusions is calculated based on both on- and off-protocol transfusions.

TABLE 4. Summary of the number of patients with different grades of WHO bleeding and mean rates of all and severe adverse events

| | All analyzable patients (n = 110) | | |
Bleeding by WHO grade and adverse event rates	PRT-PLTs (n = 56)	Reference (n = 54)	p value
Number of subjects with bleeding episodes			
Grade 1	28	19	0.128
Grade 2	11	5	0.176
Grade 3	4	2	0.679
Grade 4	2	1	1.000
Grade 1-4	33	23	0.127
Grades 2-4	12	7	0.315
Grades 3 and 4	6	3	0.490
Mean (SD) rate*			
All adverse events	0.28 (0.19)	0.23 (0.16)	0.144
Severe adverse events	0.07 (0.17)	0.04 (0.06)	0.171

* Total number of events/duration of treatment period and follow-up period.

bleeding, two had Grade 3, and one had Grade 4. The Grade 4 bleed was genitourinary and the patient completed 41 study days. The numbers of bleeding events by grade are summarized in Table 4.

The results of the primary and secondary endpoints are also summarized for the 95 patients that were included in the post hoc subgroup analysis (see Supporting Appendix S4, available as supporting information in the online version of this paper). For the primary endpoint (CCI_{1hour}) noninferiority was not demonstrated. For all secondary endpoints the results were very similar to the analysis including all 110 patients.

differences in the proportion of patients with one or more infections (p = 0.5731; Fisher's exact test), and the mean number of infections/patients/group (p = 0.4571). Table 4 summarizes rates of adverse events and SAEs.

Bleeding outcomes

Bleeding assessments were only performed for on-protocol PLT transfusions with assessments done before transfusion, after transfusion at 1 and 24 hours, and on the final follow-up visit. There were 19 patients with WHO bleeding of Grade 2 or higher: 12 patients in the PRT-PLT arm (21.4%) and seven patients (13.0%) in the reference group. Eleven subjects receiving PRT-PLTs had Grade 2 bleeding, four had Grade 3, and two had Grade 4 (both central nervous system bleeding: one patient died on Day 17 and one patient completed the study). In the reference group there were five patients with Grade 2

Safety outcomes

All patients receiving PRT-PLTs and 98.1% (53/54) of patients in the reference group had at least one reported adverse event; however, the majority of adverse events were not related to the PLT products transfused (Table 5). There were five adverse events (five patients) in the PRT-PLT group that were categorized as "possible, likely, or very likely" and eight adverse events (five patients) in the reference group that fell into these categories. For the severe adverse events two patients in the PRT-PLT group (1.8%) had events that were "very likely" related to a transfusion and two patients in the reference arm had events categorized as "very likely" related. These patients developed anaphylactic shock (one reference patient), hypersensitivity plus eyelid edema (one reference patient), and refractoriness to PLT transfusions (one PRT-PLT patient). There

DISCUSSION

This study was designed to determine whether the CCI_{1hour} for PRT-PLTs was noninferior to untreated PLT products. CCI was selected as the primary outcome because this has been the outcome historically used for licensing of new PLT products treated with PRT methods in Europe.[11] When planning the study, noninferiority would be claimed if the mean CCI_{1hour} of the pathogen-inactivated product did not exceed a reduction in mean CCI of more than 20% of the value observed with untreated PLTs. The study failed to demonstrate noninferiority for either the CCI_{1hour} (primary outcome) or the CCI_{24hour} (secondary outcome). Why pathogen inactivation of PLTs results in a lower CCI is not clear; however, this has been a consistent finding in several other studies. In a crossover RCT enrolling normal subjects, Aubuchon and colleagues[26] found that PRT-PLTs had a reduced mean survival (16.5% lower) and recovery (38 hr less) compared to untreated PLT product. The SPRINT study using amotosalen HCL (S-59) and UVA light to pathogen inactivate also reported lower CCIs at both 1 and 24 hours with the pathogen-inactivated PLT products. The mean CCIs per treatment group reported in the SPRINT study were almost identical to the values observed in this study.[12]

Metabolic activity and expression of activation markers increase in PRT-PLTs during storage;[26] hence, one could hypothesize more rapid utilization of these cells at sites of injury or damage, due to their increased activation status. Similar effects have been seen with dimethyl sulfoxide–cryopreserved PLTs; however, despite demonstrating highly elevated levels of P-selectin expression and other activation markers,[36-40] significantly increased degranulation,[41] and significantly lower levels of recovery in circulation,[42] the cryopreserved PLTs were associated with less bleeding, fewer transfusion support needs, and fewer complications compared to conventional, liquid-stored PLTs.[36,38,43,44] These findings emphasize the need for studies assessing the clinical impact of pathogen-inactivated PLTs that can clearly elucidate the relevance of the in vitro findings.

TABLE 5. The number and frequency of adverse events, severe adverse events, and SAEs by relationship to transfusion*

Adverse events categorized by relationship to transfusion	PRT-PLTs (n = 56)	Reference (n = 54)
Adverse events		
Subjects with at least one adverse event	56 (100)	53 (98.1)
Total number of adverse events	654	507
Relationship of adverse event to study transfusion†		
None	596 (91.1)	477 (94.1)
Unlikely	53 (8.1)	22 (4.3)
Possible	3 (0.5)	2 (0.4)
Likely	0 (0.0)	3 (0.6)
Very likely	2 (0.3)	3 (0.6)
Severe adverse events		
Subjects with at least one adverse event	38 (67.8)	30 (55.6)
Total number of adverse events	110	90
Relationship of adverse event to study transfusion‡		
None	100 (90.9)	86 (95.6)
Unlikely	7 (6.4)	1 (1.1)
Possible	1 (0.9)	0 (0.0)
Likely	0 (0.0)	0 (0.0)
Very likely	2 (1.8)§	3 (3.3)‖
SAEs		
Subjects with at least one adverse event	13 (23.2)	11 (20.4)
Total number of adverse events	17	14
Relationship of adverse event to study transfusion¶		
None	12 (70.6)	12 (85.7)
Unlikely	5 (29.4)	1 (7.1)
Possible	0 (0.0)	0 (0.0)
Likely	0 (0.0)	0 (0.0)
Very likely	0 (0.0)	1 (7.1)**

* Data are reported as number (%). A severe adverse event was defined as any untoward medical occurrence in a subject causing severe discomfort and significant impact on the patient's usual activities and requiring treatment. A SAE included one or more of the following: death; serious deterioration in the subject's health resulting in life-threatening illness or injury, permanent impairment of body structure or function, prolonged hospitalization, or medical/surgical intervention; and failure to complete the transfusion.
† As reported by the investigator; percentage based on the number of adverse events reported in each treatment arm.
‡ As reported by the investigator; percentage based on the number of severe adverse events reported in each treatment arm.
§ Refractoriness to PLT transfusion.
‖ One patient developed anaphylactic shock during the transfusion; one patient developed hypersensitivity during one transfusion and eyelid edema during another transfusion.
¶ As reported by the investigator; percentage based on the number of SAEs reported in each treatment arm.
** One patient developed anaphylactic shock during the transfusion.

were five thrombotic events reported; however, none was related to study transfusions: one event occurred in the PRT-PLT arm (pulmonary embolism) and four occurred in patients receiving reference PLTs (cerebral vascular thrombosis [1], myocardial infarct [1], jugular vein thrombosis [1], and veno-occlusive disease [1]). A summary of all adverse events and severe adverse events categorized by organ system are summarized in Table 6. The frequencies of all adverse events and SAEs were similar between both treatment arms. Most adverse events were categorized according to the following organ systems: gastrointestinal, general disorders and administrative site conditions, and blood and lymphatic disorders.

TABLE 6. Summary of the number of adverse events by organ system/disorder for the PRT-PLT and reference groups by treatment and follow-up period

Adverse event by organ system/disorders	Treatment period*				Follow-up period†				Overall			
	Reference		PRT-PLTs		Reference		PRT-PLTs		Reference		PRT-PLTs	
	Number of AEs	Number of patients	Number of AEs	Number of patients	Number of AEs	Number of patients	Number of AEs	Number of patients	Number of AEs	Number of patients	Number of AEs	Number of patients
Any organ system	384	51	506	56	123	41	148	37	507	53	654	56
Blood and lymphatic	46	25	59	33	8	6	7	6	54	30	66	36
Cardiac	6	4	15	13	6	6	1	1	12	9	16	14
Congenital/familial/genetic	0	0	1	1	1	1	1	1	1	1	2	2
Ear and labyrinth	3	3	0	0	0	0	0	0	3	3	0	0
Endocrine	0	0	0	0	1	1	0	0	1	1	0	0
Eye	5	5	4	3	2	2	3	3	7	7	7	6
Gastrointestinal	88	40	102	38	27	15	26	12	115	45	128	41
General/administrative site	77	43	91	43	18	15	22	12	95	46	113	46
Hepatobiliary	3	3	2	2	0	0	1	1	3	3	3	3
Immune system	5	5§	0	0§	1	1	1	1	6	6	1	1
Infections and infestations	28	21	32	22	15	14	13	9	43	30	45	28
Injury/poisoning/procedural	2	1§	9	8§	0	0	1	1	2	1§	10	9§
Investigations‡	0	0	5	5	0	0	0	0	0	0	5	5
Metabolism and nutrition	19	15	31	21	5	3	13	10	24	18	44	29
Musculoskeletal and connective tissue	6	4	6	6	7	6	4	3	13	8	10	9
Nervous system	11	8	15	13	7	5	5	4	18	12	20	14
Psychiatric	13	9	26	17	7	3	8	7	16	12	34	21
Renal and urinary	9	8	6	5	3	2	1	1	11	10	7	6
Reproductive and breast	1	1	1	1	2	1	3	2	2	2	4	3
Respiratory, thoracic, and mediastinal	19	14	39	23	5	5	12	6	24	16	51	26
Skin and subcutaneous tissue	29	22	36	26	10	7	12	9	39	27	48	31
Vascular	14	11	26	13	4	4	14	12	18	13	40	19

* Treatment period = randomization to day of last on-protocol PLT transfusions.
† Follow-up period = day after the last on-protocol PLT transfusions and up to study discontinuation/completion date.
‡ Investigations = bleeding time prolonged; blood creatine increase; weight increase.
§ Comparisons between reference and PRT-PLTs reached significance.
AE = adverse event.

Although the mean CCI values for both 1 and 24 hours were lower with PRT-PLTs, the mean values for both determinations were above the 7500 and 4500 thresholds, respectively, that have been used to define successful transfusions.[45] At 1 hour posttransfusion, 71.3% of the pathogen-inactivated products resulted in successful transfusion increments compared to 84.1% in the reference group. At 24 hours posttransfusion the proportions successful were 58.9% for PRT-PLTs and 68.1% for reference PLTs. Although the percentages of successful transfusions are lower than desired (both groups), they are within the ranges reported in other PLT transfusion studies raising questions as to why 30% to 40% of PLT transfusions are not considered successful based on current established thresholds.[46,47] Patient factors that affect increments and product variability may explain part of this failure; however, our understanding of these poor responses is still limited. Because of this observation, the sensitivity of the CCI as a clinical outcome measure could be questioned and indeed many studies have now used bleeding as their primary outcome.[12,48,49]

The time to next transfusion and overall blood product utilization analyses provided information about the resource implications of using PRT-PLTs. The time to next transfusion was determined for both study groups; however, there were limitations with this analysis as on-protocol transfusions during the treatment period were not always consecutive; hence, the interpretation was problematic. The overall PLT and RBC utilization in the two study groups was not significantly different, suggesting that the lower CCIs with the PRT-PLTs did not translate into significantly higher blood product use.

Safety information using PRT-PLTs was also obtained from this study. The study was designed to capture all adverse events regardless of whether they were related or unrelated to the transfusion of PLTs. Over 1100 adverse events occurred during the treatment and follow-up phases of the study, indicating the severe degree of illness and complications that occur in this patient population. However, only four patients had adverse events (two with PRT-PLTs and two with reference) that were categorized as having a very likely relationship to PLT transfusion. The two events in the PRT-PLT group were refractoriness to PLT transfusions. The events in the reference group included anaphylactic shock during a transfusion, hypersensitivity, and eyelid edema. All adverse events were categorized by organ system and/or disorder. The most frequently reported events in both treatment arms were gastrointestinal, general disorders and administrative site problems, blood and lymphatic disorders, and infections and infestations. These events occurred with similar frequency in both treatment groups suggesting an acceptable safety profile with PRT; however, additional safety data would be useful collected either as postmarketing surveillance or as part of a larger clinical trial where bleeding could be used as the primary outcome. Bleeding data were collected as a secondary outcome during this study but they were only actively assessed during the 24-hour time period around on-protocol transfusions. Each treatment group had Grade 4 bleeding events (two in the PRT-PLT group and one in the reference group). The study was not powered to show difference in bleeding and given the paucity of data we do not attempt to make conclusions related to risk of bleeding.

There were a number of additional limitations to this study. The frequent use of off-protocol transfusions made it difficult to analyze some of the secondary outcomes that involved measures over time. The reasons for the off-protocol transfusions were not documented. This information would have been useful to understand some of the logistical considerations when using PRT-PLTs and to provide further insight into the challenges with producing a standardized product volume and dose. The responses to off-protocol transfusions were not available, which also precluded traditional intention-to-treat analyses. These data would have been helpful to provide a more complete representation of the full transfusion history. There were also a number of protocol violations where posttransfusion samples for CCI determination were collected outside of the time-compliant period: 17.4% (86/493) for the CCI_{1hour} and 22.8% (109/478) for $CCI_{24hours}$. To avoid excluding these data, we prespecified an extended time period in addition to the time-compliant period and analyzed the data both ways; however, this compliance issue illustrates the challenges with getting CCI measurements posttransfusion in this complex patient population.

In conclusion, the noninferiority of PRT-PLTs compared to reference PLTs using the surrogate outcome measure of CCI_{1hour} was not demonstrated in this controlled clinical trial in 110 patients. Safety data did not identify any major adverse effects associated with the transfusion of PRT-PLTs. Overall PLT and RBC utilization in the two study groups was not significantly different, suggesting that the lower CCIs with the PRT-PLTs did not translate into significantly higher blood product use. Further studies are needed to show whether the lower CCI observed with PRT-PLTs is associated with any change in the risk of bleeding.

CONFLICT OF INTEREST

Raymond P. Goodrich is an employee of CaridianBCT Biotechnologies, LLC. Dr Goodrich assumes full responsibility for the overall content and integrity of the manuscript.

REFERENCES

1. Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti ML, Smaill F. Pathogen inactivation: making decisions about new technologies. Report of a consensus conference. Transfusion 2007;47:2338-47.

2. Dodd R, Roth WK, Ashford P, Dax EM, Vyas G. Transfusion medicine and safety. Biologicals 2009;37:62-70.

3. Seghatchian J, de Sousa G. Pathogen-reduction systems for blood components: the current position and future trends. Transfus Apher Sci 2006;35:189-96.

4. Lin L, Cook DN, Wiesehahn GP, Alfonso R, Behrman B, Cimino GD, Corten L, Damonte PB, Dikeman R, Dupuis K, Fang YM, Hanson CV, Hearst JE, Lin CY, Londe HF, Metchette K, Nerio AT, Pu JT, Reames AA, Rheinschmidt M, Tessman J, Isaacs ST, Wollowitz S, Corash L. Photochemical inactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. Transfusion 1997;37:423-35.

5. Ben-Hur E, Horowitz B. Advances in photochemical approaches for blood sterilization. Photochem Photobiol 1995;62:383-8.

6. Lambrecht B, Mohr H, Knuver-Hopf J, Schmitt H. Photoinactivation of viruses in human fresh plasma by phenothiazine dyes in combination with visible light. Vox Sang 1991;60:207-13.

7. Wagner SJ, Skripchenko A, Robinette D, Foley JW, Cincotta L. Factors affecting virus photoinactivation by a series of phenothiazine dyes. Photochem Photobiol 1998;67:343-9.

8. Matthews JL, Sogandares-Bernal F, Judy MM, Marengo-Rowe AJ, Leveson JE, Skiles H, Newman JT, Chanh TC. Preliminary studies of photoinactivation of human immunodeficiency virus in blood. Transfusion 1991;31:636-41.

9. Matthews JL, Newman JT, Sogandares-Bernal F, Judy MM, Skiles H, Leveson JE, Marengo-Rowe AJ, Chanh TC. Photodynamic therapy of viral contaminants with potential for blood banking applications. Transfusion 1988;28:81-3.

10. Li J, de KD, Woolum MD, Ruane PH, Keil SD, Lockerbie O, McLean R, Goodrich RP. Pathogen reduction of buffy coat platelet concentrates using riboflavin and light: comparisons with pathogen-reduction technology-treated apheresis platelet products. Vox Sang 2004;87:82-90.

11. van Rhenen D, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Kluter H, Vermeij H, Kappers-Klunne M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Lin L, Metzel P, Buchholz D, Corash L; euroSPRITE trial. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood 2003;101:2426-33.

12. McCullough J, Vesole DH, Benjamin RJ, Slichter SJ, Pineda A, Snyder E, Stadtmauer EA, Lopez-Plaza I, Coutre S, Strauss RG, Goodnough LT, Fridey JL, Raife T, Cable R, Murphy S, Howard F 4th, Davis K, Lin JS, Metzel P, Corash L, Koutsoukos A, Lin L, Buchholz DH, Conlan MG. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT trial. Blood 2004;104:1534-41.

13. Goodrich RP, Edrich RA, Li J, Seghatchian J. The Mirasol PRT system for pathogen reduction of platelets and plasma: an overview of current status and future trends. Transfus Apher Sci 2006;35:5-17.

14. Reddy HL, Dayan AD, Cavagnaro J, Gad S, Li J, Goodrich RP. Toxicity testing of a novel riboflavin-based technology for pathogen reduction and white blood cell inactivation. Transfus Med Rev 2008;22:133-53.

15. Engelfriet CP, Reesink HW, Blajchman MA, Muylle L, Kjeldsen-Kragh J, Kekomaki R, Yomtovian R, Hocker P, Stiegler G, Klein HG, Soldan K, Barbara J, Slopecki A, Robinson A, Seyfried H. Bacterial contamination of blood components. Vox Sang 2000;78:59-67.

16. Ruane PH, Edrich R, Gampp D, Keil SD, Leonard RL, Goodrich RP. Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. Transfusion 2004;44:877-85.

17. Goodrich RP, Edrich RA, Goodrich LL, Scott CA, Manica KJ, Hlavinka DJ, Hovenga DH, Hansen ET, Gampp D, Keil SD, Gilmour DI, Li J, Martin CB, Platz MS. The antiviral and antibacterial properties of riboflavin and light: applications to blood safety and transfusion medicine. In: Silva E, Edwards AM, editors. Comprehensive series in photochemistry and photobiology. Vol 6. Flavins: photochemistry and photobiology. Cambridge, UK: The Royal Society of Chemistry; 2006. p. 83-113.

18. Cardo LJ, Rentas FJ, Ketchum L, Salata J, Harman R, Melvin W, Weina PJ, Mendez J, Reddy H, Goodrich R. Pathogen inactivation of Leishmania donovani infantum in plasma and platelet concentrates using riboflavin and ultraviolet light. Vox Sang 2006;90:85-91.

19. Cardo LJ, Salata J, Mendez J, Reddy H, Goodrich R. Pathogen inactivation of *Trypanosoma cruzi* in plasma and platelet concentrates using riboflavin and ultraviolet light. Transfus Apher Sci 2007;37:131-7.

20. Rentas F, Harman R, Gomez C, Salata J, Childs J, Silva T, Lippert L, Montgomery J, Richards A, Chan C, Jiang J, Reddy H, Li J, Goodrich R. Inactivation of Orientia tsutsugamushi in red blood cells, plasma, and platelets with riboflavin and light, as demonstrated in an animal model. Transfusion 2007;47:240-7.

21. Goodrich RP, Gilmour D, Hovenga N, Keil SD. A laboratory comparison of pathogen reduction technology treatment and culture of platelet products for addressing bacterial contamination concerns. Transfusion 2009;49:1205-16.

22. Mintz PD, Wehrli G. Irradiation eradication and pathogen reduction. Ceasing cesium irradiation of blood products. Bone Marrow Transplant 2009;44:205-11.

23. Fast LD, DiLeone G, Cardarelli G, Li J, Goodrich R. Mirasol PRT treatment of donor white blood cells prevents the development of xenogeneic graft-versus-host disease in Rag2-/-gamma c-/- double knockout mice. Transfusion 2006;46:1553-60.

24. Fast LD, DiLeone G, Li J, Goodrich R. Functional inactivation of white blood cells by Mirasol treatment. Transfusion 2006;46:642-8.

25. Asano H, Lee CY, Fox-Talbot K, Koh CM, Erdinc MM, Marschner S, Keil S, Goodrich RP, Baldwin WM III. Treatment with riboflavin and ultraviolet light prevents alloimmunization to platelet transfusions and cardiac transplants. Transplantation 2007;84:1174-82.

26. AuBuchon JP, Herschel L, Roger J, Taylor H, Whitley P, Li J, Edrich R, Goodrich RP. Efficacy of apheresis platelets treated with riboflavin and ultraviolet light for pathogen reduction. Transfusion 2005;45:1335-41.

27. Picker SM, Steisel A, Gathof BS. Effects of Mirasol PRT treatment on storage lesion development in plasma-stored apheresis-derived platelets compared to untreated and irradiated units. Transfusion 2008;48:1685-92.

28. Ambruso DR, Thurman G, Marschner S, Goodrich RP. Lack of antibody formation to platelet neoantigens after transfusion of riboflavin and ultraviolet light-treated platelet concentrates. Transfusion 2009;49:2631-6.

29. Andreu G, Vasse J, Tardivel R, Semana G. Platelet transfusion: products, indications, does, threshold and efficacy. Transfus Clin Biol 2009;16:118-33.

30. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981;47:207-14.

31. TRAP. Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. The Trial to Reduce Alloimmunization to Platelets Study Group. N Engl J Med 1997;337:1861-9.

32. McCulloch CE, Searle SR, Nehaus JM. Generalized, linear and mixed models. 2nd ed. New York: John Wiley & Sons Inc; 2008. p. 1.

33. Wu L. Mixed effects models for complex data. Boca Raton (FL): Chapman and Hall/CRC; 2009. p. 1-431.

34. Guidelines for the use of platelet transfusions. Br J Haematol 2003;122:10-23.

35. Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model. 1st, 2nd Printing ed. New York: Springer-Verlag; 2000. p. 1-350.

36. Khuri SF, Healey N, MacGregor H, Barnard MR, Szymanski IO, Birjiniuk V, Michelson AD, Gagnon DR, Valeri C. Comparison of the effects of transfusions of cryopreserved and liquid-preserved platelets on hemostasis and blood loss after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1999;117:172-85.

37. Spector JI, Skrabut EM, Valeri CR. Oxygen consumption, platelet aggregation and release reactions in platelets freeze-preserved with dimethylsulfoxide. Transfusion 1977;17:99-109.

38. Melaragno AJ, Carciero R, Feingold H, Talarico L, Weintraub L, Valeri CR. Cryopreservation of human platelets using 6% dimethyl sulfoxide and storage at –80 degrees C. Effects of 2 years of frozen storage at –80 degrees C and transportation in dry ice. Vox Sang 1985;49:245-58.

39. Owens M, Cimino C, Donnelly J. Cryopreserved platelets have decreased adhesive capacity. Transfusion 1991;31:160-3.

40. Shepherd KM, Sage RE, Barber S, O'Brien E. Platelet cryopreservation. 1. In vitro aggregation studies. Cryobiology 1984;21:39-43.

41. Dullemond-Westland AC, van Prooijen HC, Riemens MI, Akkerman JW. Cryopreservation disturbs stimulus-response coupling in a platelet subpopulation. Br J Haematol 1987;67:325-33.

42. Valeri CR. Hemostatic effectiveness of liquid-preserved and previously frozen human platelets. N Engl J Med 1974;290:353-8.

43. Schiffer CA, Aisner J, Wiemik PH. Frozen autologous platelet transfusion for patients with leukemia. N Engl J Med 1978;299:7-12.

44. Blauhut B, Gross C, Necek S, Doran JE, Spath P, Lundsgaard-Hansen P. Effects of high-dose aprotinin on blood loss, platelet function, fibrinolysis, complement, and renal function after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1991;101:958-67.

45. Bishop JF, Matthews JP, Yuen K, McGrath K, Wolf MM, Szer J. The definition of refractoriness to platelet transfusions. Transfus Med 1992;2:35-41.

46. Heddle NM, Blajchman MA, Meyer RM, Lipton JH, Walker IR, Sher GD, Constantini LA, Patterson B, Roberts RS, Thorpe KE, Levine MN. A randomized controlled trial comparing the frequency of acute reactions to plasma-removed platelets and prestorage WBC-reduced platelets. Transfusion 2002;42:556-66.

47. Heddle NM, Cook RJ, Blajchman MA, Barty RL, Sigouin CS, Boye DM, Nelson EJ, Kelton JG. Assessing the effectiveness of whole blood-derived platelets stored as a pool: a randomized block noninferiority trial. Transfusion 2005;45:896-903.

48. Rebulla P, Finazzi G, Marangoni F, Avvisati G, Gugliotta L, Tognoni G, Barbui T, Mandelli F, Sirchia G. The threshold for prophylactic platelet transfusions in adults with acute myeloid leukemia. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto. N Engl J Med 1997;337:1870-5.

49. Heddle NM, Cook RJ, Tinmouth A, Kouroukis CT, Hervig T, Klapper E, Brandwein JM, Szczepiorkowski ZM, AuBuchon JP, Barty RL, Lee KA; SToP Study Investigators of the BEST Collaborative. A randomized controlled trial comparing standard- and low-dose strategies for transfusion of platelets (SToP) to patients with thrombocytopenia. Blood 2009; 113:1564-73.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Patients were not eligible for the study if the following medications had been taken within 14 days of randomization.
Appendix S2. Product withdrawal criteria.
Appendix S3. Categories of adverse event severity used.
Appendix S4. Ninety-five patient post hoc subgroup analysis.

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