参考資料１

血小板製剤に対する感染症因子低減化血小板の臨床試験に関する文献集

メタアナリシス 文献

ORIGINAL ARTICLE

Meta-analysis of the randomized controlled trials of the hemostatic efficacy and capacity of pathogen-reduced platelets

Eleftherios C. Vamvakas

BACKGROUND: A recent independently funded randomized controlled trial (RCT; Br J Haematol 2010;150: 209-17) questioned prevailing opinion concerning the hemostatic capacity of pathogen-reduced platelets (PLTs). Meta-analysis was used to calculate the effect of pathogen reduction (PR) of PLTs on hemostatic efficacy and capacity based on all available data and to investigate possible reasons for the variation in reported findings.

STUDY DESIGN AND METHODS: RCTs allocating patients to receive routine PLT transfusions with pathogen-reduced or untreated PLTs and reporting on at least one of six hemostasis endpoints were eligible for analysis. Five RCTs of hemato-oncology patients met eligibility criteria. Endpoints determined by similar criteria in all RCTs were integrated by fixed-effects methods. Endpoints determined by different criteria were integrated by random-effects methods.

RESULTS: Studies were statistically homogeneous in all analyses. Pathogen-reduced PLTs were associated with a significant ($p < 0.05$) reduction in 1- and 24-hour posttransfusion corrected count increments (summary mean difference, 3260; 95% confidence interval [CI], 2450-4791; and summary mean difference, 3315; 95% CI, 2027-4603) as well as a significant increase in all and in clinically significant bleeding complications (summary odds ratio [OR], 1.58; 95% CI, 1.11-2.26; and summary OR, 1.54; 95% CI, 1.11-2.13). The frequency of severe bleeding complications did not differ.

CONCLUSION: The results of the recent RCT are not inconsistent with those of the earlier studies. Introduction of PR technologies in their current stage of development would result in an increase in mild and moderate (albeit not severe) bleeding complications, which the transfusion-medicine community must explicitly tolerate to reap the benefits from PR.

Pathogen reduction (PR) of platelets (PLTs) results in a predictable loss of PLTs.[1-3] Because fewer PLTs are transfused, there is a decrease in post-transfusion PLT recovery and survival,[4,5] as well as an increase in the number of transfused PLT concentrates and a shortening of the interval between PLT transfusions.[1] However, if the reduction in effective PLT dose can be overcome by increasing the number of transfused concentrates,[6-9] because these PLTs are pathogen reduced, prevailing opinion[6-9] has held that the advantages of PR (i.e., the elimination of the residual risk of transfusion-associated sepsis[10,11] and the prevention of most emerging transfusion-transmitted infections[6-9]) more than compensate for any disadvantages.[6-9] The finding that half-dose PLT transfusions may prevent bleeding as effectively as standard-dose transfusions in patients with hypoproliferative thrombocytopenia (provided that more frequent transfusions are administered)[12] strengthened the belief that patients receiving pathogen-reduced PLTs would not be at increased risk of bleeding because they receive a lower PLT dose. Observational studies after the implementation of PR in some countries[13,14] did not record any increased bleeding in the recipients of treated PLTs, but neither did they document the expected increase in the number of transfused concentrates. Most likely, PLT

ABBREVIATIONS: CTC = Common Toxicity Criteria; CTCAE = Common Terminology Criteria for Adverse Events; DIC = disseminated intravascular coagulation; PAS = platelet additive solution; PR = pathogen reduction; PWBD = pooled whole blood–derived.

From the Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, California.

Address reprint requests to: Eleftherios C. Vamvakas, MD, PhD, MPH, Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, 8700 Beverly Avenue, South Tower, Room 3733, Los Angeles, CA 90048; e-mail: vamvakase@cshs.org.

Received for publication August 13, 2010; revision received September 9, 2010, and accepted September 14, 2010.

doi: 10.1111/j.1537-2995.2010.02925.x

TRANSFUSION **;**:**-**.

transfusions had been previously overutilized, so that the effective reduction in PLT dose did not result in a visibly increased need for PLTs.[9] It is also possible that an increase in mild and moderate bleeding complications might have gone unnoticed outside the framework of a randomized controlled trial (RCT).

To derive the benefits of PR,[6-11] transfusion medicine professionals are willing to accept a margin of inferiority in the hemostatic efficacy (or even capacity) of the pathogen-reduced PLTs.[9] Hemostatic efficacy refers to the 1- (and/or 24-) hour posttransfusion corrected count increments (CCIs); hemostatic capacity refers to the ability to prevent bleeding, that is, to the frequency of bleeding complications in patients receiving pathogen-reduced PLTs versus controls. The RCT of the riboflavin/ultraviolet (UV)A light (Mirasol, CaridianBCT, Lakewood, CO) technology[15] defined "noninferiority" as a 20% reduction in the 1-hour CCI after transfusion of pathogen-reduced (compared to untreated) PLTs.

"The proof of the pudding for a PLT component is its ability to prevent and treat hemorrhage."[9] A recent independently funded RCT[16] disputed whether such proof had been provided for the pathogen-reduced PLTs by the initial (manufacturer-sponsored) RCTs.[1,17] In the recent study,[16] compared with patients receiving untreated PLTs also stored in PLT additive solution (PAS) III, subjects receiving pathogen-reduced PLTs had a more than threefold (15.3% vs. 4.3%) higher risk of Common Terminology Criteria for Adverse Events (CTCAE) and Common Toxicity Criteria (CTC) (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm) Grade 2 or greater hemorrhagic events. This difference was both significant ($p < 0.05$) and clinically too big to be ascribed only to the reduction in effective PLT dose secondary to PR. Since patients receiving pathogen-reduced PLTs were given prophylactic transfusions for counts of 10×10^9/L or less,[16] and since the effective PLT dose in the PR arm[16] was higher than half the standard PLT dose,[12] enrolled subjects should not have had such an increase in bleeding complications. Nonetheless, an independent Data Safety Monitoring Board interrupted enrollment into the PR arm owing to the increased frequency of Grade 2 or higher bleeding ($p < 0.05$ compared to a third [reference] arm of patients receiving PLTs stored in plasma).[16]

Because these results[16] have questioned the basis of our prevailing assumption[6-9] that the reduction in effective PLT dose owing to PR can be overcome by simply increasing the number of transfused concentrates, a meta-analysis of all available RCTs of the efficacy of pathogen-reduced concentrates was undertaken to: 1) produce an estimate of the inferiority of pathogen-reduced PLTs in terms of hemostatic efficacy and capacity based on all available data and 2) investigate possible reasons for the variation in the findings of the reported studies.

MATERIALS AND METHODS

Three PR procedures for PLTs have reached or are approaching clinical application.[9] All three are photoinactivation methods sharing the use of UV light, and two of them (amotosalen-HCl/UVA light [Intercept, Cerus Corp., Concord, CA] and riboflavin/UVA light [Mirasol]) have undergone testing of their effects on the hemostatic efficacy and capacity of transfused PLTs. Treatment of PLTs with UV light alone (MacoPharma, Tourcoing, France) has not yet proceeded to the clinical trial stage.[9]

Study retrieval

A computerized (PubMed) search of the English literature targeted studies that had appeared between January 1, 2001, and June 30, 2010 (Table 1). The electronic search was supplemented by a manual search for abstracts published in the supplements of transfusion medicine and hematology journals between January 1, 2008, and June 30, 2010, and the reference lists of retrieved articles were reviewed for pertinent citations. RCTs allocating patients to receive routine PLT transfusions with either pathogen-reduced or untreated PLTs were eligible for inclusion in the meta-analysis if they had reported on at least one of six (hemostatic efficacy and/or capacity) outcomes.

PLT transfusions were regarded as routine if: 1) transfusion decisions were made by the patients' treating physicians based on the standard criteria used at each participating institution; and 2) transfused components were not manipulated in ways other than PR. A study was included in the meta-analysis if data on one of the

TABLE 1. PubMed search strategy

Search statement	Search terms	Number of citations
#1	Platelet AND transfusion	12,764
#2	Pathogen reduction OR pathogen inactivation	411
#3	Amotosalen OR "S 59" OR S59	902
#4	Riboflavin	12,919
#5	"Ultraviolet-A light" OR "UVA light"	547
#6	Intercept OR Mirasol	5,264
#7	#2 OR #3 OR #4 OR #5 OR #6	19,817
#8	#1 AND #7	191
#9	#1 AND #7 Limits: English; publication date from January 1, 2001, to June 30, 2010	159
#10	#1 AND #9 Limits: clinical trial, randomized controlled trial, controlled clinical trial	13

following outcomes had been reported: 1) 1-hour CCI (mean ± SD); 2) 24-hour CCI (mean ± SD); 3) PLT transfusion interval (days, mean ± SD); 4) number of PLT transfusions (number of therapeutic doses, mean ± SD); 5) number of red blood cell (RBC) transfusions (number of units, mean ± SD); and/or 6) proportion of patients having bleeding complications (mild or moderate versus severe, number [%]). When the information needed for meta-analysis had been presented in separate reports of the same RCT, information for the meta-analysis could be retrieved from all the reports of that RCT.

All studies meeting the aforementioned eligibility criteria were to be included in the meta-analysis regardless of study quality. Delaney and colleagues[18] proposed a list of items for assessing the quality of reporting by RCTs of PLT transfusion, based on both the CONSORT-Statement checklist and a checklist specific for PLT transfusion. PLT transfusion–specific items most pertinent to the quality of the RCTs included in this meta-analysis related to the information reported on the following study descriptors investigated here as sources of variation in the presented results.

Study descriptors

PLT concentrate transfused to the treatment arm

Pathogen-reduced PLTs are stored in a mixture of approximately 35% plasma and 65% PAS.[1,17] Because psoralens bind noncovalently to plasma proteins and lipids, the plasma ratio must be maintained within tightly controlled limits (30%-45%). Apheresis and pooled whole blood–derived (PWBD) PLTs prepared by the PLT-rich plasma method in the United States, and some of the apheresis and PWBD PLTs prepared by the buffy coat method in Europe, are not stored in PAS. For this reason, the hemostatic efficacy and capacity of pathogen-reduced PLTs prepared by each method must be demonstrated separately. Buffy coat and apheresis PLTs differ in many quality control measures,[19] raising the possibility that one or the other component might incur a greater reduction in hemostatic efficacy and/or capacity after PR.

Storage medium of PLTs transfused to the control arm

In vitro studies have shown significant differences in metabolic, functional, and flow cytometric variables for PLTs stored in PAS II compared with plasma,[20-22] and an RCT reported reduced 1- and 24-hour CCIs along with reduced pH for buffy coat PLTs stored in PAS II versus plasma.[23] The lower PLT content could be ascribed to less efficient separation owing to a viscosity-related difference in the PLT distribution during centrifugation;[24] the lower pH to the lower intrinsic pH of PAS II, the lower buffering capacity, and higher lactate production.[24] One paired radiolabeled PLT survival study showed a significant decrease in both the recovery and the survival of PLTs stored in PAS II versus plasma.[25] In contrast, PAS III has been shown to perform equally to plasma.[16]

Length of storage and number of PLTs contained in transfused components

If PLTs given to the control arm have been stored in a PAS medium whose performance is inferior to that of plasma, 1- and 24-hour CCIs in the control arm may be reduced, thereby reducing or concealing any decrease in the hemostatic efficacy of the treated PLTs.[16] Increased storage time correlates with a reduction in both the 1- and 24-hour CCIs.[16,23,26] Extended storage of PLTs for 6 to 7 days results in a modest decrease in 1- and 24-hour CCIs when PLTs are stored in plasma[27,28] and in significantly lower CCIs and a shortened interval between PLT transfusions when PLTs are stored in PAS II.[29] The number of PLTs contained in components pathogen reduced by nonoptimized methods could also be reduced to a level associated with reduced CCIs and/or increased bleeding complications in the treatment arm.

Patient population and indications for transfusion

More than 80% of PLT transfusions are given to prevent bleeding complications.[1,16] Patients enrolled in the reported RCTs could consist of *unselected* hemato-oncology patients needing prophylactic PLT transfusions at a given trigger (either $\leq 20 \times 10^9$ or $\leq 10 \times 10^9$/L) or before invasive procedures or needing PLT transfusions to treat bleeding; alternatively, they could consist of hemato-oncology patients selected in a manner that excludes subjects with factors predisposing to PLT consumption.[23] The latter may have included history of immune thrombocytopenia; history of alloimmunization; or refractoriness to PLT transfusion, acute surgical condition, and psoralen UVA therapy as well as splenomegaly and disseminated intravascular coagulation (DIC) or fever. Such factors predispose to PLT transfusion failure.[30-33] Exclusion from RCTs of the thrombocytopenic subjects with these clinical factors (i.e., the subjects most likely to bleed or need increased PLT transfusions while receiving PLT transfusion support) could reduce or conceal any decreased hemostatic capacity of PLTs treated with PR. Depending on the listed exclusion criteria, hemato-oncology patients were categorized as *selected* or *moderately* selected. For included patients to be categorized as *unselected*, only the following exclusions were permitted: 1) patients with HLA or PLT-specific alloantibodies and current refractoriness to PLT transfusions requiring support with apheresis components from matched donors and 2) pregnant or pediatric patients.

Period under observation

If patients were under observation for a longer period, they could manifest bleeding complications over a longer interval, with the RCT potentially recording a higher frequency of bleeding complications. By the same token, if the period over which bleeding complications were recorded had been less than the total study period, the trial could have recorded a lower frequency of bleeding complications. For example, the only fatal cerebral hemorrhage occurring in the RCT of Kerkhoffs and colleagues[16] happened after a subject from the PR arm of the trial had gone off protocol; therefore, this bleeding event was not included in the analysis of the RCT. Accordingly, the meta-analysis extracted information on hemorrhagic events pertaining to the maximal period during which this information had been made available, recording the length of study follow-up, the total number of PLT transfusions, the interval between PLT transfusions, and the period during which bleeding complications were recorded.

Ascertainment and frequency of bleeding complications

Ascertainment of bleeding was made by different methods and bleeding was recorded at different intervals and by blinded versus unblinded assessors. Thus, a trial could have recorded more frequent bleeding complications if ascertainment of bleeding events had taken place more frequently or it could have recorded bleeding differentially between the arms if it had used unblinded assessors.

Definition of bleeding severity

Bleeding was recorded as "all" versus "severe,"[17] by World Health Organization (WHO) grade,[34] or based on the CTCAE/CTC, Version 3.0 scale (or Version 2.0 of the same scale).[35] In the SPRINT trial,[1] bleeding occurring during the transfusion period was initially presented by WHO grade.[1] Subsequently,[36] bleeding occurring during both the transfusion and the surveillance periods was graded by blinded personnel on a scale from 1 to 4 based on the CTC Version 2.0 scale.[35] The more recent version of this scale was used by Kerkhoffs and colleagues.[16] Briefly, Grade 1 bleeding comprises petechiae and minimal or microscopic bleeding not requiring interventions. Grade 2 bleeding comprises symptomatic bleeding for which minimal intervention (e.g., cauterization) may be needed. Grade 3 bleeding requires RBC transfusion or other intervention; it also includes generalized petechiae and/or purpura and retinal bleeding with visual impairment. Grade 4 bleeding is catastrophic bleeding, such as intracranial bleeding causing neurologic deficit or disability. The distinction between Grade 1 and Grade 2 bleeding can be subtle. For example "mild" rectal bleeding is Grade 1, whereas "persistent" rectal bleeding requiring medication (e.g., steroid suppositories) is Grade 2; vaginal bleeding is Grade 1 if it requires fewer than two pads per day, but it is Grade 2 if it requires two or more pads per day; and so on. When the similar WHO grading scale[34] is used, Grade 2 or greater bleeding is considered "clinically significant."[37]

Bleeding is a complex outcome because it is a composite (three types of bleeding: Grade 2, Grade 3, and Grade 4).[38] Two recent RCTs of PLT dose[12,39] recorded an absolute difference of approximately 20% (70%[12] vs. 50%[39]) in the frequency of WHO Grade 2 or greater bleeding. Measuring and grading bleeding is difficult, and observers often disagree.[38,39] The WHO bleeding scale has not been validated using measurement methodology, and its reproducibility, accuracy, and face validity remain to be established.[38] Accordingly, the meta-analysis distinguished between "all" bleeding, clinically significant bleeding, and "severe" bleeding. Bleeding categorized as "severe" by the investigators[17] or as Grade 3 or greater on the CTC Version 2.0[36] or Version 3.0[16] scale (or the WHO scale if grading on the CTC scale was unavailable) was recorded as severe in the meta-analysis. Similarly, Grade 2 or greater bleeding on the CTC Version 2.0[36] or Version 3.0[16] scale (or the WHO scale if grading on the CTC scale was unavailable) was recorded as clinically significant.

Proportion of transfusions administered in violation of study protocol

Protocol violations could have occurred because of errors or because a PLT transfusion was needed when the specially prepared component required by the protocol was not available in the inventory. Violations occurring equally in both arms would reduce, and possibly conceal, any adverse effect of PR on hemostatic efficacy and/or capacity. Violations occurring selectively in the treatment arm (because pathogen-reduced PLTs were unavailable when needed) would have had the same effect (provided that the component given to the control arm did not have reduced hemostatic efficacy, as would be the case for PLTs stored in PAS II).

Statistical analysis

Results of studies were integrated if the variation in reported findings was sufficiently modest to be attributed to chance.[40] The magnitude of the variation in reported findings was evaluated by a Q test statistic. The p value calculated for the Q test statistic represents the probability that the noted variation could have arisen by chance. The hypothesis of homogeneity was not rejected if the p value was 0.10 or greater for the Q test statistic, that is, if there was at least a 10% probability that the disagreements among the studies might have arisen by chance.

When RCTs determining the occurrence of a hemostatic efficacy or capacity endpoint by similar criteria were integrated, study results were combined by fixed-effects methods. When the outcome was reported as mean ± SD (i.e., for the 1- or 24-hour CCI, the number of PLT or RBC

transfusions, and the PLT transfusion interval), a summary mean difference between patients receiving pathogen-reduced versus untreated PLTs was calculated across the RCTs by the method of Cochran.[41] For the frequency of bleeding complications, a summary odds ratio (OR) in subjects receiving pathogen-reduced versus untreated PLTs was calculated across the RCTs by the method of Peto.[42] When RCTs determining the occurrence of an endpoint by different criteria were integrated, summary ORs were calculated by the random-effects method of DerSimonian and Laird.[43] Subgroup analyses were conducted to investigate the effects of the sources of variation (or study descriptors) listed above. In the subgroup analyses, RCTs were stratified according to the levels of each study descriptor.

RESULTS

Study retrieval

Five RCTs[1,15-17,44] met the eligibility criteria of the meta-analysis. Four[1,16,17,44] had evaluated the amotosalen-HCl/UVA light technology and were retrieved through the electronic search of the literature. These four studies tested treated PLTs suspended in approximately 35% plasma and 65% PAS III (InterSol, Baxter, Lessines, Belgium;[17,44] Baxter, Deerfield, IL;[1] or Fenwal, Lake Zurich, IL[16]). Two reports of the SPRINT trial[1,36] were used to retrieve the information needed for the meta-analysis (Table 2). One study of the riboflavin/UVA light technology was retrieved through the manual search of abstracts and has since been made available in complete form.[15] All five RCTs[1,15-17,44] enrolled hemato-oncology patients. All randomly assigned patients receiving at least one PLT transfusion were included in the reported analyses.

Two RCTs[45,46] ($n = 20$[45] and $n = 10$[46]) retrieved electronically were excluded because they were crossover studies in which patients had received both pathogen-reduced and untreated PLTs in different time periods. A significant treatment-by-period interaction had been observed in one study,[45] while in the other study[46] the investigators had transfused RBCs to maintain a hematocrit (Hct) of at least 25% (because Hct affects bleeding time—the study's primary outcome). Two RCTs[45,47] ($n = 20$[45] and $n = 201$[47]) retrieved electronically[45] or through the manual search of abstracts[47] were excluded because they had transfused only PLTs stored for 6 (20%) or 7 (80%) days[47] or 7 days[45]—that is, past the allowed storage period of PLTs. Rather than evaluating efficacy, these studies[45,47] targeted cost-effectiveness and aimed to demonstrate that the introduction of PR with the Intercept technology could permit extension of the PLT storage period to 7 days (so as to offset the cost of PR by reducing PLT outdating). Length of storage reduces PLT efficacy,[27-29,48,49] making the intervention evaluated in these studies[45,47] different from that in the other studies (Table 2). The other reports located by the last step of the electronic search (Table 1) were observational studies,[13] further analyses of the SPRINT trial,[2,36] experimental studies of normal volunteers,[5] or studies unrelated to the purpose of the search. Review of the abstracts of all studies located in the step before the last step of the search (Table 1) did not identify any other RCT meeting the eligibility criteria.

Regarding study quality, three RCTs[1,17,44] were double-blind and two[15,16] were open-label investigations. The studies[1,15-17,44] had reported adequately on the descriptors relevant to the meta-analysis (Table 2). Only two studies,[1,16] however, had optimally determined the occurrence of bleeding complications (Table 2): the SPRINT trial[1,36] and the RCT of Kerkhoffs and coworkers[16] and of these only the SPRINT trial[1,36] had used blinded assessors. The euroSPRITE trial[17] and the Mirasol study[15] supplemented the observations made by the investigators (Table 2) with hemorrhagic events recorded as part of the documentation of adverse events, while Janetzko and coworkers[44] relied on the recording of adverse events because bleeding was not an endpoint in their trial. Because the available RCTs had used different criteria for determining the occurrence of bleeding complications, the meta-analyses of the bleeding complications used random-effects methods for the synthesis unless otherwise stated.

Hemostatic efficacy

Table 3 shows the results of the meta-analysis integrating four RCTs[15-17,44] juxtaposed with the corresponding findings of the SPRINT trial.[1] The SPRINT trial[1] had reported a mean without the associated SD for each endpoint listed in Table 3; thus its findings could not be integrated with the results of the other RCTs.[15-17,44] Both the meta-analysis of the four RCTs[15-17,44] and the SPRINT trial[1] demonstrated a significant ($p < 0.05$) reduction in the 1-hour CCI, a significant reduction in the 24-hour CCI, a significant increase in total PLT transfusions, and a significant reduction in the interval between PLT transfusions in association with PR.

For the 1-hour CCI, the summary mean difference from the meta-analysis of three RCTs of the Intercept technology[16,17,44] was 3156 (Table 4), compared with 4900 in the SPRINT trial[1] (Table 3) and 5214 in the Mirasol study[15] (Table 4). Although the reported effect of PR was greater in the SPRINT trial[1] and the Mirasol study[15] than in the three other studies[16,17,44] combined, all three findings were significant. The Mirasol study[15] presented a detailed noninferiority analysis. Limiting the analysis to first eight PLT transfusions for which a 1-hour CCI had been obtained within 30 to 90 minutes posttransfusion, the study[15] failed to show noninferiority of the PR-treated PLTs based on predetermined criteria. The difference between subjects receiving PR-treated PLTs and controls was –5214 (95% confidence interval [CI], –2887 to –7542). The 95% CI

TABLE 2. RCTs investigating effects of PR on the hemostatic efficacy and/or capacity of the transfused PLTs

Study descriptor (source of variation in results)	euroSPRITE trial[17] (n = 103)	SPRINT trial[1,36] (n = 645)	Janetzko et al.[44] (n = 43)	Kerkhoffs et al.[16] (n = 278)	Mirasol Clinical Evaluation Study Group[15] (n = 110)
PLT concentrate transfused to the treatment arm	Pools of five or six WBD buffy coat concentrates; the number of concentrates in the pool depended on the participating center	Apheresis concentrates collected on the Amicus separator (Baxter Healthcare, Round Lake, IL)	Apheresis concentrates collected on the Amicus separator with an optimized integrated set to minimize PLT losses (compared with the processing of treated PLTs in the SPRINT trial[1])	Pools of five WBD buffy coat concentrates	Apheresis concentrates collected on Trima Version 5.0 (CaridianBCT, Lakewood, CO; 73.9% of transfusions); or pools of six WBD buffy coat concentrates (26.1% of transfusions)
PLT concentrate transfused to the control arm	Pools of four or five WBD buffy coat concentrates (depending on the participating center), suspended in 35% plasma and 65% PAS II (T-Sol; Baxter, Lessines, Belgium—two centers) or 100% plasma (two centers)	Apheresis concentrates collected on the Amicus separator and suspended in 100% plasma	Apheresis concentrates collected on the Amicus separator and suspended in 100% plasma	Pools of five WBD buffy coat concentrates suspended in PAS III like the pathogen-reduced PLTs (n = 94; control arm used in the meta-analysis); additional reference arm (n = 99) in which the untreated PLTs were suspended in 100% plasma	Apheresis concentrates collected on Trima (74.8%) or pools of six WBD buffy coat concentrates suspended in 100% plasma (25.2%)
Mean ± SD length of storage of transfused PLT concentrates (days)	Treatment arm: 3.5 ± 1.1 Control arm: 3.4 ± 1.2	Treatment arm: 3.4 ± NR Control arm: 3.6 ± NR	Treatment arm: 3.1 ± 1.0 Control arm: 3.2 ± 0.8	Treatment arm: 4.0 ± 1.6* Control arm: 3.8 ± 1.8*	Treatment arm: 2.7 ± 1.1 Control arm: 2.6 ± 1.1
Number of PLTs (mean ± SD) contained in the PLT components ($\times 10^{11}$)	Treatment arm: 3.9 ± 1.0 Control arm: 4.3 ± 1.2	Treatment arm: 3.7 ± NR (20% contained $< 3.0 \times 10^{11}$) Control arm: 4.0 ± NR (12% contained $< 3.0 \times 10^{11}$)	Treatment arm: 4.1 ± 1.2 Control arm: 3.8 ± 0.4	Treatment arm: 3.4 ± 0.8 Control arm: 3.6 ± 0.8 Reference (plasma) arm: 3.9 ± 1.0‡	Treatment arm: 5.2 ± 2.1† Control arm: 5.2 ± 2.0†
Enrolled population of hemato-oncology patients	*Selected*: splenomegaly (>18 cm) and DIC listed among the factors predisposing to PLT consumption (and representing exclusion criteria)	*Moderately* selected: splenomegaly and DIC *not* listed among the exclusion criteria; patients with clinical factors "that could potentially interfere with the assessment of study endpoints" were excluded	*Selected*: splenomegaly, fever, and DIC listed among the factors predisposing to PLT consumption (and representing exclusion criteria)	*Unselected*	*Selected*: splenomegaly, DIC, and infection/fever listed among the exclusion criteria
Trigger for prophylactic PLT transfusions in hypoproliferative thrombocytopenia	$\leq 20 \times 10^9$/L	$\leq 10 \times 10^9$/L at most participating centers	$\leq 20 \times 10^9$/L	$\leq 10 \times 10^9$/L	$\leq 10 \times 10^9$/L in the absence of risk factors for bleeding $\leq 20 \times 10^9$/L in the presence of risk factors
Period under observation	Up to 56 days, followed by a 28-day surveillance period§ (Cycle 1)	Up to 28 days or until transfusion independence (defined as 7 days without PLT transfusions); a 7-day surveillance period followed	Up to 28 days; efficacy analyses pertained to the first eight PLT transfusions	Up to 42 days or up to a maximum of five PLT transfusions	Up to 28 days or until transfusion independence; a 28-day surveillance period followed; efficacy analyses pertained to the first eight PLT transfusions

TABLE 2. *Continued*

Study descriptor (source of variation in results)	euroSPRITE trial[17] (n = 103)	SPRINT trial[1,36] (n = 645)	Janetzko et al.[44] (n = 43)	Kerkhoffs et al.[16] (n = 278)	Mirasol Clinical Evaluation Study Group[15] (n = 110)
Number of PLT transfusions (mean ± SD)	Treatment arm: 7.5 ± 5.8‖ Control arm: 5.6 ± 5.5‖	Treatment arm: 8.4 ± NR Control arm: 6.2 ± NR	Treatment arm: 4.7 ± 3.3 Control arm: 5.5 ± 4.7	Treatment arm: 5 ± 3‖ Control arm: 4 ± 3‖	Treatment arm: 4.5 (1-21)¶ Control arm: 3.0 (1-19)¶
Interval between PLT transfusions (days, mean ± SD)	Treatment arm: 3.0 ± 1.2 Control arm: 3.4 ± 1.2	Treatment arm: 1.9 ± NR Control arm: 2.4 ± NR	Treatment arm: 2.4 ± 1.0 Control arm: 2.8 ± 1.0	Treatment arm: 2.5 ± 2.0 Control arm: 3.2 ± 1.8	Treatment arm: 2.2 ± 1.7 Control arm: 2.3 ± 1.5
Period during which bleeding complications were reported	Cycle 1	Period of PLT transfusion support[1] or both transfusion and surveillance periods[36]	Entire study period during which safety monitoring for adverse events occurred	Same as period under observation	Same as period under observation, but with bleeding assessments performed only on the "on-protocol" PLT transfusions
Ascertainment of bleeding complications**	Twelve potential bleeding sites evaluated by a blinded observer within 6 hr before and 6 hr after each PLT transfusion	Patients evaluated daily by trained observers blinded to the treatment assignment; at each assessment, each of eight potential bleeding sites were assigned a WHO bleeding grade††	Bleeding complications not included among the study endpoints; method of ascertaining bleeding complications not described	Patients evaluated daily by trained *non*blinded assessors to grade bleeding complications at eight potential bleeding sites	Bleeding assessments performed by *non*blinded assessors before and after each "on-protocol" transfusion (at 1 and 24 hr posttransfusion) and on the final follow-up visit
Definition of bleeding severity	All bleeding vs. severe bleeding; severe bleeding events "required treatment intervention and change in patient activity status"‡‡	WHO scale Grade 1 through 4;[1] and CTC Version 2.0 scale Grade 1 through 4[36]	All bleeding vs. severe bleeding; only one episode of hemoptysis was categorized as "severe"	CTCAE/CTC Version 3.0 scale Grade 1 through 5§§	WHO scale Grade 1 through 4
Frequency of bleeding complications in the entire study population (number [%] of patients)	All: 79 (76.7%) Severe: 6 (5.8%)	All: 562 (87.1%) Grade ≥ 2: 251 (38.9%) Grade ≥ 3: 138 (21.4%)	All: 29 (67.4%) Severe: 1 (2.3%)	All: 60 (21.6%) Grade ≥ 2: 22 (7.9%) Grade ≥ 3: 6 (2.2%)	All: 56 (50.9%) Grade ≥ 2: 19 (17.3%) Grade ≥ 3: 9 (8.2%)
Proportion of PLT transfusions administered in violation of study protocol	Treatment arm: 20% Control arm: 10%	Treatment arm: 8.5% Control arm: 7.5%	Treatment arm: 17% Control arm: 7%	26.7% (302 of 1129)	Treatment arm: 17.7% Control arm: 23.2%

* Extended storage for up to 7 days was permitted in all study arms, but PLTs were issued for transfusion as per routine procedure. Samples of all PLT components were cultured for 7 days using the BacT/Alert culturing system (bioMérieux, Boxtel, the Netherlands). Twenty-six percent of the PLTs transfused to the treatment arm and 21% of the PLTs given to the control arm had been stored for 6 or 7 days.
† Prepared PLT components that did not meet French requirements for minimum PLT content (3.0×10^{11} per transfused component) were not used in the study.
‡ The reference (plasma) arm was also associated with a better CCI, although the difference in CCI between the control arm and the reference arm was not significant. Only the control (PAS III) arm was used as control arm in the meta-analysis.
§ If additional PLT transfusion support was required, patients were asked to re-enroll for a second 56-day transfusion period and an additional 28-day adverse event surveillance period (Cycle 2).
‖ Multiple PLT transfusions (given without PLT counts between them to treat bleeding) were counted as one transfusion episode.
¶ Median (range). Mean ± SD of PLT transfusions per *patient-day* during the transfusion period was 0.24 ± 0.16 for the treatment arm and 0.20 ± 0.19 for the control arm.
** In addition to the recording of hemorrhagic events as part of the recording of all adverse events for safety assessment purposes.
†† Blinded personnel of the study also coded all hemorrhagic events occurring during the transfusion and surveillance periods as Grade 1 through 4 on the CTC Version 2.0 scale.[35]
‡‡ They included three fatal cerebral hemorrhages, one retinal hemorrhage, one epistaxis, and one gastrointestinal hemorrhage. For the latter three severe bleeding episodes, it is noted that of 4, 44, and 33 episodes, respectively, of retinal hemorrhage, epistaxis, and gastrointestinal hemorrhage recorded during Cycle 1 of the trial, only the one episode noted here was categorized by the investigators as severe bleeding.
§§ Grade 5 is fatal bleeding. Except for the addition of Grade 5, there are only minimal differences between CTC Version 3.0[16] and CTC Version 2.0.[36]
DIC = disseminated intravascular coagulation; NR = not reported.

TABLE 3. Effects of PR on the hemostatic efficacy of transfused PLTs

	Meta-analysis of four RCTs[15-17,44]				SPRINT trial[1]	
	Q test for homogeneity:	Summary mean difference*				
Effect of PR	p value	Mean†	95% CI‡	p value	Mean difference	p value
Reduction in 1-hr CCI	>0.10	3260	2450-4791	<0.05	4900	<0.001
Reduction in 24-hr CCI	>0.75§	3315	2027-4603	<0.05	3500	<0.001
Increase in total PLT transfusions	>0.25‖	0.93	0.16-1.70	<0.05	2.2	<0.001
Reduction in the interval between PLT transfusions (number of days)	>0.50	0.41	0.13-0.67	<0.05	0.5	<0.001

* The direction of the difference is indicated in the first column (under "effect of PR").
† The results integrated from the Mirasol study[15] pertain to all "on-protocol" transfusions given within the 28-day study period. Substitution of the (selected) results pertaining to the first eight "on-protocol" transfusions for which a 1-hr CCI had been obtained within 30 to 90 min did not alter the results of the meta-analysis.
‡ When the 95% CI does not include the null value of 0, the corresponding summary mean difference is statistically significant ($p < 0.05$).
§ Across three[15-17] RCTs. Janetzko and colleagues[44] did not report 24-hr CCIs.
‖ Across three[16,17,44] RCTs. The Mirasol study[15] did not report this outcome.

TABLE 4. Effects of PR on the hemostatic efficacy of transfused PLTs stratified by PR technology

	Amotosalen-HCl/UVA light technology				Riboflavin/UVA light technology	
	Meta-analysis of three RCTs[16,17,44]					
	Q test for homogeneity	Summary mean difference*			Mirasol trial[15]	
Effect of PR	p value	Mean	95% CI†	p value	Mean difference‡	p value
Reduction in 1-hr CCI	>0.25	3156	1856-4457	<0.05	5214	<0.0001
Reduction in 24-hr CCI	>0.50§	3512	2006-5018	<0.05	3210	0.001
Increase in total PLT transfusions	>0.25	0.93	0.16-1.70	<0.05	NS‖	NS‖
Reduction in the interval between PLT transfusions (number of days)	>0.50	0.48	0.17-0.79	<0.05	0.4	0.01

* The direction of the difference is indicated in the first column (under "effect of PR").
† When the 95% CI does not include the null value of 0, the corresponding summary mean difference is statistically significant ($p < 0.05$).
‡ Pertaining to the first eight "on-protocol" transfusions for which a 1-hr CCI had been obtained within 30 to 90 min (see text).
§ Across two[16,17] RCTs. Janetzko and colleagues[44] did not report 24-hr CCIs.
‖ NS = not significant. The mean ± SD per patient-day (as opposed to mean ± SD for the entire study period) was reported (see Table 1).

included the prespecified upper limit of the zone of noninferiority (–2940), which had been set at 20% of the mean CCI anticipated in the control arm. Similarly, the odds of achieving a successful 1-hour CCI (set at 7500[50]) were significantly ($p = 0.01$) lower in subjects receiving PR-treated PLTs compared with controls (OR, 0.28; 95% CI, 0.11-0.77). When the odds of achieving a successful 24-hour CCI (set at 4500[50]) were considered, the difference between the arms was not significant ($p = 0.08$) when the analysis was limited to the transfusions for which a CCI had been obtained within 18 to 26 hours posttransfusion. However, the difference became significant ($p = 0.04$) and favored the control arm, when all transfusions for which a CCI had been obtained within 15 to 30 hours posttransfusion were included.

Hemostatic capacity

Information on hemorrhagic events in the SPRINT trial was extracted from the expanded safety report of that study.[36] Figure 1 shows the OR of all, clinically significant, or severe bleeding complications in each of the five RCTs[15-17,36,44] that had reported the corresponding outcome, along with the results of the meta-analyses integrating the frequency of bleeding complications across the studies. In all analyses, the studies were statistically homogeneous ($p \geq 0.50$ for the Q-test statistic). Across the studies, PR was associated with a significant increase in all bleeding complications (summary OR, 1.58; 95% CI, 1.11-2.26) and in clinically significant bleeding complications (summary OR, 1.54; 95% CI, 1.11-2.13), but not in severe bleeding complications (summary OR, 1.25; 95% CI, 0.86-1.81). The lack of a difference across the studies in severe bleeding complications was confirmed by the integration of the number of RBC transfusions administered in each RCT. The SPRINT trial[1] had observed no difference ($p = 0.13$) in the number of RBC transfusions given to the treatment versus the control arm. Similarly, across the four other (statistically homogeneous, $p > 0.10$ for the Q-test statistic) RCTs,[15-17,44] there was no difference between the arms (summary mean difference, 0.31; 95% CI, –0.84 to 0.24; $p > 0.05$).

Table 5 shows that when the analysis was stratified by the PR technology employed in each study, the differences in all and in clinically significant bleeding complications—albeit similar to the results of the overall

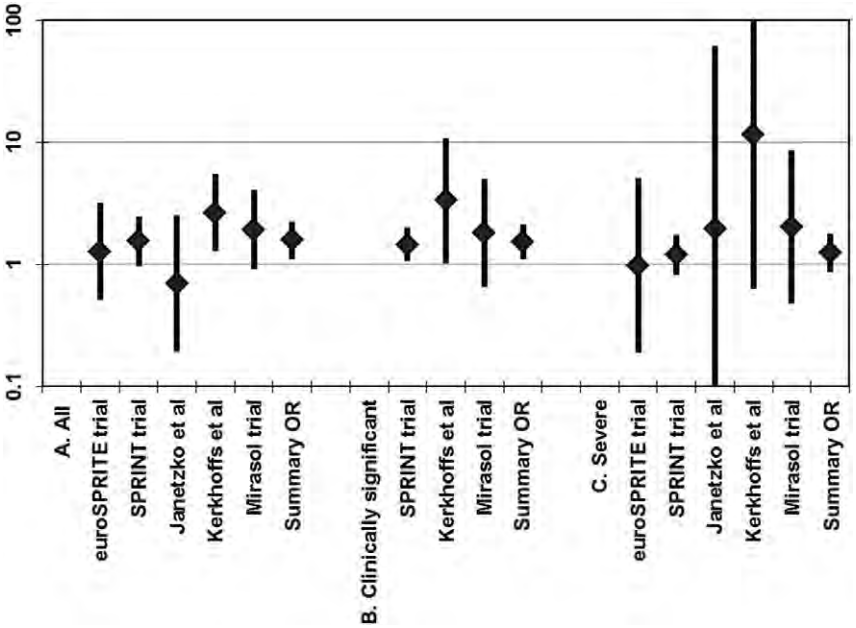

Fig. 1. ORs of all bleeding complications, clinically significant bleeding complications, and severe bleeding complications in patients receiving pathogen-reduced versus untreated PLTs in the RCTs included in the meta-analysis,[15-17,36,44] and summary ORs of bleeding complications across the studies that had presented each of these outcomes. When the 95% CI for the OR does not include the null value of 1, the difference is significant ($p < 0.05$).

meta-analysis—did not attain significance. The summary OR of severe bleeding complications across the Intercept studies[16,17,36,44] was 1.22 (95% CI, 0.83-1.78); the OR in the Mirasol study[15] was 2.04 (95% CI, 0.48-8.61). When the two studies[16,36] that had used daily ascertainment of bleeding complications by trained assessors were integrated, PR was associated with an increase in all and in clinically significant bleeding complications. Individually, both the blinded[36] and the unblinded[16] study had shown an increase in clinically significant bleeding complications, but only the unblinded study[16] had reported an increase in all bleeding complications.

Table 5 also shows that, when the RCT of Kerkhoffs and colleagues[16] (which differed from the other RCTs in five study descriptors—Table 2) was not included in the meta-analysis, there was no increase in all bleeding complications across the four other RCTs.[15,17,36,44] When the analysis was limited to the clinically significant bleeding complications though, PR was associated with a significant increase in bleeding complications even when the RCT of Kerkhoffs and coworkers[16] was not included in the analysis. The meta-analysis further showed a significant increase in all and in clinically significant bleeding complications in association with PR across RCTs using a 10×10^9/L prophylactic PLT transfusion trigger.[16,36]

DISCUSSION

The principal finding of this meta-analysis is that the results of Kerkhoffs and colleagues[16]—which questioned our previous assumptions[6-9]—are not inconsistent with the earlier literature. The five RCTs[1,15-17,44] integrated in this meta-analysis were consistently homogeneous. The hypothesis of homogeneity of effects[40] was not rejected in any analysis, that is, variation in the findings of the available studies in all meta-analyses could always have arisen by chance. Concerning the studies of the amotosalen-HCl/UVA light technology, the results of the studies represented a continuum, from the findings of the euroSPRITE trial[17] and of Janetzko and coworkers[44]—who observed no difference in the 1-hour CCI or the bleeding complications—to the middle-of-the-road results of the SPRINT trial[1,36] and to the recent findings of Kerkhoffs and colleagues[16]—who observed larger differences between the arms than had been reported by the SPRINT trial.[1,36] The results of the single RCT of riboflavin/UVA light technology[15] were similar to the findings of the SPRINT trial.[1,36] Accordingly, because the five available RCTs[1,15-17,44] represent a continuum, in the future the accumulated experience from all 1080 subjects[1,15-17,44] analyzed here should be considered in its entirety. Only when all these subjects are considered together is the entire population of hemato-oncology patients represented (Table 2). Based on the totality of the evidence hitherto adduced, transfusion of pathogen-reduced (compared with untreated) PLTs is associated with a 58% increase in all bleeding complications and a 54% increase in clinically significant bleeding complications. This is the reduction in the hemostatic capacity of the treated PLTs that the transfusion medicine community must explicitly tolerate to reap the benefits[6-11] of PR.

Concerning the sources of variation in reported findings, two RCTs[17,44] at one end of the spectrum had enrolled *selected* hemato-oncology patients, excluding patients with splenomegaly and DIC,[17,44] as well as fever,[44] in addition to excluding subjects with other factors predisposing to PLT consumption. The euroSPRITE trial[17] increased the PLT dose given to the treatment arm (five to six PWBD concentrates, compared with four to five concentrates given to the control arm) to compensate for the PLT losses secondary to PR, and transfused PLTs with possibly impaired hemostatic efficacy (stored in PAS II[20-25]) to

TABLE 5. Effects of employed PR technology and study descriptors on bleeding complications

Possible source of variation in reported results	Levels of study descriptor	All bleeding complications			Clinically significant bleeding complications		
		Q test for homogeneity p value	Summary OR		Q test for homogeneity p value	Summary OR	
			Point estimate	95% CI*		Point estimate	95% CI*
Employed PR technology	Intercept technology[16,17,36,44]	>0.25	1.51	0.99-2.31	>0.50	1.57	0.97-2.54
	Mirasol technology[15]	NA†[15]	1.93	0.91-4.13	NA†[15]	1.83	0.66-5.07
Daily ascertainment of bleeding complications by trained assessors	All[16,36]	>0.10‡	1.81	1.23-2.66*	>0.10‡	1.54	1.14-2.09*
	Blinded[36]	NA†[36]	1.56	0.97-2.49	NA†[36]	1.45	1.06-2.0*
	Unblinded[16]	NA†[16]	2.66	1.28-5.51*	NA†[16]	3.34	1.02-10.9*
Five study descriptors§ differing between the RCT of Kerkhoffs et al.[16] and the other RCTs[15,17,36,44] (Table 2)	Kerkhoffs et al.[16]	NA†[16]	2.66	1.28-5.51*	NA†[16]	3.34	1.02-10.9*
	Other RCTs[15,17,36,44]	>0.25	1.44	0.98-2.12	>0.75	1.48	1.06-2.07*
Transfused PLT concentrate	Buffy coat pool[16,17]	>0.25	1.87	0.90-3.89	NA†[16]	3.34	1.02-10.9*
	Apheresis[36,44]	>0.10	1.25	0.61-2.53	NA†[36]	1.45	1.06-2.0*
Trigger for prophylactic PLT transfusions for hypoproliferative thrombocytopenia	$\leq 10 \times 10^9$/L[16,36]	>0.10‡	1.81	1.23-2.66*	>0.10‡	1.54	1.14-2.09*
	$\leq 20 \times 10^9$/L[17,44]	>0.25	1.00	0.47-2.11	NA‖		

* When the 95% CI does not include the null value of 1, the difference is significant ($p < 0.05$).
† NA = not applicable, because only one RCT was available for analysis.
‡ Fixed-effects analysis because the assessment of bleeding complications was made by similar criteria.
§ Kerkhoffs et al.[16]: 1) had transfused PLTs to the treatment arm stored for a longer period (>3.5 days compared to ≤3.5 days); 2) had transfused a lower mean PLT dose per component given to the treatment arm ($<3.5 \times 10^{11}$ versus $>3.5 \times 10^{11}$); 3) had enrolled unselected hemato-oncology patients; 4) had committed a higher proportion of protocol violations (>25% compared with ≤25%); and 5) had recorded a lower frequency of bleeding complications (<30% compared with ≥50%—Table 2).
‖ Not applicable, because no RCTs reported on this outcome.

approximately half of the control patients. Both the euroSPRITE trial[17] and Janetzko and coworkers[44] transfused platelets prophylactically when the PLT count fell to or below 20×10^9. Taken together, these four study descriptors may account for the lack of impairment in the hemostatic efficacy or capacity of the PLTs given to the PR arm. At the other end of the spectrum, Kerkhoffs and coworkers[16] enrolled *unselected* hemato-oncology patients, transfused fewer PLTs per component given to the PR arm, and stored PLTs for longer[27-29] than the other studies. These factors could exacerbate any bleeding complications secondary to PR, although the higher proportion of protocol violations compared with the other studies would diminish any differences between the arms.

In the middle of the road, the SPRINT trial[1] enrolled *moderately* selected hemato-oncology patients and avoided the design features seen at the two ends of the spectrum. All measures of hemostatic efficacy (Table 3) were significantly compromised in the PR (compared with the control) arm.[1] During the transfusion period, the proportion of patients having WHO Grade 2 bleeding did not differ between the arms, although Grade 2 bleeding occurred on a mean of 3.2 days in the PR arm versus 2.5 days in the control arm ($p < 0.05$).[1] During both the transfusion and the surveillance periods, comparisons of bleeding complications separately for each CTC Version 2.0 grade yielded no significant difference.[36] The investigators reported significant differences only in *individual* minor hemorrhagic events, such as petechiae and fecal occult blood.[36] The results of the single Mirasol study[15] were similar to those of the SPRINT trial.[1] This is especially important because the Mirasol trial:[15] 1) had exclusively transfused pathogen-reduced PLT components containing at least 3.0×10^{11} PLTs and yet 2) failed to show noninferiority of the pathogen-reduced PLTs.

The categorization of hemorrhagic events in the SPRINT trial by system-organ-class MedDRA (Medical Dictionary for Regulatory Activities, Version 3.3[51]) category,[36] in addition to CTC Version 2.0 grade,[36] as well as WHO grade,[1] and the reporting of information on bleeding complications in two different time periods, underscores the complexity of using bleeding as an endpoint.[38,39] To address this complexity, the meta-analysis extracted the bleeding information that could be considered most comparable from the reports of the five RCTs, and it did so over a period of observation that could also be regarded as the most comparable. The latter was the maximal period of observation during which information on hemorrhagic events had been made available (Table 2). The SPRINT trial[36] and Kerkhoffs and coworkers[16] had reported bleeding complications on the same scale (CTC[35]), albeit different versions of it (Version 2.0[36] vs. Version 3.0[16]). Hemorrhagic events described as "severe" by the investigators of the euroSPRITE trial[17] and by Janetzko and coworkers[44] (Table 2) corresponded to Grade 3 or Grade 4 events on the CTC Version 2.0 scale.[35] Events described as "not severe," however, could be either Grade 1 or Grade 2 on the CTC scale. Differentiation between "mild" (Grade 1) versus "clinically significant" (Grade 2) bleeding complications was not possible for these two RCTs[17,44] based on the available information.

The limitation of this meta-analysis therefore is that results pertaining to clinically significant bleeding complications (middle panel in Fig. 1) are likely to be less reliable than the findings shown for all and for severe bleeding complications (top and lower panels in Fig. 1). Differentiation between Grade 1 and Grade 2 bleeding events could have differed between the studies, as it often differs between observers trained to participate in the same trial.[38,39] Results relying on the assignment of a "Grade 2" bleeding category are especially prone to the lack of reproducibility and accuracy inherent in the use of bleeding as an endpoint.[38,39]

The strength of this meta-analysis is that for all and for severe bleeding complications, comparable data could be extracted from all five RCTs.[15-17,36,44] With the reporting of data on 1080 subjects,[15-17,36,44] the difference in all bleeding complications between recipients of pathogen-reduced and untreated PLTs attained significance here for the first time. (As shown in the analysis stratified by PR technology in Table 5, the difference in all bleeding complications across the four RCTs of the Intercept technology[16,17,36,44]—which had enrolled 970 subjects—is only marginally significant: OR, 1.51; 95% CI, 0.99-2.31; $p \leq 0.05$.) The meta-analysis demonstrated a significant increase in all bleeding complications secondary to PR, and it also found that severe bleeding complications do not differ between subjects transfused with pathogen-reduced versus untreated PLTs. (The failure to detect a 25% increase in the risk of severe bleeding complications, however, may be due to the inadequacy of a sample of 1080 subjects for establishing such a small difference in risk.) A second strength of the meta-analysis is that, when measures of hemostatic efficacy rather than capacity are employed, the results of the meta-analysis are unequivocal (Table 3), just as the SPRINT trial[1] findings had been unequivocal. Because of the difficulties in using bleeding as an endpoint,[38,39] only the SPRINT trial[1] used WHO Grade 2 bleeding as its primary outcome; the four[15-17,44] other studies used the 1-hour CCI as their primary endpoint.[52] This outcome is both accurate and reproducible, and it was also consistently presented in all reports.[1,15-17,44]

The question that cannot be answered from the available data is whether the reduced hemostatic capacity secondary to PR is due only to cellular losses of a proportion of the treated PLTs[2,53] or to functional impairment of all treated PLTs as well.[54,55] Because the cellular losses reported by in vitro studies do not exceed one-third of the total PLTs, they should not have resulted in the bleeding complications observed across the five RCTs[15-17,36,44]

(Fig. 1) if a PLT dose equal to half the standard PLT dose does not increase bleeding complications.[12] For this reason, it is possible that the damage to the PLT mitochondrial nucleic acids induced by PR does not result only in loss of viability of a proportion of the PLTs but entails functional impairment of all treated PLTs as well.[16]

The nature of the PR-induced damage needs to be elucidated before these technologies are recommended for routine use. If the damage is loss of viability of a proportion of the treated PLTs, it can be overcome by increasing the PLT dose, as we had previously assumed.[6-9] If the damage is functional impairment of all treated PLTs, it cannot be overcome by merely increasing the PLT dose. Although PR is starting to be implemented in some European countries,[56] the research question remains one of efficacy; investigations of cost-effectiveness[45,47] are premature. At the current stage of research and development, the transfusion medicine community would have to tolerate an increase in mild and moderate (albeit not severe) bleeding complications if it opted to implement these technologies before they are further developed.

CONFLICT OF INTEREST

I have no conflict of interest of any kind.

REFERENCES

1. 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.
2. Murphy S, Snyder E, Cable R, Slichter SJ, Strauss RG, McCullough J, Lin JS, Corash L, Conlan MG, SPRINT Study Group. Platelet dose consistency and its effect on the number of platelet transfusions for support of thrombocytopenia: an analysis of the SPRINT trial of platelets photochemically treated with amotosalen HCl and ultraviolet A light. Transfusion 2006;46:24-33.
3. Pineda A, McCullough J, Benjamin RJ, Cable R, Strauss RG, Burgstaler E, Porter S, Lin L, Metzel P, Conlan MG, SPRINT Study Group. Pathogen inactivation of platelets with a photochemical treatment with amotosalen HCl and ultraviolet light: process used in the SPRINT trial. Transfusion 2006;46:526-71.
4. Snyder E, Raife T, Lin L, Cimino G, Metzel P, Rheinschmidt M, Baril L, Davis K, Buchholz DH, Corash L, Conlan MG. Recovery and lifespan of ^{111}Indium-radiolabeled platelets treated with pathogen inactivation with amotosalen HCl (S-59) and ultraviolet light. Transfusion 2004;44:1732-40.
5. 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.
6. McCullough J. Pathogen inactivation: a new paradigm for blood safety. Transfusion 2007;47:2180-4.
7. 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.
8. Vamvakas EC, Blajchman MA. Transfusion-related mortality: the on-going risks of allogeneic blood transfusion and the available strategies for their prevention. Blood 2009;113:3406-17.
9. AuBuchon JP. Current status of pathogen inactivation methods. ISBT Sci Ser 2010;5:125-33.
10. Eder AF, Kennedy JM, Dy BA, Notari EP, Weiss JW, Fang CT, Wagner S, Dodd RY, Benjamin RJ, American Red Cross Regional Blood Centers. Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: The American Red Cross experience (2004-2006). Transfusion 2007;47:1134-42.
11. Eder AF, Kennedy JM, Dy BA, Notari EP, Skeate R, Bachowski G, Mair DC, Webb JS, Wagner SJ, Dodd RY, Benjamin RJ, The American Red Cross Regional Blood Centers. Limiting and detecting bacterial contamination of apheresis platelets: inlet-line diversion and increased culture volume improve component safety. Transfusion 2009;49: 1554-63.
12. Slichter SJ, Kaufman RM, Assmann SF, McCullough J, Triulzi DJ, Strauss RG, Gernsheimer TB, Ness PM, Brecher ME, Josephson CD, Konkle BA, Woodson RD, Ortel TL, Hillyer CD, Skerrett DL, McCrae KR, Sloan SR, Uhl L, George JN, Aquino VM, Manno CS, McFarland JG, Hess JR, Leissinger C, Granger S. Dose of prophylactic platelet transfusions and prevention of hemorrhage. N Engl J Med 2010;362:600-13.
13. Osselaer JC, Messe N, Hervig T, Bueno J, Castro E, Espinosa A, Accorsi P, Junge K, Jacquet M, Flament J, Corash L. A prospective observational cohort safety study of 5106 platelet transfusions with components prepared with photochemical pathogen inactivation treatment. Transfusion 2008;48:1061-71.
14. Osselaer JC, Cazenave JP, Lambermont M, Garraud O, Hidajat M, Barbolla L, Tardivel R, Defoin L, Waller C, Mendel I, Raidot JP, Kandel G, De Menter R, Fabrigli P, Dehenau D, Arroyo JL, Pardon F, Grouezec M, Corral M, Jacquet M, Sundin D, Lin L, Corash L. An active hemovigilance program characterizing the safety profile of 7437 platelet transfusions prepared with amotosalen photochemical treatment. Vox Sang 2008;94:315-23.
15. The Mirasol Clinical Evaluation Study Group. A randomized controlled clinical trial evaluating the performance and safety of platelets treated with MIRASOL pathogen reduction technology. Transfusion 2010;50:2362-75.

16. Kerkhoffs JL, van Putten WL, Novotny VM, Te Boekhorst PA, Schipperus MR, Zwaginga JJ, van Pampus LC, de Greef GE, Luten M, Huijgens PC, Brand A, van Rhenen DJ, Dutch—Belgian HOVON Cooperative Group. Clinical effectiveness of leukoreduced, pooled donor platelet concentrates, stored in plasma or additive solution with and without pathogen reduction. Br J Haematol 2010;150:209-17.
17. van Rhenen D, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Klüter 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 a photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood 2003;101:2426-33.
18. Delaney M, Meyer E, Cserti-Gazdewich C, Haspel RL, Lin Y, Morris A, Pavenski K, Dzik WH, Murphy M, Slichter S, Wang G, Dumont LJ, Heddle N. A systematic assessment of the quality of reporting for platelet transfusion studies. Transfusion 2010;50:2135-44.
19. Schrezenmeier H, Seifried E. Buffy-coat-derived pooled platelet concentrates and apheresis platelet concentrates: which product type should be preferred? Vox Sang 2010;99: 1-15.
20. van der Meer PF, Pietersz RNI, Reesink HW. Storage of platelets in additive solution up to 12 days with maintenance of good in-vitro quality. Transfusion 2004;44:1204-11.
21. deWildt-Eggen J, Gulliksson H. In-vivo and in-vitro comparison of platelets stored in either synthetic media or plasma. Vox Sang 2003;84:256-64.
22. Wagner T, Vetter A, Dimonic N, Guber SE, Helmberg W, Kröll W, Lanzer G, Mayr WR, Neumüller J. Ultrastructural changes and activation differences in platelet concentrates stored in plasma and additive solution. Transfusion 2002; 42:719-27.
23. Kerkhoffs JLH, Eikenboom JC, Schipperus MS, van Wordragen-Vlaswinkel RJ, Brand R, Harvey MS, de Vries RR, Barge R, van Rhenen DJ, Brand A. A multicenter randomized study of the efficacy of transfusions with platelets stored in platelet additive solution II versus plasma. Blood 2006;108:3210-15.
24. van der Meer PF, Pietersz RN, Tiekstra MJ, Huijgens PC, Dekker WJ, Reesink HW. WBC-reduced platelet concentrates from pooled buffy coats in additive solution: an evaluation of in-vitro and in-vivo measures. Transfusion 2001;41:917-22.
25. Turner VS, Mitchell SG, Hawker RJ. More on the comparison of Plasma-Lyte A and PAS 2 as platelet additive solutions. Transfusion 1996;36:1033-4.
26. van Der Meer PF, Kerkhoffs JL, Carvers J, Scharenberg J, de Korte D, Brand A, de Wildt-Eggen J. In-vitro comparison of platelet storage in plasma and in four platelet additive solutions, and the effect of pathogen reduction: a proposal for an in-vitro rating system. Vox Sang 2010;98:517-24.
27. Dijkstra-Tiekstra MJ, Pietersz RN, Hendriks EC, Reesink HW, Huijgens PC. In-vivo PLT increments after transfusions of WBC-reduced PLT concentrates stored for up to 7 days. Transfusion 2004;44:330-6.
28. Hogge DE, Thompson BW, Schiffer CA. Platelet storage for 7 days in second-generation blood bags. Transfusion 1986; 26:131-5.
29. Diedrich B, Ringden O, Watz E, Shanwell A. A randomized study of buffy-coat platelets in platelet additive solution stored 1-5 versus 6-7 days prior to prophylactic transfusion of allogeneic hematopoietic progenitor cell transplant recipients. Vox Sang 2009;97:254-9.
30. Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, Kao KJ, Kickler T, Lee E, McFarland J, McCullough J, Rodey G, Schiffer CA, Woodson R. Factors effecting post-transfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood 2005;105:4106-14.
31. Klumpp TR, Herman JH, Innis S, Pearlman E, Culling N, Kotz KW, Slachta C, Goldberg SL, Mangan KF. Factors associated with response to platelet transfusion following hematopoietic stem-cell transplantation. Bone Marrow Transplant 1996;17:1035-41.
32. Norol F, Kuentz M, Cordonnier C, Beaujean F, Haiouin C, Vernant JP, Duedari N. Influence of clinical status on the efficacy of stored platelet transfusion. Br J Hematol 1994; 86:125-9.
33. Bishop JF, Matthews JP, McGrath K, Yuen K, Wolf MM, Szer J. Factors influencing 24-hour increments after platelet transfusion. Transfusion 1991;31:392-6.
34. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981;47:207-14.
35. Trotti A, Byhardt R, Stetz J, Gwede C, Corn B, Fu K, Gunderson L, McCormick B, Morrisintegral M, Rich T, Shipley W, Curran W. Common toxicity criteria version 2.0. An improved reference for grading the acute effects of cancer treatment: impact on radiotherapy. Int J Radiat Oncol Biol Phys 2000;47:13-47.
36. Snyder E, McCullough J, Slichter SJ, Strauss RG, Lopez-Plaza I, Lin JS, Corash L, Conlan MG, SPRINT Study Group. Clinical safety of platelets photochemically-treated with amotosalen HCl and ultraviolet A light for pathogen inactivation: the SPRINT trial. Transfusion 2005;45:1864-75.
37. Webert KE, Cook RJ, Sigouin CS, Rebulla P, Heddle NM. The risk of bleeding in thrombocytopenic patients with acute myeloid leukemia. Haematologica 2006;91:1530-7.
38. Heddle NM. Optimal timing and dosing of platelet transfusions. ISBT Sci Ser 2010;5:88-94.
39. Heddle NK, 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.

40. Cooper H, Hedges LV, editors. The handbook of research synthesis. New York, NY: Russell Sage Foundation; 1994.
41. Cochran WG. The combination of estimates from different experiments. Biometrics 1954;10:101-29.
42. Yusuf S, Peto R, Lewis J, Collins R, Sleight P. Beta blockade during and after myocardial infarction: an overview of the randomized trials. Prog Cardiovasc Dis 1985;27:335-71.
43. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled Clin Trials 1986;7:177-88.
44. Janetzko K, Cazenave JP, Kluter H, Kientz D, Michel M, Beris P, Lioure B, Hastka J, Marblie S, Mayaudon V, Lin L, Lin JS, Conlan MG, Flament J. Therapeutic efficacy and safety of photochemically-treated apheresis platelets processed with an optimized integrated set. Transfusion 2005; 45:1443-52.
45. Simonsen AC, Johansson PI, Conlan MG, Jacquet M, Lin JS, Junge K, Lin L, Sørensen H, Borregaard N, Flament J. Transfusion of 7-day-old amotosalen photochemically-treated buffy-coat platelets to patients with thrombocytopenia: a pilot study. Transfusion 2006;46:424-33.
46. Slichter SJ, Raife TJ, Davis K, Rheinschmidt M, Buchholz DH, Corash L, Conlan MG. Platelets photochemically treated with amotosalen HCl and ultraviolet A light correct prolonged bleeding times in patients with thrombocytopenia. Transfusion 2006;46:731-40.
47. Lozano M, Knutson N, Tardivel R, Cid J, Maymo R, Lof HM, Roddie HP, Pelly JP, Dochery AR, Sherman CD, Lin L, Propst M, Corash L, Prowse CV. A multicenter study of therapeutic efficacy and safety of platelet components prepared with pathogen inactivation (Intercept) stored for 6-7 days prior to transfusion. Vox Sang 2010;99(Suppl 1):13.
48. Truilzi DJ, Assmann SF, Strauss RG, Ness PM, Hess J, Granger S, Slichter SJ. The effect of platelet characteristics on the platelet increment and HLA alloimmunization in hypoproliferative thrombocytopenia. Transfusion 2010; 50(Suppl. 2):3A.
49. Dumont LJ, Dumont DF, Unger ZM, Siegel A, Szczepiorkowski ZM, Corson JS, Jones M, Christoffel T, Pellham E, Slichter SJ. A randomized controlled trial comparing autologous radiolabeled in vivo platelet recoveries and survivals of 7-day stored PRP and buffy coat platelets from the same donors. Transfusion 2010;50(Suppl 2):1A.
50. British Committee for Standards in Haematology, Blood Transfusion Task Force. Guidelines for the use of platelet transfusions. Br J Haematol 2003;122:10-23.
51. MedDRA MSSO. MedDRA™ Introductory Guide. Version 3.3. Reston, VA: MedDRA MSSO; 2002 Dec.
52. Vostal JG, Reid TJ, Mondoro TH. Summary of a workshop on in-vivo efficacy of transfused platelet components and platelet substitutes. Transfusion 2000;40:742-50.
53. Picker SM, Schneider V, Oustianskaia L, Gathof BS. Cell viability during platelet storage in correlation to cellular metabolism after different pathogen reduction technologies. Transfusion 2009;49:2311-8.
54. Apelseth TO, Bruserud O, Wentzel-Larsen T, Bakken AM, Bjorsvik S, Hervig T. In-vitro evaluation of metabolic changes and residual platelet responsiveness in photochemically-treated and gamma-irradiated single-donor platelet concentrates during long-term storage. Transfusion 2007;47:657-65.
55. Keuren JF, Cawenberghs S, Heeremans J, de Kort W, Heemskerk JW, Curvers J. Platelet ADP response deteriorates in synthetic storage media. Transfusion 2006;46:204-12.
56. Reesink HW, Panzer S, McQuilten ZK, Wood EM, Marks DC, Wendel S, Trigo F, Biagini S, Olyntho S, Devine DV, Mumford I, Cazenave JP, Rasonglès P, Garraud O, Richard P, Schooneman F, Vezon G, Al Radwan R, Brand A, Hervig T, Castro E, Lozano M, Navarro L, Puig L, Almazán C, MacLennan S, Cardigan R, Franklin IM, Prowse C. Pathogen inactivation of platelet concentrates. Vox Sang 2010;99:85-95.

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

*The Mirasol Clinical Evaluation Study Group**

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, CaridianBCT 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

ABBREVIATIONS: DSMB = Data Safety Monitoring Board; LS = least square; PRT-PLT(s) = pathogen reduction of platelet(s); RCT = randomized controlled trial; SAE(s) = serious adverse event(s).

From CaridianBCT Biotechnologies, LLC, Lakewood, Colorado.

Address reprint requests to: Raymond P. Goodrich, PhD, CaridianBCT Biotechnologies, LLC, 1215 Quail Street, Lakewood, CO 80215; e-mail: ray.goodrich@caridianbct.com.

*The members of the Mirasol Clinical Evaluation (MIRACLE) trial are listed in the Acknowledgments.

Received for publication December 4, 2009; revision received March 5, 2010, and accepted March 11, 2010.

doi: 10.1111/j.1537-2995.2010.02694.x

TRANSFUSION 2010;50:2362-2375.

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.

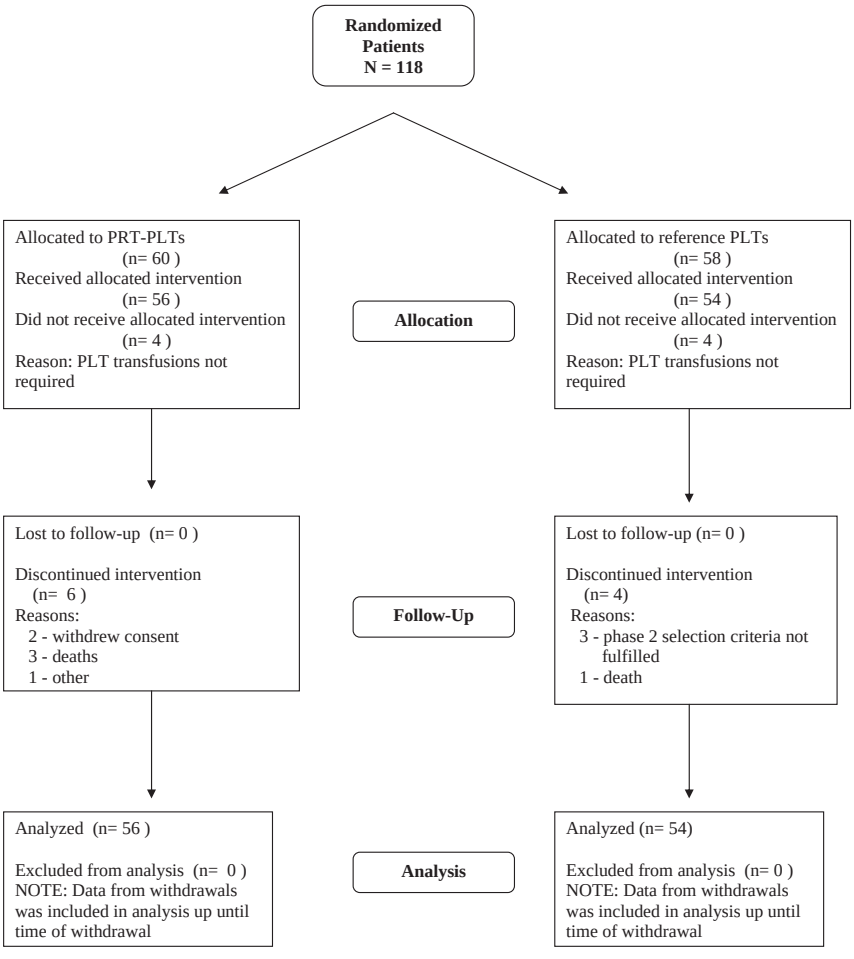

Fig. 1. CONSORT flow diagram showing the flow of patients through the study from the time of randomization to analysis.

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

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

Demographic characteristic	Treatment arm: PRT-PLTs (n = 56)	Treatment arm: 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.

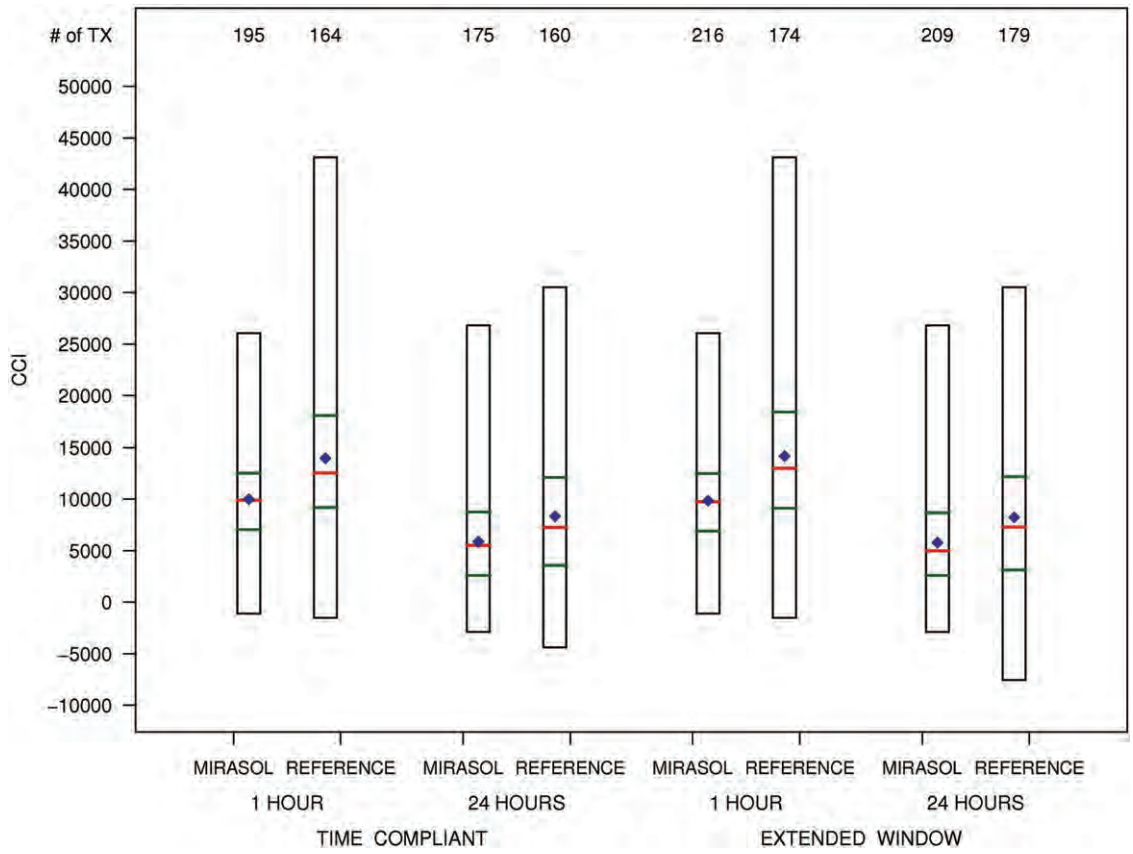

Fig. 2. Box plots of 1- and 24-hour CCIs for transfusions in the time-compliant and extended time periods by treatment group. The lines within the boxes represent the Q_{75} (upper line), median (middle line) and Q_{25} (lower line). The diamond indicates the raw means (the mean of the raw CCI values). 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.

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 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.

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

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.

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 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

	Treatment period*				Follow-up period†				Overall			
	Reference		PRT-PLTs		Reference		PRT-PLTs		Reference		PRT-PLTs	
Adverse event by organ system/disorders	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	3	3	8	7	16	12	34	21
Renal and urinary	9	8	6	5	2	2	1	1	11	10	7	6
Reproductive and breast	1	1	1	1	1	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.

ACKNOWLEDGMENTS

The following persons and institutions participated in the MIRACLE study:

Blood Transfusion Centers:

EFS Alsace, J-P. Cazenave; EFS Pays de la Loire, G. Folléa; EFS Bourgogne-Franche Comté, L. Bardiaux; EFS Aquitaine-Limousin, J.-M. Boiron; EFS Rhône-Alpes, B. Lafeuillade, M. Debost

Clinical Study Sites:
CHU Hautepierre, Hôpitaux Universitaires de Strasbourg, B. Lioure; CHU Hôtel Dieu, Nantes, J-L. Harousseau; CHU Jean Minjoz, Besançon, E. Deconinck; CHU Bordeaux, R. Tabrizi; CHU Grenoble, J.-Y. Cahn; CHU Lyon, M. Michallet
Neoantigenicity Testing Site:
Bonfils Blood Center, D. Ambruso
Medical Monitor:
Universitair Zieckenhuis Brussel, Brussels, R. Schots
Data Safety Monitoring Board (DSMB):
SRTS VD-CHUV, Lausanne, J.-D. Tissot; EFS-Centre Atlantique, L. Sensebé; Hôpital Broussais-Hôtel Dieu, T. Kondo
Data Monitoring Committee (DMC):
University of Minnesota Medical School, J. McCullough; Centro di Medicina Transfusionale, Terapia Cellulare e Criobiologia Dipartimento di Medicina Rigeneritiva Fondazione Ospedale Maggiore Policlinico, Paolo Rebulla; University of Barcelona, Gines Escolar; University of Virginia Health System, P. Mintz
Publication Committee:
McMaster University, N.M. Heddle; CaridianBCT Biotechnologies, R.P. Goodrich
Statistics:
DSMB-Squarepoint-Pointcarré, J. Bruhwyler; DMC-University of Minnesota, C. Le
Publication Committee:
University of Waterloo, R.J. Cook; Philadelphia College of Medicine, B. Stouch

We also acknowledge the technical assistance of Dana Fletcher and Liesbeth Deberdt in the conduct of the study and preparation of the manuscript.

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.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Mirasol 感染性因子低減化技術により処理した血小板の輸血効果および安全性を評価したランダム化比較臨床試験　（仮訳）

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

*The Mirasol Clinical Evaluation Study Group**

背景：リボフラビンと紫外線照射を用いる血小板の感染性因子低減化（PRT-血小板）については、有効性および安全性を検討する第 I 相および第 II 相試験を実施した。今回のランダム化対照臨床試験（RCT）では、輸血 1 時間後の補正血小板増加数（CCI_{1hour}）を主要評価項目として、PRT-血小板の輸血効果および安全性を評価した。

試験デザインおよび方法：非劣性 RCT を実施し、化学療法により血小板減少症を来した患者（6 施設）を PRT-血小板群（Mirasol PRT、Caridian-BCT Biotechnologies）と対照血小板製剤群にランダムに割り付けた。28 日間の投与期間の後、28 日間の（安全性）追跡調査期間を設けた。主要評価項目は CCI_{1hour} とし、算出には投与期間中に施行した治験実施計画書に適合する血小板輸血のうち 1～8 回目までのデータを用いた。

結果：計 118 例の患者をランダムに割り付けた（PRT-血小板群 60 例、対照群 58 例）。うち各群 4 例は血小板輸血を必要としなかったため、残り 110 例が解析対象例であった（PRT-血小板群 56 例、対照群 54 例）。治験実施計画書に適合する血小板輸血は計 541 回行われた（PRT-血小板群 303 回、対照群 238 回）。CCI の最小二乗平均は、PRT-血小板群 11,725［標準誤差（SE）、1,140］、対照群 16,939（SE、1,149）であった（群間差-5,214、95% 信頼区間-7,542～-2,887、2 群間に差がないという帰無仮説の検定結果は $p < 0.0001$）。

結論：本試験では、事前に規定した CCI 基準に基づき、PRT-血小板の非劣性は示されなかった。血小板および赤血球の使用量に有意な群間差がみられなかったことから、PRT-血小板群の CCI は対照群よりわずかに低かったものの、これにより血液製剤使用量は増加しなかったことが示唆される。安全性データに関する群間差はみられなかった。PRT-血小板群で認められた CCI 低値が出血リスク上昇につながるか否かを明らかにするためには、

さらなる研究が必要である。

略語：DSMB = データ安全性モニタリング委員会、LS =最小二乗、PRT-血小板 =感染性因子低減化処理血小板、RCT = ランダム化比較試験、SAE = 重篤な有害事象

輸血によるウイルスおよび細菌伝播の予防対策は、この 20 年間に大幅に進歩した（献血時のドナースクリーニングの向上、ウイルス検出への核酸検査法の導入、細菌スクリーニング、献血時の初流血除去による細菌混入低減など）[1,2]。このような改善措置にもかかわらず、一部の血液媒介感染性因子の伝播リスクは依然として大きい。ウイルス伝播は、検査法の検出感度が十分でないため感染性因子の量が少なく検出できないウインドウピリオドの時期や、既知の感染性因子に対して実用的かつ有効なドナースクリーニング法が使えない場合に発生する可能性がある。

細菌検査が一律に実施されていないことや、汚染製剤を確認しようとする場合、現在の検出システムに限界があることから、血液製剤中の細菌に起因する輸血関連敗血症も発生している。しかし、感染性因子伝播の新規予防技術の開発を駆り立てている最大の問題は、現時点で検査法が存在しない新たな感染性因子または既知の感染性因子の変異株による血液製剤の汚染リスクである[1]。

この 10 年以上の研究で重要と考えられたことは、既知の感染性因子の伝播のみならず、新規または変異細菌株、あるいは新規または変異ウイルス株の伝播に対する予防効果も期待される安全かつ有効な感染性因子低減化技術の開発である。

赤血球、血小板および血漿に対する感染性因子低減化技術は現在開発段階にあり、そのうちの一部は欧州ですでに臨床使用されている[3]。これらの技術のいくつかは光増感剤を使用する。光増感剤は紫外線により活性化されると、DNA および RNA を化学的に修飾しその複製を阻止する[4-12]。このような処理により、血液製剤中の感染性因子は保存期間中に複製できないだけでなく、輸血後も感染合併症を発症できなくなる。血小板に対する感染性因子低減化処理の 1 法である Mirasol 感染性因子低減化技術（PRT、CaridianBCT、コロラド州レークウッド）は、リボフラビン存在下で紫外線を照射し、核酸に修復不能な損傷を引き起こすことにより、感染性因子および白血球の複製を阻害する[13]。リボフラビ

ンは毒性および変異原性を有さない化合物であるため、処理後の除去は不要である[14]。本技術は、血小板製剤に含まれる病原性を有する感染性因子量を大幅に減少させるとともに[15-21]、血液成分中に残存するとみられる白血球にも不活化効果があることから、輸血関連移植片対宿主病を予防するという強力なエビデンスが得られている[22-25]。実験室での研究において、Mirasol 処理された血小板の代謝および品質（一連の *in vitro* 血小板機能試験により測定）が許容できるものであることも実証されている[26,27]。そのため、本技術が感染性因子の伝播低減化法として安全かつ有効であることが第 I 相および第 II 相試験により示唆されたことから、より大規模な臨床試験を実施することとした。

我々は、感染性因子低減化処理済みの血小板（PRT-血小板）が化学療法による血小板減少症患者に輸血された場合、輸血 1 時間後の補正血小板増加数（CCI_{1hour}）を指標とする有効性が、標準的な非処理血小板製剤と同等であるか否かを明らかにするため、ランダム化比較試験（RCT）を実施したので報告する。本試験では、全有害事象を記録することにより、PRT-血小板の安全性情報も入手可能な計画とした。

材料および方法

試験デザイン

本試験は、フランス国内で Etablissement Francais du Sang と大学病院（6 施設）が実施した多施設共同、非盲検、並行群間、非劣性 RCT であり、血小板減少症を伴う血液疾患および／または腫瘍患者に、PRT-血小板と標準（対照）血小板製剤を輸血した場合の効果を比較した。本試験は、参加施設の中央研究倫理委員会により承認され、http://www.clinicaltrials.gov（NCT00263809）および AFSSAPS の公式治験サイトに登録された。

試験対象集団

患者の適格性評価は 2 段階のプロセスで行った。第一段階で、次の組み入れ基準を満たす患者を選択し、第二段階の評価適格対象者とした：1）年齢が 16 歳以上であること、2）化学療法、悪性血液疾患、同種または自家造血幹細胞移植による血小板減少症、あるいは固形腫瘍と診断され、2 回以上の血小板輸血が予想されていること、3）入院治療中であること。適格者のうち、次の基準に 1 項目以上に該当する者は除外した：1）妊娠中である

こと、2）授乳中であること、3）脾腫がみられること、4）止血機能に影響を及ぼす自己免疫疾患の病歴があるか、そのような疾患と診断されていること。第一段階の適格基準を満たした患者を対象に、インフォームド・コンセントの取得を開始した。本試験を行う理由および目的は、各施設の治験責任医師または治験分担医師が患者に説明した。全患者からのインフォームド・コンセントは、ヘルシンキ宣言に従って取得しなければならないものとした。試験参加に同意した患者に対し、第二段階のスクリーニングプロセスを実施し、適格性を確認した。次の基準のいずれかに該当する患者を除外した：1）ランダム化前 72 時間以内に血清または尿妊娠検査が陽性であった場合、2）リボフラビンまたはその代謝物に対する過敏症の既往がある場合、3）血小板輸血に対する不応状態の既往がある場合（連続 2 回の輸血で $CCI_{1hour} < 5{,}000$ であった場合）、4）抗 HLA 抗体を保有、リンパ球傷害性試験が陽性、過去に血小板に対する同種免疫が記録されている（各施設の検査プロトコールに準拠）のいずれかに該当する場合、5）出血症状があり 1 回以上の赤血球輸血を必要とする場合、6）急性または慢性の播種性血管内凝固と診断されている場合、7）免疫性／特発性血小板減少性紫斑病、血栓性血小板減少性紫斑病または溶血性尿毒症症候群の既往があるか、これらの疾患と診断されている場合、8）固形臓器移植歴がある場合、9）静脈閉塞症の徴候を有する場合、10）体温が 39.5ºC を超えているか、感染の徴候ありのいずれか一方または両方に該当する場合、11）過去 6 ヵ月以内に感染性因子低減化技術に関する臨床試験に登録された場合、12）ランダム化前 30 日以内に他の治験薬に曝露された場合、13）ランダム化前 14 日以内に本試験の使用禁止薬を使用した場合（本稿オンライン版に参考情報として示す補遺 S1 を参照）、14）慢性的なアルコール乱用の確証がある場合[28]、15）本試験への参加を妨げうる他の何らかの医学的状態にある場合。

第二段階の適格基準を満たした患者は、対照血小板群または PRT-血小板群にランダムに割り付けた。施設およびブロック化による層別化を含む処理群へのランダム割付計画は、本試験の調整担当施設（MedPass International、パリ、フランス）がコンピュータによって作成した。患者の割付は、各施設にて、投与割付が入った封筒法で行った。PRT-血小板がわずかに黄色を帯びていたため、本試験は二重盲検法とはならなかった。しかし、血小板数評価および患者評価の担当者は、患者の投与割付について盲検化した。

ランダム化後、初回来院時に、次のデータを収集した：身長、体重、バイタルサイン、

併用治療および臨床検査値［D-ダイマー、アルブミン、アルカリホスファターゼ、アラニンアミノトランスフェラーゼ（ALT）、血中尿素窒素、乳酸デヒドロゲナーゼ（LDH）、カリウム、総蛋白、フィブリノゲン、クレアチニン、尿素、ビリルビン、全血算およびリンパ球傷害性試験］。光化学反応の生成物および新抗原生成の検出用にも血液検体を採取した（結果は別報に報告）[28]。

介入

対照血小板製剤および PRT-血小板製剤は、アフェレーシス（Trima Version 5.0、CaridianBCT）により採取するか、6 人分の全血プールを用いて分離したバフィーコートから、top-and-bottom 方式による血液分離装置 OptiPress（Fenwal, Inc、イリノイ州ラウンドレーク）および従来の血小板プール法を用いて調製した。全血小板製剤とも、フランス国内の要求基準（97%を超える製剤で 1 バッグ中の残存白血球数が 10^6 個未満であること）に従って白血球を除去した。製剤の要求基準は、容量 170～360 mL、血漿中の血小板数 1,180 x 10^9～2,100 x 10^9 個/L、血小板数の最小値 3.0 x 10^{11} 個および最大値 5.1 x 10^{11} 個であった。全製剤とも血漿に懸濁し、撹拌しながら 22ºC で最長 5 日間保存した。要求基準（本稿オンライン版に参考情報として示す補遺 S2 を参照）に適合しなかった製剤は、本試験に使用しなかった。

PRT-血小板は、MIRASOL PRT を用いて調製した。静置期間（アフェレーシス血小板は採取後 2 時間、バフィーコート血小板は調製後 1 時間）の後、血小板を照射／保存用バッグに移し、リボフラビン溶液を添加した（500 μmol/L、35 ± 5 mL）。バッグは MIRASOL PRT Welder（融着装置）を用いて密封した。製剤を照射装置に入れ、6.24 J/mL で紫外線を照射後 [26]、「臨床試験専用」と記載されたラベルを貼付した。

推奨される血小板輸血のトリガー値は、臨床的な危険因子がない場合には 10 x 10^9/L、発熱、高血圧、グレード 2 の粘膜炎、出血の可能性のある病変、および／または 72 時間以内に急激な血小板減少がみられた場合には 20 x 10^9 /L、抗血栓薬が投与されている場合、線溶または血液凝固障害の既往がある場合、あるいは侵襲的手術が必要な場合には 50 x 10^9/L とした [29]。患者は本試験をいつでも中止できるものとした。また、本試験への参加が患者にとって最善でないことを示唆する臨床所見または臨床検査値が得られた場合には、

担当医が中止を決定できるものとした。

投与期間はランダム化時（第 0 日）を開始時点とし、最長 28 日間継続した。第 28 日より前に患者が試験終了に至った理由としては、治験実施計画書に適合する血小板輸血が不要となった、有害事象による中止、同意の撤回、追跡不能、転科（集中治療室など）または死亡があった。投与期間の終了後、安全性の追跡調査期間を第 1 日［28 日間（23～42 日間は許容範囲とみなす）の投与期間中の治験実施計画書に適合する最終輸血の翌日、試験中止日（有害事象／同意の撤回）、追跡不能または死亡のいずれか最も早い日］に開始した。治験実施計画書に適合しない輸血とは、1）血小板製剤が事前に規定した基準（上記）を満たさなかった場合、2）PRT-血小板群へのランダム割付例に PRT-血小板以外の製剤が輸血された場合、あるいは 3）血小板輸血が投与期間（28 日間）外で行われた場合とした。

治験実施計画書に適合する各輸血時に収集した製剤情報は、重量（g）、血小板数、ABO 型、採取および／または製造方法、γ線照射の有無、輸血量、輸血日時であった。治験実施計画書に適合する各輸血時の患者情報は、輸血前および輸血 24 時間後に収集し、体重、バイタルサイン、出血の徴候、併用治療、クレアチニン、尿素、ビリルビンおよび血算値とした。クレアチニン、尿素、ビリルビンを除く項目の記録は輸血 1 時間後にも行った。安全性の追跡調査期間終了時にも、輸血前評価と同じ評価を実施した。治験実施計画書に適合する輸血時の出血評価は、WHO の出血評価基準に基づくスコア化に関して適切な訓練を受けた病院スタッフ（医師または看護師）が実施した[30]。これには、出血徴候および症状の身体診察と、出血記録に関するカルテレビューが含まれた。出血評価は、追跡調査のための最終来院時にも実施した。

試験の評価項目

主要有効性評価項目は CCI_{1hour} とし、28 日間の投与期間中に施行した治験実施計画書に適合する患者あたり最高 8 回の各血小板輸血後（輸血 30～90 分後）に測定した。CCI_{1hour} 算出のためには、当該患者の輸血前 12 時間以内の輸血前血小板数測定が必要であった。輸血 30～90 分後の CCI_{1hour} が算出された輸血は、測定時点が遵守されたとみなした。輸血 0～120 分後の CCI_{1hour} が算出された場合も、測定時点がより広範囲の CCI_{1hour} とみなして解析した。輸血 120 分後より後の CCI_{1hour} が算出された輸血は、これらの解析には含

めなかった。CCI は次の式を用いて算出した。

$$CCI = \frac{\text{輸血後血小板数} - \text{輸血前血小板数}\ (\times 10^9 / L)}{\text{輸血された血小板総数}\ (\times 10^{11})} \times BSA^*$$

*BSA（体表面積）
$= 0.0202457 \times \text{身長}_{\text{m}}^{0.725} \times \text{体重}_{\text{kg}}^{0.425}$

副次評価項目は、CCI_{24hour}（輸血 18～26 時間後に採血された場合には測定時点が遵守された CCI_{24hour} とみなし、輸血 15～30 時間後に採血された場合には測定時点がより広範囲の CCI_{24hour} と定義）、輸血間隔、投与期間中の患者あたりの血小板輸血回数および赤血球輸血回数、体表面積および投与日（投与期間の何日目に投与されたか）により標準化した輸血血小板数、不応状態の徴候（連続 2 回の輸血で $CCI_{1hour} < 5{,}000$ であった場合）、輸血関連感染症の発生頻度、出血（WHO グレード 1～4）とした。

安全性評価項目は有害事象、重篤な有害事象（SAE）、血小板輸血日の出血状態、輸血関連感染症および死亡とし、投与期間中および追跡調査期間中に評価した。有害事象は軽度、中等度または重度に分類した。輸血との因果関係は、「関連性がない」、「関連性が弱い」、「関連性が示唆される」、「関連性が強い」または「関連性がきわめて強い」に分類した（本稿オンライン版に参考情報として示す補遺 S3 を参照）。有害事象は、有害事象共通用語規準［CTCAE Version 3.0/医薬品規制用語集（MedDRA）Version 6.0、MedDRA MSSO、バージニア州シャンティリー）に従ってコード化した。全 SAE は当該事象の確認後 24 時間以内に、本試験の調整担当施設および他の関係当局に報告した。新抗原に対する同種免疫も評価し、結果を別報に報告した[28]。

症例数

対照群の CCI_{1hour} は平均 14,700［標準偏差（SD）5,200、TRAP 試験の結果に基づく］と推定された[31]。PRT-血小板が通常の血小板と比較して非劣性であることを主張するために必要な症例数は、非劣性限界値を 20%（CCI の群間差 2,940）と設定し、第 1 種過誤率 2.5%、検出力 80%とした場合、1 群 50 例と算出された。この症例数は、複数の追跡不能

例が生じることを考慮して、118 例まで増やした。CCI_{1hour} 平均値の群間差（PRT-血小板群から対照群を減じた値）の両側 95%信頼区間（CI）下限が-2,940 より大きい場合に、非劣性が実証できると考えられた。

データ安全性モニタリング委員会

データ安全性モニタリング委員会（DSMB）は輸血専門家 2 名、生物統計学者 1 名、医師 1 名で構成した。全員が治験依頼者とは無関係であった。DSMB は、盲検化された安全性および有効性データをモニターし、治験実施計画書の変更および治験継続／中止に関する勧告を行うとともに、全 SAE を再調査し最終判定を下した。中間解析は治験前に計画し、ランダム化例 54 例の追跡調査完了後に独立したグループにより行われた。しかし、正式な中止規則は事前に規定しなかった。

統計解析

人口統計学的変数および臨床変数については、記述的解析を実施した。連続変数は平均値および SD により要約し、カテゴリー変数は頻度および百分率により要約した。治験実施計画書に適合する輸血および適合しない輸血の頻度を表にした。

主要および副次評価項目（それぞれ CCI_{1hour} および CCI_{24hour}）は、複数回の輸血による患者の輸血応答と、輸血前血小板数および PRT 処理群との関連を調整するため、患者情報を変量とする混合効果共分散分析モデルにより解析した[32]。各処理群の最小二乗（LS）平均および標準誤差（SE）は、コンピュータソフトウェア（PROC MIXED、SAS 9.1.3、SAS Institute, Inc.、ノースカロライナ州ケアリー）を用いた適合度によって算出し、処理群間で比較した。このアプローチは、同一患者における一連の輸血に対する反応が無関係なものでないとの認識により、すべての変動要因を認識し、確実な推論を得るものである。解析対象としたのは、輸血を 1 回以上受けたすべてのランダム化例に対する投与期間中の治験実施計画書に適合する血小板輸血のうち、測定時点が守られた 1～8 回目の輸血とした。副次解析では、輸血後測定時点がより広範囲の輸血も対象とした。

処理群と輸血前血小板数の交互作用の検定を行い、輸血前血小板数に差があることで PRT-血小板の効果が変化するとの検討と併せ、輸血効果と施設間の交互作用の検定から全

施設のデータが統合可能であるか否についても検討した。

また、混合継時的ロジスティック回帰モデル[33]は、PRT-血小板製剤と対照血小板製剤の輸血効果（CCI_{1hour}およびCCI_{24hour}がそれぞれ7,500および4,500以上であること）[34]の比較評価に適している。このモデルでは輸血前血小板数と、輸血応答の経時変化における関連性を明らかにするため、患者情報を変量として組み込んだ。Frailty（脆弱性）モデルは、輸血間隔が空くことが患者内の変動要因の主な原因ではあるものの、輸血間隔の評価に適している[35]。副次評価項目の比較に関するp値はいずれも両側検定値とした。有害事象データは表形式に要約し、記述的に解析した。

主要解析および副次解析は、患者95例を対象とした事後の部分集団解析でも実施した。この部分集団は、DSMBとの話し合い後にデータ不完全例15例（対照血小板群8例およびPRT-血小板群7例）を除外することにより得た。

結果

2005年12月～2007年9月に、6施設において患者118例が本試験に登録された。うち60例をPRT-血小板群、58例を対照血小板群とした。各処理群4例は血小板輸血を受けなかったため、110例がintention-to-treat解析の対象例であった。投与期間の第28日より前に本試験を中止した患者は110例中10例であった（PRT-血小板群6例、対照血小板群4例）。したがって、投与期間を完了した患者の割合は、PRT-血小板群91.1%（56例中51例）、対照血小板群98.1%（54例中53例）であった。これら10例のデータは、各患者の中止時点まで解析した。安全性の追跡調査期間を完了した患者の割合は、PRT-血小板群73.2%（56例中41例）、対照血小板群81.5%（54例中44例）であった（両群の試験期間中央値はそれぞれ45日および44日）。本試験をとおしての患者の流れを図1にまとめる。

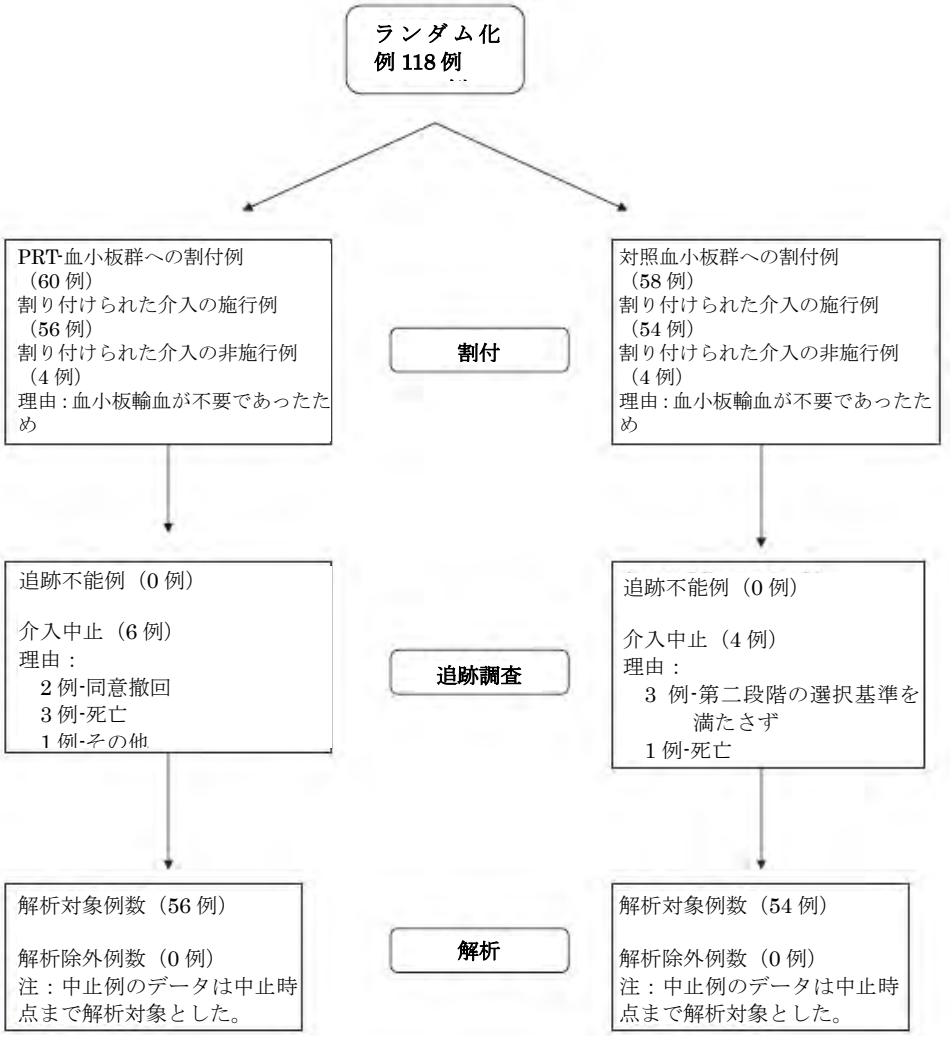

図 1.　試験（ランダム化から解析まで）を通じての患者の流れを示した CONSORT 声明によるフローチャート

試験対象患者の輸血前の人口統計学的特性は 2 群間で同等であり、表 1 に要約する。記録したその他のベースライン特性（データは示さず）も両群間で同等の分布を示した（身体所見、バイタルサイン、全血算、フィブリノゲン、アルブミン、アルカリホスファターゼ、ALT、クレアチニン、尿素、直接および総ビリルビン、血中尿素窒素、LDH、カリウム、総蛋白）。

試験期間中の血小板輸血回数は計 678 回であり、PRT-血小板群 368 回（治験実施計画書に適合する輸血 303 例、適合しない輸血 65 回）、対照血小板群 310 回（治験実施計画書に適合する輸血 238 例、適合しない輸血 72 回）であった。治験実施計画書に適合しない血小板輸血の頻度は、PRT-血小板群 17.7%、対照血小板群 23.2%であった。適合しない輸

血の基準は治験実施計画書に事前に規定したが、データ収集プロセスではその理由は記録されなかった。

事前に規定した主要評価項目 CCI_{1hour} の解析は、28 日間の投与期間中に施行した患者あたり最高 8 回の血小板輸血（PRT-血小板群 258 回、対照血小板群 209 回、計 467 回）のデータに基づき実施した。治療効果 CCI_{1hour} の施設間での均質性を検定したところ、有意な結果が得られなかったことから（$p = 0.1728$）、治療効果を推定する際、全施設のデータを統合できると考えられた。CCI_{1hour} の LS 平均は、PRT-血小板群が 11,725（SE、1,140）、対照群が 16,939（SE、1,149）で、群間差は-5,214（95% CI、-7,542～-2,887、$p < 0.0001$）であった。群間差の CI には事前に規定した非劣性上限値（対照群における平均 CCI 推定値の 20%、すなわち 2,940 に設定）が含まれた。非劣性であるためには、この CI 下限が -2,940 より大きくなければならないため、非劣性であると主張することはできなかった。測定時点がより広範囲の CCI_{1hour} も算出し、輸血前血小板数（連続変数）および施設について補正した（表 2）。測定時点が遵守された CCI_{1hour} データおよび測定時点がより広範囲の CCI_{1hour} データを、箱ひげ図を用いて図 2 に示す。

副次評価項目

測定時点が遵守された CCI_{24hour} および測定時点がより広範囲の CCI_{24hour} を解析対象とし、輸血前血小板数（連続変数）および施設について補正した。治療効果 CCI_{24hour} の施設間での均質性を検定したところ、有意な結果が得られなかったことから（$p = 0.1336$）、全施設のデータを統合できると考えられた。測定時点が遵守された CCI_{24hour} の LS 平均は、PRT-血小板群が 6,676（SE、883）、対照群が 9,886（SE、915）で、群間差は-3,210（95% CI、-5,160～-1,260）であった。CCI_{24hour} の結果を表 2 および図 2 に要約する。表 2 には、混合ロジスティック回帰モデルによる解析結果も示し、目標 CCI 値（CCI_{1hou} は 7,500、CCI_{24hour} は 4,500）達成のオッズ比（OR）を記載した。目標とする反応が得られる確率は、測定時点が遵守された CCI_{1hour} を解析対象とした場合には、PRT-血小板群の方が有意に低かった（OR、0.284；95% CI、0.105～0.767；$p = 0.0130$）。一方、測定時点が遵守された CCI_{24hour} を解析対象とした場合には、PRT-血小板群の方が低かったが有意差はみられなかった（OR、0.481；95% CI、0.211～1.098；$p = 0.0822$）。測定時点がより広範囲の CCI を対象とした解析でも同様の結果が得られたが、CCI_{24hour} 目標値が得られるオッズ比

は PRT-血小板群が有意に低かった。

両投与群の患者とも、投与期間中に治験実施計画書に適合しない輸血を施行されたため、有意の輸血間隔を算出することは難しかった。

表1. PRT-血小板群および対照群の患者の輸血前の特性

	処理群	
人口統計学的特性	PRT-血小板群（56 例）	対照群（54 例）
年齢中央値、歳（範囲）	58 (20-73)	53 (20-74)
性別（男/女）	32/24	34/20
身長中央値、m（範囲）	1.7 (1.5-1.86)	1.7 (1.51-1.93)
体重中央値、kg（範囲）	71.5 (46.3-121.0)	73.6 (45.0-110.3)
ABO 血液型、例数（%）		
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)
診断、例数（%）		
急性リンパ性白血病	2 (3.6)	2 (3.7)
急性骨髄性白血病	26 (46.4)	27 (50.0)
多発性骨髄腫	4 (7.1)	2 (3.7)
非ホジキンリンパ腫	19 (33.9)	18 (33.3)
ホジキンリンパ腫	1 (1.8)	3 (5.6)
その他*	4 (7.1)	2 (3.7)
平均（SD、血小板減少症の期間）、日†	16.3 (7.2)	14.8 (7.0)
ベースライン時の検査値、中央値（範囲）		
ヘモグロビン（g/dL）	9.5 (8-14)	9.5 (7-15)
血小板数（x 10^9/L）	42.5 (8-479)	43.0 (6-206)
白血球数（x 10^9/L）	1.05 (0-14)	1.30 (0-51)

* その他には、重度の特発性延髄無形成（1 例）、急性混合型白血病（1 例）、慢性リンパ性白血病（1 例）、骨髄異形成-過剰芽球を伴う不応性貧血（2 例）、縦隔奇形癌（1 例）があった。

† 投与期間中

表2. 投与群別のCCI値の要約：治験実施計画書に適合する1～8回目の輸血に基づく解析（主要評価項目）および投与期間中の治験実施計画書に適合する全血小板輸血を対象とした解析*

評価項目	PRT-血小板群		対照群		PRT-血小板群－対照群		
CCI—連続変数	回数	LS平均（SE）	回数	LS平均（SE）	差	95% CI	*p*値
28日間の投与期間中の治験実施計画書に適合する1～8回目の輸血を対象とした解析							
CCI_{1hour}							
測定時点が遵守された値	195	11,725 (1,140)	164	16,939 (1,149)	-5214	(-7542 ~ -2887)	<0.0001
測定時点がより広範囲の値	216	11,766 (1,072)	174	17,170 (1,057)	-5404	(-7721 ~ -3088)	<0.0001
CCI_{24hour}							
測定時点が遵守された値	175	6,676 (883)	160	9,886 (915)	-3210	(-5160 ~ -1260)	0.0014
測定時点がより広範囲の値	209	6,998 (811)	179	10,385 (811)	-3387	(-5232 ~ -1542)	0.0004
28日間の投与期間中の治験実施計画書に適合する全輸血を対象とした解析							
CCI_{1hour}	273	11,005 (962)	220	16,614 (977)	-5609	(-7791 ~ -3427)	<0.0001
CCI_{24hour}	267	7,162 (831)	211	10,070 (839)	-2907	(-4802 ~ -1013)	0.0027
CCI—2値変数	回数	回数（%）	回数	回数（%）	OR	95% CI	*p*値
28日間の投与期間中の治験実施計画書に適合する1～8回目の輸血を対象とした解析							
測定時点が遵守された値							
CCI_{1hour}> 7,500	195	139 (71.3)	164	138 (84.1)	0.284	(0.105 ~ 0.767)	0.0130
CCI_{24hour}> 4,500	175	103 (58.9)	160	109 (68.1)	0.481	(0.211 ~ 1.098)	0.0822
測定時点がより広範囲の値							
CCI_{1hour}> 7,500	216	151 (69.9)	174	147 (84.5)	0.233	(0.081 ~ 0.667)	0.0067
CCI_{24hour} > 4,500	209	118 (56.5)	179	120 (67.0)	0.423	(0.189 ~ 0.945)	0.0360

* CCIを2値変数とし、治験実施計画書に適合する1～8回目の輸血を対象とした解析結果も示す。

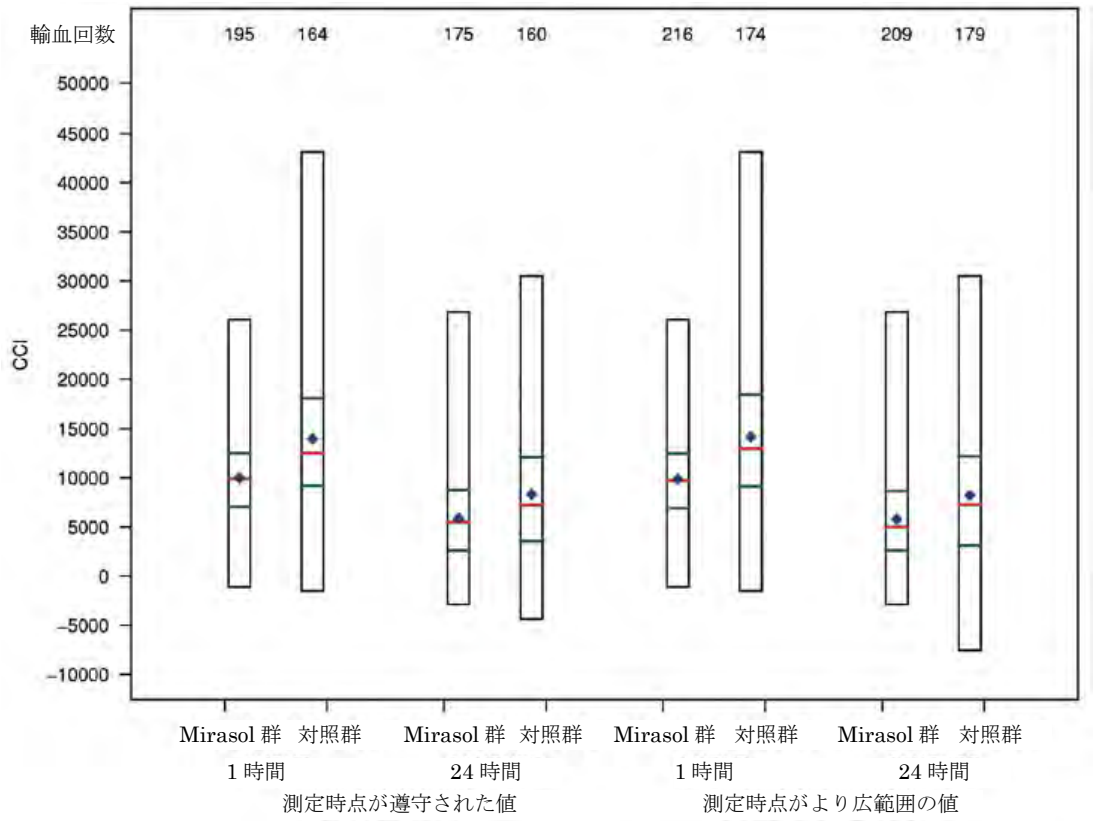

図 2.　各投与群における測定時点が遵守された CCI_{1hour} および CCI_{24hour} と、測定時点がより広範囲の CCI_{1hour} および CCI_{24hour} の箱ひげ図。

ボックス内の横線は、第 3 四分位数（Q_{75}：上側の線）、中央値（中間の線）および第 1 四分位数（Q_{25}：下側の線）を示す。菱形は、生データの平均値（CCI 生データの平均値）を示す。ボックスの高さは最小値および最大値を示す。

28 日間の投与期間中の治験実施計画書に適合する輸血と適合しない輸血の両方を本解析の対象とした結果、平均輸血間隔は PRT-血小板群が 2.16 日（SD、1.69 日）、対照群が 2.30 日（SD、1.48 日）であった（$p = 0.2903$）。投与期間中の患者-日あたりの平均血小板輸血回数（治験実施計画書に適合する輸血と適合しない輸血の両方を解析対象とした場合）は、PRT-血小板群が 0.24 回（SD、0.16 回）、対照群が 0.20 回（SD、0.19 回）で、有意差はみられなかった（$p = 0.2046$）。副次評価項目の要約を表 3 に示す。有意な群間差は認められなかった。

赤血球輸血必要量にも 2 群間で差はみられなかった。PRT-血小板群における赤血球輸血

量は、投与期間および追跡調査期間の合計が 183 単位、投与期間が 155 単位で、患者あたりの平均輸血量（SD）は 2.8 単位（1.7 単位）であった。対照群における赤血球輸血量は、投与期間および追跡調査期間の合計が 160 単位、投与期間が 142 単位で、患者あたりの平均輸血量（SD）は 2.6 単位（2.4 単位）であった（$p = 0.7257$）。

同種免疫および不応状態

PRT-血小板群では 2 例（3.6%）に同種免疫の応答がみられ、対照群では 4 例（7.45%）に HLA 抗体が産生した（$p = 0.4336$、Fisher の直接法）。試験期間中に不応状態となった患者は 110 例中 5 例（4.5%）のみで、内訳は PRT 血小板群 3 例（5.4%）、対照群 2 例（3.7%）であった（$p = 1.0000$、Fisher の直接法）。

感染症

感染症に関する有害事象は計 88 件が患者 58 例に報告された。PRT-血小板群では、感染症 45 件が患者 28 例に報告された（1 例あたり 1.61 件）。感染症 6 件は重度の有害事象に分類され、その内訳は、サイトメガロウイルス（CMV、1 例）、クレブシエラ（2 例）、大腸菌性尿路感染（1 例）、感染（1 例）および敗血症（1 例）であった。CMV 感染症を来した患者 1 例は、幹細胞移植および輸血前の CMV の血清学的検査が陽性であった。対照群では、感染症 43 件が患者 30 例に報告された（1 例あたり 1.42 件）。うち 9 件が重度の有害事象に分類され、その内訳は、細菌感染（1 例）、細菌性敗血症（1 例）、気管支肺アスペルギルス症（1 例）、*Clostridium* 性大腸炎（1 例）、*Clostridium difficile* 大腸炎（1 例）、ヘルペスウイルス感染（1 例）、腸球菌性尿路感染（1 例）、敗血症（1 例）、敗血症性ショック（1 例）であったが、いずれも輸血に関連しないと判断された。1 件以上の感染を来した患者の割合には有意な群間差はみられず（$p = 0.5731$、Fisher の直接法）、患者 1 例あたりの平均感染件数にも有意な群間差はみられなかった（$p = 0.4571$）。表 4 に、有害事象および SAE の発生率を要約する。

表3. 血小板輸血の特性および血小板輸血に関連する副次評価項目の要約

	血小板輸血							
	28日間の投与期間中の治験実施計画書に適合する1～8回目の輸血のみ				28日間の投与期間中の治験実施計画書に適合する輸血			
評価項目／特性	PRT-血小板群		対照群	*p*値	PRT-血小板群		対照群	*p*値
合計輸血回数	258		209		303		238	
アフェレーシス血小板の輸血回数（%）	180	(69.8)	149	(71.3)	224	(73.9)	178	(74.8)
バフィーコート血小板の輸血回数（%）	78	(30.2)	60	(28.7)	79	(26.1)	60	(25.2)
患者1例あたりの血小板輸血回数の中央値（範囲）	4.0	(1-8)	3.0	(1-8)	4.5	(1-21)	3.0	(1-19)
平均輸血血小板総数（SD）（x 10^{11}）	5.37	(2.14)	5.38	(2.10) 0.9615	5.23	(2.09)	5.22	(2.02) 0.9867
輸血時の製剤の平均保存期間（日）	2.8	(1.1)	2.6	(1.1) 0.0891	2.7	(1.1)	2.6	(1.1) 0.2210
ABO 不適合輸血の合計回数（%）	41	(15.9)	37	(17.7)	50	(16.5)	40	(16.8)
メジャー不適合	39	(15.1)	32	(15.3)	46	(15.2)	35	(14.7)
マイナー不適合	2	(0.8)	5	(2.4)	4	(1.3)	5	(2.1)
CCI_{1hour} 測定時点が遵守された輸血回数(%)	195	(81.9)	164	(85.4)	222	(81.3)	185	(84.1)
CCI_{24hour} 測定時点が遵守された輸血回数（%）	175	(75.8)	160	(83.3)	196	(73.4)	173	(82.0)
血小板輸血の平均間隔（SD）*	2.32	(1.79)	2.72	(1.44) 0.0107	2.16	(1.69)	2.3	(1.48) 0.2903

***** 血小板輸血の平均間隔（SD）は、治験実施計画書に適合する輸血および適合しない輸血の両方を解析対象として算出している。

表4. 各WHOグレードの出血を来した患者数と、全有害事象および重度の有害事象の平均発生率の要約

	全解析対象例（110例）		
WHO グレード別の出血と有害事象発生率	PRT-血小板群（56 例）	対照群（54 例）	*p* 値
出血を来した患者数			
グレード1	28	19	0.128
グレード2	11	5	0.176
グレード3	4	2	0.679
グレード4	2	1	1.000
グレード1～4	33	23	0.127
グレード2～4	12	7	0.315
グレード3および4	6	3	0.490
平均発生率（SD）*			
全有害事象	0.28 (0.19)	0.23 (0.16)	0.144
重度の有害事象	0.07 (0.17)	0.04 (0.06)	0.171

* 有害事象の合計発生件数を、投与期間および追跡調査期間の日数の和で除した値

出血評価項目

出血の評価は、治験実施計画書に適合する血小板輸血のみを対象として実施し、評価時点は輸血前、輸血 1 時間後および 24 時間後、ならびに追跡調査のための最終来院時とした。WHO グレード 2 以上の出血を来した患者数は 19 例で、内訳は PRT-血小板群 12 例（21.4%）、対照群 7 例（13.0%）であった。PRT-血小板群におけるグレード 2、3、4 の出血の発生患者数は、それぞれ 11 例、4 例、2 例であった（グレード 4 の 2 例はいずれも中枢神経系出血で、1 例は第 17 日に死亡し、もう 1 例は本試験を完了した）。対照群におけるグレード 2、3、4 の出血の発生患者数は、それぞれ 5 例、2 例、1 例であった。グレード 4 の出血は泌尿生殖器の出血で、同患者は 41 日間の試験を完了した。グレード別の出血事象発生件数を、表 4 に要約する。

事後の部分集団解析の対象とした患者 95 例における主要および副次エンドポイントの結果も要約する（本稿オンライン版に参考情報として示す補遺 S4 を参照）。主要エンドポイント（CCI_{1hour}）の非劣性は実証されなかった。副次エンドポイントに関する結果はいずれも、全 110 例を解析対象とした結果とほぼ等しかった。

安全性評価項目

有害事象を 1 件以上報告した患者の割合は、PRT-血小板群が全例、対照群が 98.1%（54 例中 53 例）であった。しかし、有害事象の大多数は、輸血された血小板製剤とは関連がないものであった（表 5）。被験輸血が「関連性が示唆される」、「関連性が強い」または「関連性がきわめて強い」に分類された有害事象の発生件数は、PRT-血小板群 5 件（患者 5 例）、対照群 8 件（患者 5 例）であった。重度の有害事象のうち、輸血に「関連性がきわめて強い」に分類された事象の発生患者数は、PRT-血小板群 2 例（1.8%）、対照群 2 例であった。これらの患者は、アナフィラキシーショック（対照群 1 例）、過敏症と眼瞼浮腫（対照群 1 例）、ならびに血小板輸血に対する不応状態（PRT-血小板群 1 例）を来した。血栓事象は 5 件報告されたが、いずれも被験輸血には関連しなかった。うち 1 件（肺塞栓症）は PRT-血小板群、4 件［脳血栓症（1 件）、心筋梗塞（1 件）、頸静脈血栓症（1 件）、静脈閉塞性疾患（1 件）］は対照群に発生した。器官別に分類した全有害事象および重度の有害事象を、表 6 にまとめる。全有害事象および SAE の発生頻度に処理群間差はみられなかった。次の器官に分類される有害事象が大半を占めた：胃腸障害、全身障害および投

与局所様態、血液およびリンパ系障害。

表5. 有害事象、重度の有害事象およびSAEの輸血との関連性別件数および頻度*

輸血との関連性により分類した有害事象	PRT-血小板群（56 例）	対照群（54 例）
有害事象		
有害事象が 1 件以上発生した患者数	56 (100)	53 (98.1)
有害事象の合計発生件数	654	507
有害事象と被験輸血の関係†		
関連性がない	596 (91.1)	477 (94.1)
関連性が弱い	53 (8.1)	22 (4.3)
関連性が示唆される	3 (0.5)	2 (0.4)
関連性が強い	0 (0.0)	3 (0.6)
関連性がきわめて強い	2 (0.3)	3 (0.6)
重度の有害事象		
有害事象が 1 件以上発生した患者数	38 (67.8)	30 (55.6)
有害事象の合計発生件数	110	90
有害事象と被験輸血の関係‡		
関連性がない	100 (90.9)	86 (95.6)
関連性が弱い	7 (6.4)	1 (1.1)
関連性が示唆される	1 (0.9)	0 (0.0)
関連性が強い	0 (0.0)	0 (0.0)
関連性がきわめて強い	2 (1.8)§	3 (3.3)||
SAE		
有害事象が 1 件以上発生した患者数	13 (23.2)	11 (20.4)
有害事象の合計発生件数	17	14
有害事象と被験輸血の関係¶		
関連性がない	12 (70.6)	12 (85.7)
関連性が弱い	5 (29.4)	1 (7.1)
関連性が示唆される	0 (0.0)	0 (0.0)
関連性が強い	0 (0.0)	0 (0.0)
関連性がきわめて強い	0 (0.0)	1 (7.1)**

* データは件数または例数（%）として報告する。重度の有害事象とは、患者に重度の苦痛を引き起こし日常活動に重大な影響を及ぼすとともに、治療を必要とするあらゆる好ましくない医療上の出来事と定義した。SAE とは、次の 1 つ以上に該当する事象とした：1）死亡、2）生命を脅かす疾患または損傷、身体構造または機能の永続的な障害、入院期間の延長または内科的／外科的治療に至る重篤な健康状態の悪化、3）輸血完了不能。

† 治験責任医師の報告通りに示す。百分率は、各処理群で報告された有害事象発生件数を分母として算出した。

‡ 治験責任医師の報告通りに示す。百分率は、各処理群で報告された重度の有害事象発生件数を分母として算出した。

§ 血小板輸血に対する不応状態

|| 患者 1 例は輸血中にアナフィラキシーショックを来し、1 例は 1 回の輸血中に過敏症、別の 1 回の輸血中に眼瞼浮腫を来した。

¶ 治験責任医師の報告通りに示す。百分率は、各処理群で報告された SAE 発生件数を分母として算出した。

** 患者 1 例は輸血中にアナフィラキシーショックを来した。.

考察

本試験は、CCI_{1hour} を指標として、PRT 血小板製剤が非処理血小板製剤に対し非劣性を示すか否かを確認する目的で計画した。CCI を主要評価項目とした理由は、PRT 処理さ

れた新規血小板製剤が欧州で認可を受ける際、従来より評価項目として使用されてきたことにあった[11]。本試験を計画する際、非劣性の基準は、感染性因子不活化処理した血小板製剤輸血群の CCI_{1hour} 平均値が、非処理血小板製剤群の CCI_{1hour} 平均値からその 20%値を減じた値を下回らない場合とした。本試験では、CCI_{1hour}（主要評価項目）、CCI_{24hour}（副次評価項目）いずれに関しても、非劣性を実証できなかった。血小板の感染性因子不活化処理により CCI が低下する原因は不明であるが、他のいくつかの試験でも一致した結果が得られている。Aubuchon ら[26]は、健常被験者を対象としたクロスオーバーRCT において、PRT-血小板の平均生存期間（38 時間短縮）および回収率（16.5%低下）が非処理血小板製剤と比較して低いことを確認した感染性因子不活化にアモトサレン塩酸塩（S-59）と UVA 照射を用いた SPRINT 試験も、感染性因子不活化処理した血小板製剤群の CCI_{1hour} と CCI_{24hour} がいずれも相対的に低いことを報告している。SPRINT 試験で報告された各処理群の平均 CCI は、本試験で得られた値とほぼ等しかった[12]。

PRT-血小板では、保存期間中に代謝活性が亢進し、活性化マーカーの発現が増加する[26]。したがって、血小板が活性亢進状態にあるため、傷害・損傷部位でより速やかに利用されるという仮説が成り立ちうる。同様の影響は、ジメチルスルホキシド（DMSO）存在下で低温保存した血小板でも認められている。しかし、P-セレクチンをはじめとする活性化マーカーの発現レベルが大幅に上昇すること[36-40]、血小板顆粒放出が有意に亢進すること[41]、血中からの回収率が有意に低下すること[42]が実証されているにもかかわらず、低温保存血小板の輸血では、従来の液状保存された血小板と比較して、出血件数、輸血必要回数および合併症発生件数が少なかった[36,38,43,44]。これらの結果は、血小板に対する感染性因子不活化処理の臨床的影響を評価する試験が必要であることを浮き彫りにしており、このような試験の実施により、*in vitro* における結果との関連性を明らかにできると考えられる。

CCI_{1hour}、CCI_{24hour} の平均値はいずれも、PRT-血小板群の方が低かったが、両平均値とも輸血が有効との定義に使用されている閾値（それぞれ 7,500 および 4,500）[45]を上回った。CCI_{1hour} が輸血成功の閾値を上回った輸血の割合は、PRT-血小板群 71.3%に対し、対照群 84.1%であった。CCI_{24hour} が輸血が有効との閾値を上回った輸血の割合は、PRT-血小板群 58.9%に対し、対照群 68.1%であった。輸血有効率は（両群とも）目標値より低いものの、血小板輸血に関する他の試験で報告された有効率の範囲内であり、現在確立され

ている閾値を基準とした場合に有効と判定されない血小板輸血が 30～40%存在するのはなぜかという疑問が生じる[46,47]。輸血が無効であった一因は、CCI に影響を与える患者側の要因と、製品のばらつきにより説明できると考えられる。しかし、輸血効果がこのように不十分である原因は未だ十分には解明できていない。このような結果から、臨床評価項目としての CCI の感度には疑問があり、実際、多くの試験では現在、出血症状を主要評価項目として用いている[12,48,49]。

輸血間隔および総血液製剤使用量の解析から、PRT-血小板製剤を使用する場合の原料血液に関する情報が得られた。輸血間隔は両被験群について求めたが、投与期間中には治験実施計画書に適合する輸血が必ずしも連続施行されなかったため、この解析には限界があり、明確な解釈はできなかった。総血小板輸血量および総赤血球輸血量には有意な群間差がみられなかったことから、PRT-血小板群の CCI が対照群より低かったことは、血液製剤使用量の有意な増加につながらなかったことを示唆している。

本試験からは、PRT-血小板を使用した場合の安全性情報も得られた。本試験では、血小板輸血との関連性の有無を問わず、すべての有害事象を記録する計画であった。本試験の投与期間中および追跡調査期間中に 1,100 件を超える有害事象が発生したことは、この患者集団には疾患および合併症がきわめて発生しやすかったことを示している。しかし、血小板輸血に「関連性がきわめて強い」に分類された有害事象を発生した患者数は、4 例（PRT-血小板群 2 例、対照群 2 例）にすぎなかった。PRT-血小板群に発生した事象 2 件は、血小板輸血に対する不応状態であった。対照群に発生した事象は、輸血中のアナフィラキシーショック、過敏症および眼瞼浮腫であった。全有害事象を器官および／または障害別に分類したところ、両処理群で特に高頻度に報告された事象は、胃腸障害、全身障害および投与局所様態、血液およびリンパ系障害、感染症および寄生虫症に属する事象であった。これらの事象の発生頻度に群間差はみられなかったことから、PRT-血小板輸血の安全性プロファイルは許容できることが示唆された。しかし、市販後調査として、あるいはより大規模な臨床試験の一貫として出血症状を主要評価項目として、追加の安全性データを収集することは有用と考えられる。本試験では、出血に関するデータは副次評価項目として収集したものの、積極的な評価が行われたのは、治験実施計画書に適合する輸血の前後 24 時間にすぎなかった。グレード 4 の出血事象は両群に発生した（PRT-血小板群 2 件、対照群

1 件)。本試験は、出血に関する群間差を示す検出力を有しておらず、また、データが不十分であったことから、出血リスクに関して結論を下す試みは行わないことにする。

本試験には、その他にもいくつかの制限があった。治験実施計画書に適合しない輸血が頻回に行われたため､経時的評価を行う副次評価項目の中には解析が困難な項目もあった。治験実施計画書に適合しない輸血が行われた理由は記録されなかった。この情報は、PRT-血小板が使用される際の実務的な検討事項の一部を理解するため、また、容量および投与量が統一された製剤の製造に伴う課題についてさらなる考察を得るために有用と考えられている。治験実施計画書に適合しない輸血に対する効果は記録されなかった。このため、従来の intention-to-treat 解析も実施できなかった。これらのデータは、全輸血歴をより完全に表現するために役立つと考えられた。また、CCI 算出用の輸血後検体の採取時間が遵守されなかったという治験実施計画書からの逸脱もみられた：CCI_{1hour} については 17.4%（493 回中 86 回)、CCI_{24hour} については 22.8%（478 回中 109 回)。これらのデータを除外しない条件で解析を行うため、測定時点が遵守された期間に加えて、より広い範囲の測定期間も事前に規定し、両条件のデータを解析した。しかし、このように測定時点の遵守に問題があったことは、この複雑な患者集団では、輸血後の CCI 測定値を得る際に問題があることを示している。

結論として、患者 110 例を対象としたこの比較臨床試験では、CCI_{1hour} を代替評価項目として用いたところ、PRT-血小板の対照血小板に対する非劣性は実証されなかった。安全性データからは、PRT-血小板輸血に関連する重大な副作用は確認されなかった。血小板および赤血球の総使用量に有意な群間差がみられなかったことから、PRT-血小板群の CCI は対照群より低かったものの、これにより血液製剤使用量は有意に増加しなかったことが示唆される。PRT-血小板群で認められた CCI 低値が出血リスクの何らかの変化につながるか否かを明らかにするためには、さらなる研究が必要である。

表6. PRT-血小板群と対照群における期間別（投与期間と追跡調査期間）、器官／障害別の有害事象発生件数の要約

	投与期間*				追跡調査期間†				全期間			
	対照群		PRT-血小板群		対照群		PRT-血小板群		対照群		PRT-血小板群	
器官／障害別の有害事象	AE 件数	患者数	AE 件数	患者数	AE 件数	患者数	AE 件数	患者数	AE 件数	患者数	AE 件数	患者数
合計	384	51	506	56	123	41	148	37	507	53	654	56
血液およびリンパ系障害	46	25	59	33	8	6	7	6	54	30	66	36
心臓障害	6	4	15	13	6	6	1	1	12	9	16	14
先天性、家族性および遺伝性障害	0	0	1	1	1	1	1	1	1	1	2	2
耳および迷路障害	3	3	0	0	0	0	0	0	3	3	0	0
内分泌障害	0	0	0	0	1	1	0	0	1	1	0	0
眼障害	5	5	4	3	2	2	3	3	7	7	7	6
胃腸障害	88	40	102	38	27	15	26	12	115	45	128	41
全身障害および投与局所様態	77	43	91	43	18	15	22	12	95	46	113	46
肝胆道系障害	3	3	2	2	0	0	1	1	3	3	3	3
免疫系障害	5	5§	0	0§	1	1	1	1	6	6	1	1
感染症および寄生虫症	28	21	32	22	15	14	13	9	43	30	45	28
傷害、中毒および処置合併症	2	1§	9	8§	0	0	1	1	2	1§	10	9§
臨床検査‡	0	0	5	5	0	0	0	0	0	0	5	5
代謝および栄養障害	19	15	31	21	5	3	13	10	24	18	44	29
筋骨格系および結合組織障害	6	4	6	6	7	6	4	3	13	8	10	9
神経系障害	11	8	15	13	7	5	5	4	18	12	20	14
精神障害	13	9	26	17	3	3	8	7	16	12	34	21
腎および尿路障害	9	8	6	5	2	2	1	1	11	10	7	6
生殖系および乳房障害	1	1	1	1	1	1	3	2	2	2	4	3
呼吸器、胸郭および縦隔障害	19	14	39	23	5	5	12	6	24	16	51	26
皮膚および皮下組織障害	29	22	36	26	10	7	12	9	39	27	48	31
血管障害	14	11	26	13	4	4	14	12	18	13	40	19

* 投与期間 = ランダム化日から、治験実施計画書に適合する血小板輸血の最終施行日まで
† 追跡調査期間 = 治験実施計画書に適合する血小板輸血の最終施行日から、試験中止／完了日まで
‡ 臨床検査 = 出血時間延長、血中クレアチニン*増加、体重増加
§ 対照群と PRT-血小板群の群間差が有意水準に達した比較
AE = 有害事象

*訳注：クレアチニンの誤りである。

Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial

Dick van Rhenen, Hans Gulliksson, Jean-Pierre Cazenave, Derwood Pamphilon, Per Ljungman, Harald Klüter, Hans Vermeij, Mies Kappers-Klunne, Georgine de Greef, Michel Laforet, Bruno Lioure, Kathryn Davis, Stephane Marblie, Veronique Mayaudon, Jocelyne Flament, Maureen Conlan, Lily Lin, Peyton Metzel, Don Buchholz, and Laurence Corash

A nucleic acid-targeted photochemical treatment (PCT) using amotosalen HCl (S-59) and ultraviolet A (UVA) light was developed to inactivate viruses, bacteria, protozoa, and leukocytes in platelet components. We conducted a controlled, randomized, double-blinded trial in thrombocytopenic patients requiring repeated platelet transfusions for up to 56 days of support to evaluate the therapeutic efficacy and safety of platelet components prepared with the buffy coat method using this pathogen inactivation process. A total of 103 patients received one or more transfusions of either PCT test (311 transfusions) or conventional reference (256 transfusions) pooled, leukoreduced platelet components stored for up to 5 days before transfusion. More than 50% of the PCT platelet components were stored for 4 to 5 days prior to transfusion. The mean 1-hour corrected count increment for up to the first 8 test and reference transfusions was not statistically significantly different between treatment groups (13 100 ± 5400 vs 14 900 ± 6200, $P = .11$). By longitudinal regression analysis for all transfusions, equal doses of test and reference components did not differ significantly with respect to the 1-hour (95% confidence interval [CI], −3.1 to 6.1 × 10^9/L, $P = .53$) and 24-hour (95% CI, −1.3 to 6.5 × 10^9/L, $P = .19$) posttransfusion platelet count. Platelet transfusion dose, pretransfusion storage duration, and patient size were significant covariates ($P < .001$) for posttransfusion platelet counts. Clinical hemostasis, hemorrhagic adverse events, and overall adverse events were not different between the treatment groups. Platelet components prepared with PCT offer the potential to further improve the safety of platelet transfusion using technology compatible with current methods to prepare buffy coat platelet components. (Blood. 2003;101: 2426-2433)

Introduction

Despite continued improvements in pretransfusion donor screening and testing to detect viruses associated with transfusion-transmitted infections, blood components continue to carry risk of infectious disease, including bacteria.[1] Nucleic acid detection methods for HIV and hepatitis C (HCV) have reduced the window period for infectious risk, but may not eliminate transfusion-associated HIV[2] or HCV infections;[3] fatal bacterial infections caused by transfusion of platelet concentrates continue to be reported.[4] To improve the safety of platelet and plasma transfusion, a nucleic acid-targeted photochemical treatment (PCT) using the psoralen compound amotosalen HCl (S-59) and long-wavelength ultraviolet A (UVA) light was developed to inactivate viruses, bacteria, and protozoa that may contaminate platelet and plasma components.[5,6] Preclinical studies have demonstrated inactivation of more than 10^6 infectious HIV particles, more than 10^5 infectious hepatitis B (HBV) and HCV particles, and a broad spectrum of gram-positive and gram-negative bacteria.[5,7,8] Furthermore, this process inactivates more than 10^5 contaminating T cells with effective inhibition of proliferation and complete suppression of leukocyte cytokine synthesis.[9,10]

To utilize this technology in blood component processing, a device consisting of a series of closed interconnected plastic containers and a microprocessor-controlled UVA light source has been integrated into a system for preparation of therapeutic doses of platelets. To evaluate the therapeutic efficacy and safety of platelet components prepared using this device, a randomized, controlled, double-blinded study was conducted on an intent-to-treat (ITT) basis for patients who required repeated platelet transfusions during multiple periods of thrombocytopenia.

Patients and methods

Patients and study design

Patients with thrombocytopenia or receiving therapy expected to cause thrombocytopenia requiring platelet transfusion (suggested transfusion

From the Sanquin Blood Bank South West Region, Rotterdam, The Netherlands; Erasmus Medical Center, Rotterdam, The Netherlands; Huddinge University Hospital Stockholm, Sweden; Institute for Transfusion Science, Bristol, United Kingdom; Établissement Français du Sang EFS-Alsace, Strasbourg, France; Institute of Transfusion Medicine and Immunology, University of Heidelberg, Faculty of Clinical Medicine, Mannheim, Germany; University of Washington, Seattle; Baxter Healthcare Corp, Deerfield, IL; and Cerus Corp, Concord, CA.

Submitted March 25, 2002; accepted October 26, 2002. Prepublished online as *Blood* First Edition Paper, November 27, 2002; DOI 10.1182/blood-2002-03-0932.

A complete list of the members of the euroSPRITE investigator group appears in the "Appendix."

Supported by Baxter Healthcare, Deerfield, IL, and Cerus, Concord, CA.

J.F., P.M., and D.B. are employees of Baxter Healthcare. M.C., L.L., and L.C. are employees of Cerus.

Reprints: Laurence Corash, Cerus Corp, 2411 Stanwell Dr, Concord, CA 94520; e-mail: larry_corash@ceruscorp.com.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

threshold of $< 20 \times 10^9$ platelets/L) gave informed consent and were randomized to receive all required platelet transfusion support of the assigned treatment type, either test or reference, for up to 56 days of support. The inclusion criteria specified enrollment of patients 12 years or older admitted for treatment of acute or chronic leukemia (acute nonlymphocytic leukemia [ANLL], acute lymphocytic leukemia [ALL], chronic myelogenous leukemia [CML], chronic lymphocytic leukemia [CLL], or chronic myelomonocytic leukemia [CMML]), lymphoma, multiple myeloma, myelodysplasia, solid tumors, and hematopoietic stem cell transplantation. Patients with the following conditions at enrollment were excluded: splenomegaly (> 18 cm), history of immune thrombocytopenia, disseminated intravascular coagulation, acute surgical condition, history of alloimmunization or refractoriness to platelet transfusion, pregnancy, or recent treatment with psoralen UVA (PUVA) therapy. Patients were enrolled at Bloodbank, Rotterdam, The Netherlands; Departments of Transfusion Medicine and Hematology, Huddinge University Hospital, Stockholm, Sweden; National Blood Service, Bristol, United Kingdom; and Établissement Français du Sang, Strasbourg, France. The study protocol was approved and informed patient consent was obtained in accordance with each study center's independent ethics committee. The study was conducted in accordance with European Council Directive 93/42/EEC, standard EN 540 1993, Good Clinical Practice Guidelines, International Committee on Harmonization Guidelines, and the Declaration of Helsinki.

The ITT population consisted of patients who were randomized and received at least one transfusion of test or reference platelet concentrate after randomization. All platelet transfusions were recorded. On-protocol transfusions consisted of platelet pools prepared according to treatment assignment. Off-protocol transfusions consisted of platelet products prepared by methods other than those specified in the study protocol. When platelet products of the assigned treatment type were unavailable, patients were transfused with conventional platelet products (off-protocol transfusions) according to the standard of care for each study center. Off-protocol platelet components were prepared by methods other than those specified in the protocol (single-donor apheresis and off-protocol random-donor methods) and samples were not taken to measure platelet dose or for culture because this was not standard practice at the study centers. Study centers were instructed to maintain an adequate inventory of study platelet products to minimize off-protocol platelet transfusion.

Primary care physicians ordered all platelet transfusions either for prophylaxis of bleeding or to treat active bleeding, per institutional standard of care, without knowledge of the patient's platelet treatment assignment. After completion of the 56-day transfusion period, patients were monitored for an additional 28 days to record adverse events. Following completion of this initial 84-day study period (cycle 1), patients were followed for need of additional platelet support while the study was open, or approximately one year, depending on study center. If additional platelet transfusion support was required, patients were asked to reregister for a second 56-day transfusion period and an additional 28-day adverse event surveillance period (cycle 2). Cycle 2 was conducted identically to cycle 1, with the same treatment assignment for each patient.

Platelet components

Test platelet concentrates were prepared at each center as pooled buffy coats from 5 or 6 whole blood collections, using the Optipress device with top and bottom separation (Baxter Healthcare Corporation, La Chatre, France) and a platelet additive solution (InterSol; Baxter Healthcare Corporation, Lessines, Belgium) resulting in a final plasma concentration of approximately 35%.[11] Pooled platelet concentrates were leukoreduced by filtration followed by photochemical treatment (PCT) with 150 μM S-59 and 3 J/cm^2 UVA treatment (Helinx Technology; Cerus Corporation, Concord, CA) for pathogen and leukocyte inactivation.[5,8,9] After PCT, pooled platelet concentrates were stored for up to 5 days before transfusion. PCT was used in place of γ irradiation for prevention of transfusion-associated graft-versus-host disease.[10] Samples were obtained from test platelet pools at time of issue for transfusion to measure platelet content, pH, and bacterial culture. The targeted platelet content of test pooled components was 3.0×10^{11} platelets.

Reference platelet concentrates were prepared in conformance with standard operating procedures at each study center as pooled buffy-coat products from 4, 5, or 6 whole blood collections using the Optipress device with top and bottom separation process, conventional platelet pooling methods, and filtration leukoreduction.[11] When feasible the number of buffy coats used to create a reference pool at each center was the same as that used for test pools. However, in one center (Bristol, United Kingdom), according to standard procedures, 4 buffy coats were used for preparation of conventional platelet pools, compared with 5 for test pools. In 2 study centers (Rotterdam and Stockholm) the reference platelet products were suspended in approximately 35% plasma and 65% platelet additive solution (T-Sol; Baxter Healthcare Corporation, Lessines, Belgium).[11] In the other 2 centers (Bristol and Strasbourg) reference platelet pools were suspended in 100% donor plasma. Reference platelet pools were stored for up to 5 days prior to transfusion and were γ-irradiated per standard of care at time of issue per specific patient requirements for prevention of transfusion-associated graft-versus-host disease. Samples were obtained from reference platelet pools at time of issue for transfusion to measure platelet content, pH, and bacterial culture. The targeted platelet content of reference pooled components was 3.0×10^{11} platelets.

Platelet transfusion

Generally, a single pooled platelet component was transfused. If a second pooled platelet product was transfused within 4 hours of completion of the preceding platelet transfusion, the 2 pools were considered to be part of a single platelet transfusion episode. Platelet counts were measured from 10 minutes to 4 hours after platelet transfusion and from 18 to 24 hours after transfusion to determine the nominal 1- and 24-hour platelet count increments, respectively.

Off-protocol transfusions were given for the following indications: acute surgical conditions during which platelet count increments could not be accurately measured; refractoriness to platelet transfusion (with evidence of alloimmunization) requiring the use of single-donor human leukocyte antigen (HLA) matched or unmatched platelets; and lack of availability of on-protocol platelet products.

If a patient developed a temperature elevation of more than 2°C or more than 1°C with rigors within 24 hours after platelet transfusion, a blood culture was obtained and a reserved aliquot from the platelet component was cultured. Bacterial isolates obtained from positive blood cultures and platelet components were analyzed for identity to confirm the diagnosis of transfusion-transmitted bacteremia.

Lymphocytotoxicity antibody assays and assay for potential S-59–related neoantigens

Sera were collected and analyzed for lymphocytotoxicity antibodies at the Laboratory of Histocompatibility and Immunogenetics, Bloodbank Rotterdam, using an HLA-typed 60-cell panel. Sera reactive with 20% or more of cells in the panel were defined as reactive sera.

Sera were collected at enrollment and every 2 weeks during both transfusion cycles for detection of immunoglobulin G (IgG) antibodies directed against potential S-59–associated neoantigens. Test and reference group samples were analyzed, without knowledge of treatment assignment, using a microtiter plate enzyme-linked immunosorbent assay (ELISA; Cerus). Platelets from a pool of 12 donors were prepared with PCT or untreated, and 5×10^6 platelets per well served as the capture antigen. After overnight incubation (2-8°C), plates were washed; blocked with IgG-depleted human serum; and test, negative control (pooled sera from 54 healthy donors), and positive control samples were added to the wells. Test samples and negative control samples were diluted 1:5. Positive controls of human anti-Pla1 serum (Immucor, Norcross, GA) were used at low (1:5) and high (1:50) dilutions. Plates containing samples were incubated for 1 hour at 37°C and washed. Fc-specific rabbit antihuman IgG was added and incubated, and plates were washed. Horseradish peroxidase conjugated to donkey antirabbit antibody was added and incubated; unbound conjugate was removed by washing. Human IgG bound to the target antigen was detected by measuring absorbance (450 nm) generated by tetramethylbenzidine substrate (Sigma-Aldrich, St Louis, MO). Samples were tested first against PCT-treated platelets. Samples that surpassed the cutoff value of the assay (2 SD beyond the reference range mean) were evaluated further by

absorbing with untreated platelets followed by repeat assay with PCT-treated platelets. Samples that remained positive after absorption with untreated platelets were quantified for titer or relative concentration of IgG. Positive results for antibody to S-59–associated neoantigen required an optical density of 2 SD beyond the reference mean value for normal sera with persistence following absorption with naive platelets. For positive samples, the relative concentration of the anti-PCT antibody was estimated by 2 methods. The assay was repeated with a trinitrophenol (TNP)–platelet/goat anti-TNP/rabbit antigoat standard curve (analogous to the PCT-platelet/human anti-PCT/rabbit antihuman complex in the assay). After the background subtraction from each format, the specific signal from the human sample was quantified in terms of the amount of goat antibody bound to TNP platelets yielding the same amount of signal. Also, positive antibody samples were diluted to estimate a relative measure of antibody titer. Two positive control samples containing antiplatelet antibodies (HPA-1) were used to characterize the sensitivity of the assay. These samples remained positive at a dilution of 1:4096. Quantitative IgG assay for these 2 samples using the TNP calibration standard indicated IgG concentrations of 9.8 ng/mL and 23.7 ng/mL for these 2 positive control sera.

Study endpoints

The 1-hour platelet count increment and the 1-hour corrected count increment (CCI) were selected as coprimary endpoints because they are used in clinical practice to evaluate the efficacy of platelet transfusion. CCI was calculated using the platelet dose at time of issue for transfusion as follows: CCI = [(Posttransfusion count − Pretransfusion count) × Body surface area (m^2)]/ Platelet dose ($\times 10^{-11}$).

Secondary study endpoints included the following parameters: the count increment and CCI 24 hours after platelet transfusion, the number of platelet transfusions during the period of platelet support, the interval between platelet transfusions, clinical hemostasis before and after platelet transfusion, the number of red cell units transfused during the period of platelet support, the proportion of patients with refractoriness to platelet transfusion (defined as 2 successive 1-hour CCIs < 5000), and the proportion of patients with alloimmunization, defined as serologic conversion of the lymphocytotoxicity assay. Specific transfusion-related adverse events, including acute transfusion reactions, platelet transfusion–associated bacteremia, and all other adverse events were recorded, coded, and summarized using the MedDRA (Medical Dictionary for Regulatory Activities) preferred term synonym and the MedDRA system organ class.[12]

To determine the hemostatic efficacy of platelet transfusion, patients were evaluated within 6 hours before and for 6 hours after each platelet transfusion to assess bleeding. Twelve potential bleeding sites were evaluated by a blinded observer and assigned a score of 0 (no bleeding), 1 (minor bleeding), or 2 (major bleeding).[13] The sites assessed were cutaneous, nasal, oropharyngeal, gastrointestinal, genital, urinary, pulmonary, retinal, and invasive sites (catheter, venipuncture, tracheostomy, surgical). For any bleeding site, if the severity grade was not recorded, the missing grade was estimated using the average score for other sites. The pre- and posttransfusion hemostatic scores were computed by summing the grades of the individual bleeding sites assessed before and after each transfusion. The mean score for all patients within each treatment group (test and reference) was computed. In addition to peritransfusion hemostatic scores, the protocol required reporting of all hemorrhagic adverse events during the active transfusion period and during the period of active surveillance. Hemorrhagic adverse events were graded for severity (nonserious and serious). All red cell transfusions during the active platelet transfusion period were recorded as a surrogate indicator of bleeding.

Statistical rationale for study design

The sample size and power calculations for this study were based on data for patients enrolled in the Trial to Reduce Alloimmunization to Platelets (TRAP) study.[14] For 150 TRAP patients randomized to receive filtered single-donor platelets, the standard deviation of the mean of the first 8 CCIs was 5532, and the standard deviation of the mean of the first 8 count increments was 14.6×10^9/L. For the present study, using a .05-level 2-sided test, a sample size of 100 provided a power of 80% to detect a difference in mean CCI of 2807, and a difference in mean count increment of 8.0×10^9/L between patients receiving test and reference platelet components.

Statistical methods

Based on the TRAP experience, a majority of patients in this study were expected to receive at least 8 platelet transfusions during the 56-day transfusion period. To minimize the effect of variable numbers of transfusions, the mean 1- and 24-hour posttransfusion count increment and CCI were computed for up to the first 8 platelet transfusions during the 56-day transfusion period and compared by t test. Comparisons were according to each patient's randomized treatment assignment.

To analyze platelet count increments for all transfusions, a stepwise linear regression model for longitudinal data using generalized estimating equations was used for all 1- and 24-hour posttransfusion platelet counts during the 56-day transfusion period of each cycle.[15] Platelet transfusion number served as the longitudinal variable to deal with the effect of variation in the number of platelet transfusions among patients. The 1- and 24-hour posttransfusion platelet counts for all on-protocol transfusions were compared for test and reference patients with transfusion number, pretransfusion platelet count, platelet dose, average age of platelet concentrate, patient weight and height, and clinical site as covariates. The analysis was done stepwise with backward elimination of variables for which P was more than .05, with retention of treatment (PCT or no PCT) at each step. Interactions with PCT for each significant covariate were added to the reduced model, and the process was repeated.

Analyses of the secondary endpoints were based on the ITT population with a significance level of .05 for the secondary analyses. No adjustment for multiplicity was made.

Results

Patient population

Between June 1998 and June 2000, 103 patients (52 test and 51 reference) received at least one study transfusion. More test patients (83%) than reference patients (67%) completed cycle 1 ($P = .06$), and more test patients (10 of 52) than reference patients (2 of 51) received transfusions during cycle 2. This difference resulted in a greater number of study observation days for test patients than for reference patients (4081 vs 3633 days).

The groups were balanced with respect to primary diagnoses, therapy, previous exposure to allogeneic blood products, and previous pregnancy (Table 1) and demographic characteristics (Table 2). Baseline hematology and coagulation profiles were

Table 1. Disease, therapy, and previous alloimmunization exposure of patients receiving platelet transfusions for thrombocytopenia

	Treatment group, no. (%)	
	Test (n = 52)	Reference (n = 51)
Primary diagnosis		
Acute leukemia	26 (50)	24 (47)
Hematopoietic tumor*/nonhematopoietic solid tumor	21 (40)	19 (37)
Other	5 (10)	8 (16)
Therapy		
Bone marrow transplantation	2 (4)	1 (2)
Peripheral blood stem cell transplantation	17 (33)	18 (35)
Chemotherapy	33 (63)	32 (63)
Alloimmunization exposure†	46 (88)	44 (86)

There were no statistical differences between groups ($P > .05$).
*Includes multiple myeloma, lymphoma, and Hodgkin lymphoma.
†Previous platelet or red blood cell transfusion or, for women, previous pregnancy.

Table 2. Demographic characteristics of patients receiving platelet transfusions for thrombocytopenia

	Treatment group, no. (%)	
	Test, n = 52	Reference, n = 51
Sex, no. (%)		
Male	30 (58)	28 (55)
Female	22 (42)	23 (45)
Age, mean ± SD, y	48.6 ± 14.1	51.1 ± 13.2
Weight, mean ± SD, kg	73.56 ± 15.30	74.25 ± 15.69
Height, mean ± SD, cm	173.09 ± 8.70	171.47 ± 8.19
BSA, mean ± SD, m^2	1.88 ± 0.23	1.89 ± 0.22

There were no statistical differences between groups ($P > .05$).

reflective of patients with neoplastic diseases requiring platelet transfusion (Table 3), and not different between groups. While on study, 102 patients (99%) were taking some form of anti-infective, 26 (25%) were exposed to amphotericin, 34 (33%) were treated with anticoagulants, 4 (4%) received fibrinolytics, 5 (5%) received antifibrinolytics, and 2 (2%) received nonsteroidal anti-inflammatory drugs (NSAIDs). There were no differences in concomitant medications between the treatment groups except for the use of antifibrinolytics; all 5 patients on this therapy were among patients assigned to reference transfusions ($P = .03$).

Platelet transfusions

During cycle 1, 311 (80%) of 390 test transfusions and 256 (90%) of 286 reference transfusions were prepared according to protocol. Patients assigned to test received a mean of 7.5 ± 5.8 platelet transfusions, inclusive of on- and off-protocol transfusions, compared with 5.6 ± 5.5 for reference patients ($P = .09$). The average number of on-protocol transfusions for test patients was 6.2 ± 4.2, compared with 5.0 ± 4.8 for reference patients ($P = .22$). The average platelet dose (10^{11}) per transfusion of on-protocol transfusions was lower for test transfusions than for reference transfusions (3.9 ± 1.0 vs 4.3 ± 1.2, $P < .001$). The mean total platelet dose of on-protocol transfusions for both treatment groups was similar (test mean total dose 22.3×10^{11} vs reference mean total dose 21.2×10^{11}; $P = .74$). Two factors contributed to the reduced platelet content of test platelet products: extra samples (10 mL) required to measure S-59 levels and loss due to platelet retention (20 mL) during transfers with use of a prototype treatment set. The average storage duration of test pools was comparable to that of reference pools (3.5 ± 1.1 vs 3.4 ± 1.2 days; $P = .28$), with median pretransfusion storage durations of 4 days for test and 3 days for reference platelet pools. Moreover, 22% of test pools and 20% of reference pools were transfused on the fifth day of storage. All test and reference platelet products were ABO compatible.

Platelet count increments 1 hour after transfusion

The mean 1-hour count increment for up to the first 8 transfusions was 27.5 ± 13.5 × 10^9/ L for test patients and 35.8 ± 23.3 for reference patients. The mean difference of 8.3 × 10^9/L (95% confidence interval [CI], 0.9-15.8) was statistically significantly different ($P = .03$). Both test and reference platelet count increments were within reported therapeutic ranges.[16,17] When the 1-hour count increment was adjusted for differences in platelet dose using the CCI, the mean 1-hour CCI was not statistically significantly different between treatment groups (13 100 ± 5400 vs 14 900 ± 6200, $P = .11$), with a mean difference of 1800 (95% CI, −400 to 4100). By longitudinal regression analysis for all transfusions, platelet concentrates prepared with and without PCT did not differ significantly with respect to the 1-hour posttransfusion platelet count in cycle 1 ($P = .53$). The estimated effect of PCT treatment was a decrease in the 1-hour posttransfusion platelet count of 1.5 × 10^9/L (95% CI, −3.1 to 6.1 × 10^9/L).

Covariates with a significant ($P < .05$) effect on the posttransfusion platelet count were, in descending order of magnitude, as follows: platelet dose ($P < .001$), storage duration of the platelet concentrate prior to transfusion ($P < .001$), pretransfusion platelet count ($P < .001$), and patient weight ($P < .001$). None of these covariates had significant interactions with PCT, including the interaction term for PCT treatment and dose ($P = .73$). By regression analysis for all transfusions, PCT-treated and non-PCT-treated platelets gave comparable 1-hour posttransfusion platelet counts for equal platelet doses in cycle 1 (Figure 1). There were no significant differences among study sites in the magnitude or the direction of the effect of PCT on the count increment ($P = .80$).

Platelet count increments 24 hours following transfusion and the interval to the next platelet transfusion

The mean 24-hour posttransfusion count increment (for up to the first 8 transfusions) for the test group (16.4 ± 9.5 × 10^9/L) was less ($P = .004$) than for the reference group (24.7 ± 17.6 × 10^9/L). The mean 24-hour posttransfusion CCI (for up to the first 8 transfusions) was less ($P = .02$) for the test group (7400 ± 5500) than for the reference group (10 600 ± 7100). By longitudinal regression analysis for all cycle 1 transfusions, PCT platelets and non-PCT platelets did not differ significantly with respect to the 24-hour platelet count in cycle 1 ($P = .19$). The estimated effect of PCT treatment was a decrease in the 24-hour platelet count of 2.6 × 10^9/L (95% CI, −1.3 to 6.5 × 10^9/L). The same covariates were significant as for the analysis of 1-hour platelet count, and none of these covariates had significant interactions with PCT. The interaction term for PCT treatment and dose was not significant

Table 3. Baseline hematology and coagulation profiles of patients receiving platelet transfusions for thrombocytopenia

	Treatment group			
	Test, n = 52		Reference, n = 51	
	No.*	Value	No.*	Value
Hematocrit, %, mean ± SD	47	0.27 ± 0.05	50	0.26 ± 0.05
Platelet count, mean ± SD, × 10^9/L	52	19.1 ± 13.3	51	16.7 ± 13.1
Hemoglobin level, mean ± SD, g/L	51	94.4 ± 15.8	48	89.0 ± 19.1
Fibrinogen level, mean ± SD, g/L	47	4.673 ± 1.647	44	4.724 ± 1.203
White blood cell count, median†, × 10^9/L	52	0.350	51	0.220
Prothrombin time, mean ± SD, sec	28	12.8 ± 4.1	26	12.1 ± 1.8
Partial thromboplastin time, mean ± SD, sec	50	35.0 ± 9.2	46	34.7 ± 9.2

There were no statistical differences between groups ($P > .05$).

*Number of patients with baseline values.

†The median is given, rather than the mean, owing to a nonnormal distribution of values.

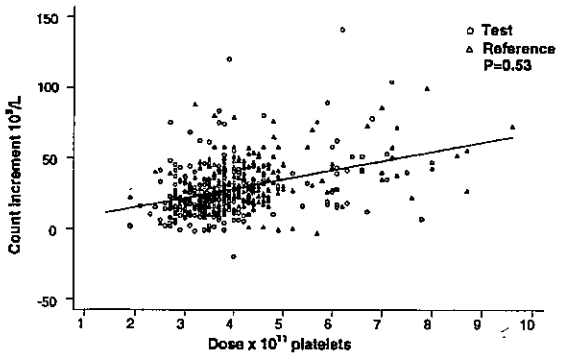

Figure 1. Effect of platelet dose on the 1-hour posttransfusion platelet count increment for test and reference platelet transfusions. The 1-hour posttransfusion count increment vs platelet transfusion dose is shown for all on-protocol (test and reference) transfusions in cycle 1. Equal doses of test and reference platelet products resulted in comparable 1-hour count increments ($P = .53$) over the range of platelet doses transfused. The regression lines for test and reference platelet transfusions appear superimposed.

($P = .31$). By regression analysis for all transfusions, PCT-treated and non-PCT-treated platelets gave comparable 24-hour posttransfusion platelet counts for equal doses in cycle 1 (Figure 2).

Because 2 study centers suspended reference components in 100% plasma and 2 used a mixture of plasma and T-Sol, we compared the 1-hour and 24-hour count increment and CCI responses for this variable within the reference patient group. No differences were observed, consistent with previously reported experience using T-Sol–plasma mixtures.[18]

There was no statistically significant difference between test and reference populations with respect to duration of the first period of platelet support or total duration of platelet support (Table 4). The average interval between platelet transfusions was computed for the first period of platelet transfusion, because all patients (n = 103) underwent the first period of platelet support, and it was not significantly different for test and reference patients (Table 4).

Posttransfusion platelet counts for patients enrolled in cycle 2

Because of the limited numbers of patients enrolled in cycle 2 (10 test and 2 reference patients), data for posttransfusion platelet counts were analyzed only by longitudinal regression. The treatment groups did not differ significantly with respect to the 1-hour platelet count ($P = .47$). The estimated effect of PCT was an increase in the 1-hour platelet count of 4×10^9/L (95% CI, −7 to 14×10^9/L). The interaction terms for PCT and dose were not significant ($P = .74$). Similarly, the treatment groups did not differ significantly with respect to the 24-hour platelet count in cycle 2 ($P = .90$). The estimated effect of PCT was an increase in the platelet count of 1×10^9/L (95% CI, −10 to 11×10^9/L). PCT-treated and non-PCT-treated platelets provided comparable 1- and 24-hour platelet counts for equal doses in cycle 2.

Bleeding, hemostatic scores, and red cell transfusions

Based on peritransfusion hemostatic assessments, 71% of test and 63% of reference patients had at least one episode of bleeding before transfusion ($P = .36$). The incidence of bleeding events was lower and similar in both groups after platelet transfusion (54% test and 49% reference; $P = .62$). There were no significant differences in hemostatic scores between test and reference patients either before or after transfusion, and average hemostatic scores were lower after transfusion in both groups (Table 5). When hemostasis during the entire transfusion period was assessed on the basis of reported hemorrhagic adverse events, a high and equal proportion of patients had at least one hemorrhagic adverse event (Table 6). The most frequent hemorrhagic adverse events were epistaxis (42% test vs 41% reference), gingival bleeding (17% test vs 12% reference), injection site hemorrhage (17% test vs 6% reference), purpura (15% test vs 14% reference), petechiae (13% test vs 16% reference), and hematoma (13% test vs 8% reference). Only 6% of patients in each group experienced severe hemorrhagic adverse events (Table 6). Bleeding also was assessed indirectly by comparing the number of red blood cell units transfused during the transfusion periods. The average number of red blood cell units transfused during the first period of platelet support in cycle 1 (test = 4.9 ± 4.2, reference = 4.5 ± 5.4) was not different between groups ($P = .68$). Additionally, the average number of red cell units transfused during the 56 days of cycle 1 (test = 9.3 ± 5.3, reference = 8.2 ± 6.3) did not differ significantly between treatment groups ($P = .34$). The average number of red blood cell units transfused per day of platelet support during the first period of platelet support in cycle 1 (test = 0.41 ± 0.35, reference = 0.56 ± 0.68) did not differ significantly between treatment groups ($P = .16$).

Refractoriness to platelet transfusion, lymphocytotoxic antibodies, and antibodies to potential S-59–associated neoantigens

Patients were classified as refractory to platelet transfusions if the 1-hour CCI was less than 5000 for 2 successive transfusions. All patients diagnosed as refractory were evaluated for lymphocytotoxic antibodies (LCAs). Seven patients—4 (8%) assigned to test and 3 (6%) assigned to reference ($P = .72$)—had at least one refractory episode to platelet transfusion during the study. Of these 7 patients, 2 from the test group and 1 from the reference group tested positive for LCAs at baseline. One patient, who received test platelet transfusions, had a positive LCA result at baseline and remained refractory throughout cycle 1. Two additional patients (one test and one reference) became LCA-positive during cycle 1. One refractory patient in each group remained LCA-negative. No patient had confirmed antibodies directed against potential S-59–related platelet neoantigens.

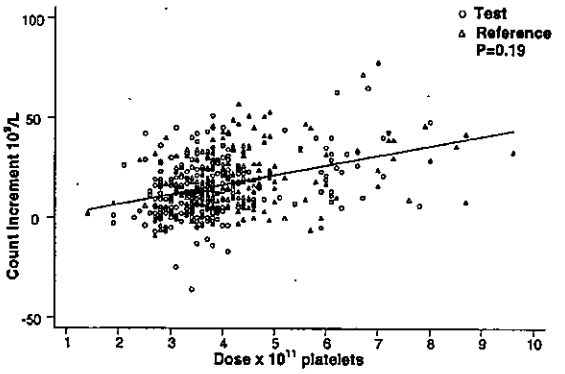

Figure 2. Effect of platelet dose on the 24-hour posttransfusion platelet count increment for test and reference platelet transfusions. The 24-hour posttransfusion count increment vs platelet transfusion dose is shown for all on-protocol (test and reference) transfusions in cycle 1. Equal doses of test and reference platelet products resulted in comparable 24-hour count increments ($P = .19$) over the range of platelet doses transfused. The regression lines for test and reference platelet transfusions appear superimposed.

Table 4. Total duration of platelet support, duration within each period of platelet support, and transfusion interval in cycle 1

	Treatment group		
	Test (n = 52)	Reference (n = 51)	*P*
Total duration of platelet support, mean ± SD, d	15.2 ± 11.2	12.7 ± 12.4	.29
Duration of platelet support by period within cycle 1, mean ± SD, d			
Period 1 (no. of patients)	11.7 ± 9.5 (52)	10.1 ± 11.7 (51)	≥.45
Period 2 (no. of patients)	7.5 ± 7.2 (22)	8.0 ± 7.9 (14)	
Period 3 (no. of patients)	15.0 (1)	5.0 ± 6.9 (3)	
Time between transfusions during first period of platelet support, mean ± SD, d	3.0 ± 1.23	3.4 ± 1.21	.13

All transfusions for the ITT population were recorded during the 56-day transfusion period. The period of platelet support was defined as the number of days from the first study transfusion to the last platelet transfusion. If an interval of 7 or more days without platelet transfusions occurred, the last platelet transfusion before the interval with no platelet transfusion was treated as the last platelet transfusion for that period of support. If platelet transfusions were resumed, the length of subsequent periods of platelet support was added to the first period of platelet support to determine the total period of platelet support during the 56-day transfusion cycle. Thus, patients could have multiple periods of pletetet transfusion during a 56-day cycle.

Acute transfusion reactions and other adverse events

All patients were evaluated following platelet transfusion for transfusion-related symptoms and signs. Posttransfusion events classified as acute transfusion reactions included fever, chills, nausea, skin rash, urticaria, bronchospasm, tachycardia, hypotension, hypertension, hemoglobinuria, hemolysis, and change in vital signs within 6 hours following platelet transfusion. In cycle 1, 6% of test transfusions were associated with acute reactions, compared with 5% of reference platelet transfusions ($P \geq .61$).

A total of 147 types of adverse events were reported in 5% or more of the patient population evaluated during cycle 1 and cycle 2. Overall, there were no differences in the incidence of adverse events by system organ class between the 2 treatment groups. Serious adverse events were reported in 14 (27%) patients randomized to the test platelet treatment group and in 13 (25%) patients randomized to the reference platelet group. Adverse events reported as related to platelet transfusion were not statistically significantly different between the groups ($P = .20$). No study transfusions demonstrated transfusion-associated bacteremia. Four test patients and 5 reference patients died while on study. No deaths were related to study transfusions.

Discussion

The duration of platelet support and the demographic characteristics of the patient population in this study provided a meaningful transfusion experience to evaluate the efficacy of platelet components prepared with a pathogen inactivation process. This study used pooled random-donor platelets prepared with the buffy coat process, a methodology commonly used in Europe.[19] Therapeutic platelet doses of test and reference products were pooled, filtered to reduce leukocyte content, and stored for up to 5 days prior to transfusion. Previous studies have shown that buffy coat platelets and platelets prepared by the platelet-rich-plasma (PRP) process provided similar 1- and 24-hour count increments.[20] Similar observations have been reported for platelets collected by apheresis and suspended in 100% plasma.[21] In addition, we, and others have observed that reference platelets suspended in 100% plasma and in mixtures of 35% plasma and 65% platelet additive solution (T-Sol)

Table 5. Pre- and posttransfusion hemostatic scores* in cycle 1

	Treatment group		
	Test (n = 52)	Reference (n = 51)	*P*
Pretransfusion score	0.43 ± 0.46	0.45 ± 0.57	.84
Posttransfusion score	0.28 ± 0.39	0.30 ± 0.43	.81

Twelve potential bleeding sites were evaluated by a blinded observer and assigned a score of 0 (no bleeding), 1 (minor bleeding), or 2 (major bleeding). The pre- and posttransfusion hemostetic scores for each transfusion were computed by summing the grades of the individual bleeding sites before and after each transfusion.

*Mean ± SD.

Table 6. Hemorrhagic adverse events by system organ class in cycle 1

	Test (n = 52), no. (%)		Reference (n = 51), no. (%)	
System organ class	Total	Severe	Total	Severe
Any hemorrhagic event	41 (79)	3 (6)	38 (79)	3 (6)
Eye disorders	7 (13)	1 (2)	0 (0)	0 (0)
Eye hemorrhage	3 (6)	0 (0)	0 (0)	0 (0)
Retinal hemorrhage	3 (6)	1 (2)	0 (0)	0 (0)
Gastrointestinal disorders	18 (35)	0 (0)	14 (27)	1 (2)
Gingival bleeding	9 (17)	0 (0)	6 (12)	0 (0)
Rectal bleeding	4 (8)	0 (0)	1 (2)	0 (0)
Gastrointestinal hemorrhage	3 (6)	0 (0)	5 (10)	1 (2)
General disorders and administration site conditions	11 (21)	0 (0)	3 (6)	0 (0)
Injection site hemorrhage	9 (17)	0 (0)	3 (6)	0 (0)
Investigations	9 (17)	0 (0)	7 (14)	0 (0)
Hematuria	7 (13)	0 (0)	6 (12)	0 (0)
Respiratory, thoracic, and mediastinal disorders	24 (46)	1 (2)	21 (41)	0 (0)
Epistaxis	22 (42)	1 (2)	21 (41)	0 (0)
Hemoptysis	6 (12)	0 (0)	2 (4)	0 (0)
Skin and subcutaneous tissue disorders	19 (37)	0 (0)	15 (29)	0 (0)
Purpura	8 (15)	0 (0)	7 (14)	0 (0)
Petechiae	7 (13)	0 (0)	8 (16)	0 (0)
Ecchymosis	3 (6)	0 (0)	0 (0)	0 (0)
Surgical and medical procedure	3 (6)	0 (0)	1 (2)	0 (0)
Postoperative hemorrhage	3 (6)	0 (0)	0 (0)	0 (0)
Vascular disorders	7 (13)	0 (0)	4 (8)	0 (0)
Hematoma	7 (13)	0 (0)	4 (8)	0 (0)

The numbers of patients with hemorrhagic adverse events with a frequency of 5% or higher are listed for system organ class and for preferred terms within each system organ class. Within some system organ classes individual events (terms) occurred with a frequency of less than 5%; these were not listed but were captured within the system organ class. Some patients had more than one type of hemorrhagic event within a system organ class. The proportions (%) were compared by χ^2 tests to determine whether statistical differences were present between the treatment groups. The proportion of severe hemorrhagic adverse events is indicated separately. Mild and moderate adverse events did not require specific treatment interventions. Severe adverse events required treatment intervention and change in patient activity status. There were no statistical differences ($P > .05$) between the treatment groups with respect to the incidence of any hemorrhagic adverse events.

provided similar count increments and CCIs.[18] PRP platelets are stored in 100% donor plasma as individual concentrates rather than as pools in a mixture of plasma with platelet additive solution. Therefore, PRP platelets prepared with photochemical treatment for pathogen inactivation may potentially demonstrate differences in clinical efficacy. Recently we reported preliminary data from a study of single-donor platelets collected by apheresis, suspended in platelet additive solution, and prepared with photochemical pathogen inactivation.[22] This study demonstrated that single-donor PCT platelets were equivalent to conventional platelets for prevention and treatment of bleeding in severely thrombocytopenic patients receiving multiple platelet transfusions. However, average 1- and 24-hour platelet count increments were significantly lower in patients supported with single-donor PCT platelets. Thus, different processing methods may result in differences in posttransfusion count increments.

The present study was designed with sufficient power to detect potentially relevant clinical differences in count increments between PCT and conventional platelets. Posttransfusion count increments are surrogate measures of platelet transfusion efficacy used in clinical practice. There are no established standards defining an adequate response to platelet transfusion; however, there is general recognition that a 1-hour CCI of 7500 or more constitutes an adequate response.[23] Earlier studies of the response to platelet transfusion demonstrated that multiple covariate factors affect the response to platelet transfusion.[24-26] In addition, the count increment response decreases with multiple transfusions, even in nonalloimmunized patients[27]; thus comparison of average count increment values for patients receiving varying numbers of transfusions may not be sufficiently robust. For this reason, we limited the analysis of average count increment and CCI values to the first 8 transfusions, since most patients were expected to receive at least 8 transfusions. Furthermore, since the CCI is a ratio measure that incorporates platelet dose and patient size, both recognized to affect count increment responses, we used linear regression analysis with count increment as the dependent variable to assess, independently, the effect of platelet dose and patient size among other potential factors, including the number of platelet transfusions used as the longitudinal variable.[28]

Analysis of the 1- and 24-hour posttransfusion platelet counts for all on-protocol study transfusions by longitudinal regression analysis demonstrated no statistically significant effects of PCT on posttransfusion platelet counts. As expected, the platelet dose, age of the platelet component, pretransfusion platelet count, and patient size were highly significant covariate factors for the posttransfusion platelet count and thus the platelet count increment. Although the doses of test and reference platelets were statistically significantly different, both preparations provided doses sufficient for support of thrombocytopenic patients. In 3 of 4 study centers the same numbers of buffy coats were used to produce pooled platelet doses of test and reference products. In one study center, to comply with standard operating procedures, reference pools could be produced from only 4 buffy coats, while test pools were composed of 5 buffy coats. Using linear regression analysis, we examined the effect of dose on count increment response, since platelet doses were determined at time of transfusion for both types of platelets. This analysis confirmed that equal doses of test and reference platelets provided comparable 1- and 24-hour count increments.

Despite platelet losses of approximately 10% due to the PCT process, the average platelet dose of test product pools was well above 3.0×10^{11}, a dose commonly recognized as sufficient for transfusion support.[29] With an integrated processing set, currently observed losses of approximately 8% are not expected to require use of additional buffy coats to prepare therapeutic doses of PCT platelets.[30] Importantly, the responses to test and reference platelet concentrates were comparable over the range of platelet doses used in clinical practice, and average test and reference 1- and 24-hour count increment and CCI were within previously reported ranges for similar patient populations.[16,17,26] In comparison with other recent studies examining the effect of platelet dose on the interval to the next transfusion,[17,29] test platelets in the present study demonstrated transfusion intervals within reported ranges.

The mean number of on-protocol transfusions and the total dose of platelets transfused during the 56-day transfusion period were higher in the test group; however, this difference was partly accounted for by the higher rate of patient withdrawal from the reference group (10 patients vs 5 patients), resulting in more days of thrombocytopenia in the test group. Withdrawal most frequently was due to enrollment in a stem cell transplantation protocol for additional therapy that did not allow for use of experimental platelet components. These patients were withdrawn from this study by their primary care physicians who remained blinded to platelet transfusion group assignment. The nonrandom distribution between groups appeared to be a chance event. When the number of transfusions during the first period of platelet support was analyzed for patients who were not withdrawn from the study (43 test and 34 reference patients), the average number of platelet transfusions was 5.5 and 4.1 ($P = .25$), respectively. For patients who required a second period of platelet support and completed cycle 1, the average number of platelet transfusions for that population (19 test and 12 reference patients) was 3.5 and 4.4, respectively.

Platelet transfusions are given to prevent and to treat bleeding associated with thrombocytopenia. In this study, assessment of hemostasis utilized integration of the pre- and posttransfusion hemostatic evaluations, all reported hemorrhagic adverse events, and red cell use during the entire period of platelet support. Although at least one hemorrhagic adverse event was observed in the majority of patients during the observation period, only 3 patients in the test group and 3 patients in the reference group experienced severe hemorrhagic adverse events. One test patient and 2 reference patients had cerebral hemorrhage resulting in death during the posttransfusion period. Five patients in the reference group and no patients in the test group received antifibrinolytics during the study. This class of medications could have resulted in reduced bleeding in these 5 patients; however, no clear trend for reduced bleeding was detected in this subset of patients. These patients were included in the analysis for all reference group patients. A recent study of single-donor platelets prepared with PCT and conventional single-donor platelets demonstrated equivalence for prevention and treatment of bleeding during profound thrombocytopenia requiring repeated platelet transfusion.[22]

The present study represents an evaluation of platelet transfusion support in a patient population at high risk for bleeding. The data support the conclusion that pooled buffy-coat platelet components treated with the S-59 pathogen inactivation device and stored for up to 5 days were comparable to conventional platelet components for transfusion support of thrombocytopenic patients. Of note, a substantial proportion of both types of platelet products were stored for 4 or more days before transfusion. In this study, the safety profile of PCT platelets was not different from that of conventional platelet components; however, the scope of our study was small. Since it is not feasible to definitively evaluate the safety of PCT platelets in pediatric or pregnant patients during a clinical trial, we conducted preclinical safety studies with pregnant and neonatal animals to address these issues. We detected no toxicity in response to transfusion of S-59 or PCT plasma in these animal

populations.[31] Furthermore, in a carcinogenicity study using mice heterozygous for the p53 mutation, S-59 was not carcinogenic at 1000 times the clinical exposure.[31]

Previously reported studies have demonstrated that the PCT technology provides robust inactivation of viruses, bacteria, and leukocytes in platelet components.[5,8,9] Based on this clinical trial, pooled platelet components prepared with PCT and stored for up to 5 days offer the potential to reduce transfusion-associated infections and inactivate residual contaminating leukocytes using a processing system that is compatible with the current method of preparing therapeutic doses of buffy coat platelets.

Acknowledgments

The authors wish to acknowledge the substantial contribution of the following collaborating investigators and study personnel, without whose efforts this study could not have been completed: S. Larsson, M. Vesterinen, M. Christenson (Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden); R. Evely, D. Marks (Avon Haematology Unit, Bristol, United Kingdom); C. Waller, P. Dufour (EFS-Alsace, Strasbourg, France); C. Oprea, A. Faradji (Department of Oncohematology, Hopital de Hautepierre, Strasbourg, France); and N. Goulden (Department of Haematology and BMT, Bristol Royal Hospital for Sick Children, Bristol, United Kingdom). Lindy Kauffman (Cerus Corporation, Concord, CA) developed the assay for detection of antibodies against potential S-59–associated neoantigens.

Appendix

The S-59 Platelet Recovery in Thrombocytopenia—Europe (euroSPRITE) investigator group consisted of: Dick van Rhenen, Hans Gulliksson, Jean-Pierre Cazenave, Derwood Pamphilon, Per Ljungman, and Harald Klüter.

References

1. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion- transmitted viral infections. N Engl J Med. 1996;334:1685-1690.
2. Ling AE, Robbins KE, Brown TM, et al. Failure of routine HIV-1 tests in a case involving transmission with preseroconversion blood components during the infectious window period. JAMA. 2000; 284:238-240.
3. Schuttler GC, Caspari G, Jursch CA, Willems WR, Gerlich WH, Schaefer S. Hepatitis C virus transmission by a blood donation negative in nucleic acid amplification tests for viral RNA. Lancet. 2000;355:41-42.
4. McDonald CP, Hartley S, Orchard K, et al. Fatal Clostridium perfringens sepsis from a pooled platelet transfusion. Transfusion Med. 1998;8:19-22.
5. Lin L, Cook DN, Wiesehahn GP, et al. Photochemical inactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. Transfusion. 1997;37:423-435.
6. Alfonso R, Lin C, Dupuis K, et al. Inactivation of viruses with preservation of coagulation function in fresh frozen plasma. Blood. 1996;88(suppl 1):526a.
7. Lin L, Corten L, Murthy KK, Corash L, Alter HJ. Photochemical inactivation of hepatitis B (HBV) and hepatitis C (HCV) virus in human platelet concentrates as assessed by a chimpanzee infectivity model. Blood. 1998;92(suppl 1):502a.
8. Knutson F, Alfonso R, Dupuis K, et al. Photochemical inactivation of bacteria and HIV in buffy-coat-derived platelet concentrates under conditions that preserve in vitro platelet function. Vox Sanguinis. 2000;78:209-216.
9. Grass JA, Hei DJ, Metchette K, et al. Inactivation of leukocytes in platelet concentrates by psoralen plus UVA. Blood. 1998;91:2180-2188.
10. Grass JA, Wafa T, Reames A, et al. Prevention of transfusion-associated graft-versus-host disease by photochemical treatment. Blood. 1999;93: 3140-3147.
11. van Rhenen DJ, Vermeij J, Mayaudon V, Hind C, Lin L, Corash L. Functional characteristics of S-59 photochemically treated platelet concentrates derived from buffy coats. Vox Sanguinis. 2000;79:206-214.
12. Brown EG, Wood L, Wood S. The medical dictionary for regulatory activities (MedDRA). Drug Saf. 1999;2:109-117.
13. Gmur J, Burger J, Schanz U, Fehr J, Schaffner A. Safety of a stringent prophylactic platelet transfusion policy for patients with acute leukemia. Lancet. 1991;338:1223.
14. TRAP Study Group. Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. N Engl J Med. 1997;337:1861-1869.
15. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika. 1986;73:13-22.
16. Sweeney JD, Kouttab NM, Penn CL, McHugh KE, Nelson EJ, Oblon DJ. A comparison of prestorage WBC-reduced whole-blood-derived platelets and bedside-filtered whole-blood-derived platelets in autologous progenitor cell transplant. Transfusion. 2000;40:794-800.
17. Klumpp TR, Herman JH, Gaughan JP, et al. Clinical consequences of alterations in platelet transfusion dose: a prospective randomized, double-blind trial. Transfusion. 1999;39:674-681.
18. van Rhenen DJ, Vermeij J, Kappers-Klunne MC, Payrat JM. Evaluation of a new citrate-acetate-NaCl platelet additive solution for the storage of white cell reduced platelet concentrates obtained from half strength CPD pooled buffy coats. Transfusion. 1995;35:50-53.
19. Murphy S, Heaton WA, Rebulla P. Platelet production in the Old World—and the New. Transfusion. 1996;36:751-754.
20. Bertolini F, Rebulla P, Riccardi D, Cortellaro M, Ranzi ML, Sirchia G. Evaluation of platelet concentrates prepared from buffy coats and stored in glucose-free crystalloid medium. Transfusion. 1989;29:605-609.
21. Eriksson L, Kristensen J, Olsson J. Evaluation of platelet function using the in vitro bleeding time and corrected count increment of transfused platelets. Comparison between platelet concentrates derived from pooled buffy coats and apheresis. Vox Sanguinis. 1996;70:69-75.
22. McCullough J, Vesole D, Benjamin RJ, et al. Pathogen inactivated platelets (plt) using Helinx technology (INTERCEPT plt) are hemostatically effective in thrombocytopenic patients (tcp pts): the SPRINT trial. Blood. 2001;98(suppl 1):450a.
23. Leach MF, AuBuchon JP. Effect of storage time on clinical efficacy of single-donor platelet units. Transfusion. 1993;33:661-664.
24. Bishop JF, Matthews JP, McGrath K, Yuen K, Wolf MM, Szer J. Factors influencing 20-hour increments after platelet transfusion. Transfusion. 1991;31:392-396.
25. Bishop JF, McGrath K, Wolf MM, et al. Clinical factors influencing efficacy of pooled platelet transfusions. Blood. 1988;71:383-387.
26. Ishida A, Handa M, Wakui M, Okamoto S, Kamakura M, Ikeda Y. Clinical factors influencing posttransfusion platelet increment in patients undergoing hematopoietic progenitor cell transplantation-a prospective analysis. Transfusion. 1998; 38:839-847.
27. Enright H, Gernsheimer T, Woodson R, et al. Factors influencing the response to platelet transfusion. Blood. 1997;90(suppl 1):268a.
28. Davis KB, Slichter SJ, Corash L. Corrected count increments and platelet recoveries as measures of post-transfusion platelet response: problems and e solution. Transfusion. 1999;39:586-592.
29. Norol F, Bierling P, Roudot-Thoraval F, et al. Platelet transfusion: a dose-response study. Blood. 1998;92:1448-1453.
30. Vermeij J, Mayaudon V, Flament J, et al. The INTERCEPT platelet system for pathogen inactivation with an integral processing set demonstrated improved process control for production of platelet concentrates. Vox Sanguinis. 2002;83(suppl 2):110.
31. Ciarevino V. Preclinical safety of a nucleic acid-targeted helinx compound: a clinical perspective. Semin Hematol. 2001;38(suppl 11):12-19.

光増感剤を用いる感染性因子不活化技術により処理されたプールバフィーコート由来血小板成分の輸血効果：euroSPRITE 試験

Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial（仮訳）

アモトサレン塩酸塩（S-59）と紫外線 A（UVA）を用いた核酸をターゲットとする光増感剤処理（PCT）は、血小板製剤に混入するウイルス、細菌、原虫および白血球の不活化を目的として開発された。我々は、バフィーコート法で調製した血小板製剤にこの感染性因子不活化処理を用いた場合の治療効果および安全性を評価するため、血小板輸血を繰り返す支持療法を、最長 56 日間必要とする血小板減少症患者を対象として、ランダム化二重盲検比較試験を実施した。患者計 103 例に対し、PCT 処理した白血球除去プール血小板製剤（被験）または従来の白血球除去プール血小板製剤（対照）を 1 回以上輸血した（それぞれ 311 回および 256 回）。輸血前保存期間は最長 5 日とした。PCT 血小板成分の 50%以上は、輸血前保存期間が 4～5 日であった。1～8 回目の輸血を対象とした比較では、輸血 1 時間後の平均補正血小板増加数に関して、被験群と対照群の間に統計学的な有意差は認められなかった（13,100 ± 5,400 vs 14,900 ± 6,200、$p = 0.11$）。全輸血を対象とした時系列回帰分析により、被験群と対照群の血小板輸血投与量を等しい条件に補正したところ、輸血 1 時間後および 24 時間後の血小板数に関して有意差は認められなかった（群間差の 95%信頼区間は、それぞれ-3.1～6.1 × 10^9/L、$p = 0.53$ および-1.3～6.5 × 10^9/L、$p = 0.19$）。血小板輸血投与量、輸血前保存期間および患者の体格は、輸血後血小板数の有意な共変量であった（$p < 0.001$）。臨床的な止血状態、出血性有害事象および全有害事象に関しては、群間差は認められなかった。血小板成分の PCT 処理は、バフィーコート由来血小板成分の現行の調製方法に追加可能な手法で行うことができ、血小板輸血の安全性をさらに向上させる可能性がある。（Blood. 2003;101: 2426-2433）

はじめに

輸血前のドナースクリーニングおよび輸血感染症に関連するウイルスの検出検査法は絶えず改善されてきたにもかかわらず、血液製剤では細菌などによる感染症のリスクが依然として存在する[1]。HIV および C 型肝炎（HCV）の核酸検査法導入により、感染リスクの恐れがあるウインドウピリオドは短縮しているものの、輸血により HIV[2] および HCV[3] 感

染が生じる可能性は残り、血小板濃厚液の輸血を原因とする致死性の細菌感染症も依然として報告されている [4]。血小板および血漿輸血の安全性を高めるため、血小板および血漿成分に混在しているるおそれのあるウイルス、細菌および原虫の不活化を目的として、合成ソラレン化合物であるアモトサレン塩酸塩（S-59）と長波長紫外線 A（UVA）を用いた核酸をターゲットとする光増感剤処理法（PCT）が開発された [5,6]。前臨床試験では、10^6 以上の HIV ウイルス、10^5 以上の感染性 B 型肝炎（HBV）および HCV ウイルスや、広範囲のグラム陽性菌およびグラム陰性菌を不活化することが明らかにされている [5,7,8]。さらに、PCT は、10^5 個以上の残存 T 細胞を不活化し、増殖を効果的に阻害するとともに、白血球によるサイトカイン生成を完全に抑制する [9,10]。

この技術を血液製剤の処理に利用するため、一連のプラスチック製バッグを接続した閉鎖系回路と、マイクロプロセッサ制御の UVA 光源からなる装置を、輸血用血小板の処理システムとして導入した。この装置を用いて調製した血小板製剤の治療効果および安全性を評価するため、血小板減少症の複数の期間で血小板輸血を繰り返す必要がある患者を対象として、intent-to-treat（ITT）解析によるランダム化二重盲検比較試験を実施した。

患者および方法

患者および試験デザイン

血小板減少症を発症しているか血小板減少症の原因になりうる治療を受けており、血小板輸血を必要とする患者（輸血の推奨閾値は 20×10^9/L 未満）からインフォームド･コンセントを取得した後､支持療法として必要な血小板輸血すべてに被験血小板を用いる群と、対照血小板を用いる群にランダムに割り付け、最長 56 日間の支持療法を行った。選択基準には、急性または慢性白血病［急性非リンパ性白血病（ANLL)、急性リンパ性白血病（ALL)、慢性骨髄性白血病（CML)、慢性リンパ性白血病（CLL)、慢性骨髄単球性白血病（CMML)］、リンパ腫、多発性骨髄腫、骨髄異形成および固形腫瘍の治療、ならびに造血幹細胞移植のため入院していた 12 歳以上の患者を組み入れるよう規定した。登録時に次の条件に該当した患者は、本試験対象から除外した：1）脾腫（> 18 cm)、2）免疫性血小板減少症の既往、3）播種性血管内凝固、4）急性の外科的疾患、5）血小板輸血に対する同種免疫または不応状態の既往、6）妊娠中、7）近々のソラレン UVA（PUVA）療法歴。患者登録施設は、血液銀行（ロッテルダム、オランダ)、Huddinge University Hospital

の Departments of Transfusion Medicine and Hematology（ストックホルム、スウェーデン）、National Blood Service（ブリストル、英国）、Établissement Français du Sang（ストラスブール、フランス）であった。治験実施計画書の承認および同意書の取得は、各試験施設の治験審査委員会の規則に従って行った。本試験は、欧州指令 93/42/EEC、欧州規格 EN 540 1993、医薬品の臨床試験の実施の基準（GCP）、日米 EU 医薬品規制調和国際会議（ICH）ガイドラインおよびヘルシンキ宣言に従って行われた。

ITT 集団は、ランダム化され、なおかつランダム化後に被験血小板濃厚液または対照血小板濃厚液の輸血を 1 回以上受けた患者で構成した。すべての血小板輸血を記録した。治験実施計画書に適合する（on-protocol）輸血とは、投与割付に規定する方法で調製されたプール血小板製剤の輸血とした。治験実施計画書に適合しない（off-protocol）輸血とは、治験実施計画書に規定されている以外の方法で調製された血小板製剤の輸血とした。割り付けられた種類の血小板製剤が利用できなかった場合には、各試験施設の治療基準に従って、従来の血小板製剤を輸血した（治験実施計画書に適合しない輸血）。治験実施計画書に適合しない血小板製剤とは、治験実施計画書に規定されている以外の方法（単一ドナーのアフェレーシスおよび治験実施計画書に適合しないランダムドナーを用いた方法）により調製されたもので、血小板輸血投与量の測定用または培養検査用のサンプル採取は、試験施設の標準的業務として行われていなかったため、実施しなかった。試験施設には、治験実施計画書に適合しない血小板輸血回数を最小限に抑えるため、被験血小板製剤の在庫を十分確保するよう指示した。

出血予防または出血中の治療用の血小板輸血はすべて、主治医が、患者の血小板輸血の割付を知らない状態で、施設の治療基準に従って指示した。56 日間の輸血期間終了後、さらに 28 日間モニターし有害事象を記録した。この最初の 84 日間の試験期間（第 1 サイクル）終了後、試験継続中または約 1 年間（試験施設毎に設定）は、追加の血小板輸血による支持療法の必要性について患者を追跡調査した。血小板輸血支持療法をさらに必要とした患者には、2 回目の輸血期間（56 日間）とその後の有害事象調査期間（28 日間）（第 2 サイクル）に再登録するよう要請した。第 2 サイクルも第 1 サイクルと同様に実施し、各患者は第 1 サイクルと同じ投与群に割り付けた。

血小板製剤

被験血小板濃厚液は、各施設にて、5 または 6 人分の全血から top and bottom 法による血液分離装置 OptiPress（Baxter Healthcare Corporation、ラ・シャトル、フランス）を用いてプールバフィーコートを調製後、血小板添加液（InterSol ; Baxter Healthcare Corporation、レシーヌ、ベルギー）を添加・処理し、血漿の最終濃度を約 35%のプール血小板濃厚液とした[11]。プール血小板濃厚液は、ろ過による白血球除去後、光増感剤 S-59（150 μM）による処理と UVA（3 J/cm^2）照射（Helinx ; Cerus Corporation、カリフォルニア州コンコード）による光化学処理、感染性因子および白血球を不活化した[5,8,9]。PCT 後のプール血小板濃厚液は、輸血まで最長 5 日間保存した。PCT は、輸血後移植片対宿主病の予防を目的としてγ線照射の代わりに使用した[10]。被験血小板プールを輸血用に供給する際、血小板数および pH の測定用および細菌培養検査用のサンプルを採取した。被験血小板プールの目標血小板数は 3.0×10^{11} 個であった。

対照血小板濃厚液は、各試験施設の標準操作手順書に従って、4、5 または 6 人分の全血から top and bottom 法による血液分離装置 OptiPress を用いてプールバフィーコートを調製後、従来の方法により血小板をプールし、白血球をろ過により除去した[11]。可能であれば、対照血小板プールと被験血小板プールの調製には、施設毎に同人数分のバフィーコートを用いた。しかし、1 施設（ブリストル、英国）では、従来血小板プールの調製には、標準手順書に従って 4 人分のバフィーコートを使用した一方、被験血小板プールの調製には、5 人分のバフィーコートを使用した。また、2 施設（ロッテルダムおよびストックホルム）では、対照血小板製剤の再浮遊に、血漿と血小板添加液（T-Sol ; Baxter Healthcare Corporation、レシーヌ、ベルギー）の 35% : 65%混合液を使用したが[11]、他の 2 施設（ブリストルおよびストラスブール）では、対照血小板製剤には 100%ドナー血漿を使用した。対照血小板プールの輸血前保存期間は最長 5 日間とし、治療基準に従って、輸血後移植片対宿主病の予防のため必要と判断された場合には、供給時にγ線を照射した。対照血小板プールを輸血用に供給する際、血小板数、pH の測定用および細菌培養検査用のサンプルを採取した。対照血小板プールの目標血小板数は 3.0×10^{11} 個であった。

血小板輸血

プール血小板成分は通常、1 バッグを輸血した。血小板輸血の完了後 4 時間以内に次の

プール血小板製剤を輸血した場合、この 2 回の輸血は 1 回の血小板輸血とみなした。血小板数は血小板輸血 10 分後～4 時間後と、18～24 時間後に測定し、それぞれ輸血 1 時間後と 24 時間後（名目値）の血小板増加数の算出に用いた。

治験実施計画書に適合しない輸血は、次の場合に行われた：急性の外科的疾患で血小板増加数が正確に測定できない場合、血小板輸血不応状態（同種抗体あり）で単一ドナー［ヒト白血球抗原（HLA）適合の有無は問わず］由来の血小板を使用する必要がある場合、治験実施計画書に適合する血小板製剤の供給量が不十分な場合。

血小板輸血後 24 時間以内に 2ºC を超える体温上昇、あるいは悪寒を伴い 1ºC を超える体温上昇を示した場合、血液培養を実施するとともに、輸血に用いた血小板製剤から分取したサンプルの培養検査を行った。血液培養が陽性であった場合には、血液培養と血小板成分から分離された菌株の同一性を分析することにより、輸血による菌血症と診断を確定した。

リンパ球傷害性抗体アッセイおよび S-59 関連新抗原形成能のアッセイ

リンパ球傷害性抗体分析用の血清を採取し、Rotterdam 血液銀行の Laboratory of Histocompatibility and Immunogenetics にて、HLA 型が明らかなパネル細胞 60 種類を用いて分析した。20%以上のパネル細胞と反応した血清を、陽性血清と定義した。

S-59 関連新抗原に対する免疫グロブリン G（IgG）を検出するため、登録時および両輸血サイクル中の 2 週間毎に血清を採取した。被験群および対照群の検体は、投与割付が判らない条件下で、マイクロタイタープレートを用いた酵素結合免疫測定法（ELISA：Cerus）により分析した。捕捉抗原として、ドナープール（12 人分）由来の血小板を PCT 処理後または未処理の状態で、1 ウェルにつき 5×10^6 個ずつ分注した。プレートは一晩インキュベーション（2～8ºC）後、洗浄し、ヒト IgG 除去血清でブロッキングした後、被験検体、陰性対照検体（健康ドナー54 人から採取したプール血清）および陽性対照検体をウェルに添加した。被験検体および陰性対照検体は 1：5 に希釈して用いた。陽性対照としたヒト抗 Pla1 血清（Immucor、ジョージア州ノークロス）は、低希釈倍率（1：5）および高希釈倍率（1：50）で使用した。検体を添加したプレートは 37ºC で 1 時間インキュベーショ

ン後、洗浄した。Fc 特異的ウサギ抗ヒト IgG を添加後、インキュベーションし洗浄した。ホースラディッシュペルオキシダーゼ（HRP）結合ロバ抗ウサギ抗体を添加し、インキュベーションした後、結合しなかった HRP 結合抗体を洗浄除去した。標的抗原に結合したヒト IgG は、HRP 基質であるテトラメチルベンジジン（Sigma-Aldrich、ミズーリ州セントルイス）の呈色反応を吸光度（450 nm）で測定することにより検出した。検体は最初、PCT 処理血小板を捕捉抗原として試験した。アッセイのカットオフ値を上回った検体（基準値平均と比較して標準偏差の 2 倍以上高かった場合）は、更に評価するため、PCT 未処理血小板にて吸着させた後に PCT 処理血小板を用いて再試験を行った。PCT 未処理血小板で吸着後も陽性であった検体は、抗体価または IgG の相対濃度を定量化した。S-59 関連の新抗原に対する抗体陽性の判定条件は、PCT 未処理血小板を捕捉抗原とした条件でも、吸光度が同様に基準値（正常血清で得られた値）平均と比較して標準偏差の 2 倍以上高かった場合とした。陽性検体については、抗 PCT 抗体の相対濃度を 2 法により推定した。アッセイは、トリニトロフェノール（TNP）血小板／ヤギ抗 TNP 抗体／ウサギ抗ヤギ抗体（本アッセイの PCT 処理血小板／ヒト抗 PCT 抗体／ウサギ抗ヒト抗体複合体と類似フォーマットのアッセイ）の検量線を用いた条件で繰り返し行った。各フォーマットからバックグラウンドを減じた後、ヒト検体の特異的信号は、等しい強度の信号を発する TNP 血小板結合ヤギ抗体量から定量した。また、抗体陽性の検体を希釈し、抗体価の相対度を推定した。抗血小板抗体（HPA-1）を含む陽性対照検体 2 例を用いて、アッセイ感度を検討した。これらの陽性検体は希釈倍率 1：4,096 でも陽性であった。TNP を校正基準としたこれら 2 検体の定量的 IgG アッセイから、これら陽性対照血清 2 例の IgG 濃度は 9.8 ng/mL および 23.7 ng/mL であることが示された。

試験のエンドポイント

臨床現場において血小板輸血の効果判定に使用されていることから、輸血 1 時間後の血小板増加数および輸血 1 時間後の補正血小板増加数（CCI）の 2 項目を主要エンドポイントとした。CCI は、輸血のための供給時に測定した血小板投与量を用いて、次式により算出した：CCI = [(輸血後血小板数– 輸血前血小板数) × 体表面積（m^2）]/血小板投与量（$\times 10^{-11}$）。

本試験の副次エンドポイントは、次のパラメータとした：血小板輸血 24 時間後の血小

板増加数および CCI、血小板輸血による支持療法期間中の血小板輸血回数、血小板輸血間隔、輸血前後の臨床的な止血状態、血小板輸血による支持療法期間中に輸血された赤血球単位数、血小板輸血不応状態となった患者の割合(輸血 1 時間後の CCI が 2 回連続で 5,000 未満となった場合と定義)、同種免疫が成立（リンパ球傷害性試験で抗体陽転が認められた場合と定義）した患者の割合。明らかに輸血に関連する有害事象（急性輸血反応、血小板輸血による菌血症など）および他のあらゆる有害事象を記録し、MedDRA（医薬規制用語集）の基本語および器官別大分類を用いてコード化・要約した[12]。

血小板輸血の止血効果を確認するため、各血小板輸血の前後それぞれ 6 時間以内に患者を観察し、出血を判定した。出血のおそれがある 12 部位を盲検化された条件で評価し、0（出血なし）、1（少量の出血）または 2（多量の出血）で採点した[13]。出血評価部位は、皮膚、鼻、口腔咽頭、胃腸、生殖器、尿路、肺、網膜および侵襲部位（カテーテル、静脈穿刺、気管切開、手術）とした。重症度グレードが記録されなかった出血部位については、他部位の平均スコアを用いて欠測グレードを推定した。輸血前後の止血状態スコアは、各輸血前後に評価した個々の出血部位のグレードを合計することにより算出した。各投与群（被験群および対照群）内の全患者の平均スコアを算出した。治験実施計画書では、輸血前後の止血状態スコアのほか、輸血期間中および調査期間中に発生したすべての出血性有害事象も報告するよう規定した。出血性有害事象は重症度（非重篤と重篤）を分類した。血小板輸血期間中に施行されたすべての赤血球輸血を出血の代替指標として記録した。

試験デザインの統計学的根拠

本試験の症例数および検出力は、Trial to Reduce Alloimmunization to Platelets（TRAP）試験の登録患者で得られたデータに基づき算出した[14]。TRAP 試験において、単一ドナー由来の白血球除去血小板輸血群にランダム化された患者 150 例では、1～8 回目の輸血後における平均 CCI の標準偏差は 5,532、1～8 回目の輸血後における平均血小板増加数の標準偏差は 14.6×10^9/L であった。本試験では、有意水準 0.05 の両側検定を用いて、被験群・対照群間での平均 CCI の差 2,807 および平均血小板増加数の差 8.0×10^9/L を、検出力 80%で検出できる症例数は 100 例と算出された。

統計手法

TRAP 試験の経験に基づき、本試験の患者の大多数は、56 日間の輸血期間中に 8 回以上の血小板輸血を施行されると予想された。輸血回数の個人差の影響を低減するため、輸血 1 時間後および 24 時間後の平均血小板増加数および平均 CCI は、56 日間の輸血期間に施行された 1～8 回目の輸血を評価対象として算出し、*t* 検定により比較した。比較は、各患者がランダム化された投与割付に従って行った。

全輸血を評価対象とした血小板増加数（56 日間の各輸血サイクル中に施行した全輸血の 1 時間後および 24 時間後の血小板数）の分析には、一般化推定方程式による経時データのステップワイズ線形回帰モデルを用いた[15]。血小板輸血回数の患者間変動の影響に対応するため、血小板輸血回数は経時変数とした。治験実施計画書に適合する全輸血の 1 時間後および 24 時間後の血小板数は、輸血回数、輸血前血小板数、血小板輸血投与量、血小板濃厚液の輸血前保存期間の平均、患者の体重および身長、ならびに臨床施設を共変量として、被験群と対照群の間で比較した。分析は、変数減少（backward elimination）法を用いて、各段階の分析に投与（PCT または非 PCT）を組み込んだ条件で、*p* 値が 0.05 を超える変数を 1 つずつ減らしていくことにより行った。有意性が認められた各共変量と PCT の交互作用を同モデルに組み込み、手順を繰り返した。

副次エンドポイントは、副次解析として、ITT 集団を解析対象として有意水準 0.05 にて解析した。多重性の補正は行わなかった。

結果

患者集団

1998 年 6 月～2000 年 6 月に、患者 103 例（被験群 52 例および対照群 51 例）に対し 1 回以上の試験輸血を施行した。第 1 サイクルの完了例数は、被験群（83%）が対照群（67%）より多く（$p = 0.06$）、第 2 サイクル中に輸血を受けた患者数も、被験群（52 例中 10 例）が対照群（51 例中 2 例）より多かった。この群間差の結果として、試験観察日数も被験群が対照群と比較して多かった（4,081 日 vs 3,633 日）。

両群は、一次診断、治療、同種血液製剤の使用歴、妊娠歴（表 1）および人口統計学的特性（表 2）に関して釣り合いが取れていた。試験開始時の血液学的検査値および凝固検

査値は、患者に腫瘍があり血小板輸血を必要としていることを示しており（表 3）、群間差は認められなかった。試験期間中、102 例（99%）が何らかの感染症治療薬、26 例（25%）がアムホテリシン、34 例（33%）が抗凝固薬、4 例（4%）が線維素溶解薬、5 例（5%）が抗線維素溶解薬、2 例（2%）が非ステロイド系抗炎症薬（NSAID）の投与を受けた。抗線維素溶解薬の使用を除き、併用薬に関する投与群間差は認められなかった。抗線維素溶解薬の投与を受けた全 5 例とも、対照群の患者であった（$p = 0.03$）。

表 1.　血小板輸血を受けた血小板減少症患者の疾患、治療および同種免疫限原への曝露歴

	投与群 例数（%）	
	被験群 （52 例）	対照群 （51 例）
一次診断		
急性白血病	26 (50)	24 (47)
造血系腫瘍*/非造血系固形腫瘍	21 (40)	19 (37)
その他	5 (10)	8 (16)
治療		
骨髄移植	2 (4)	1 (2)
末梢血幹細胞移植	17 (33)	18 (35)
化学療法	33 (63)	32 (63)
同種免疫原への曝露	46 (88)	44 (86)

統計学的に有意な群間差は認められなかった（$p > 0.05$）。
*多発性骨髄腫、リンパ腫、ホジキンリンパ腫など。
†血小板または赤血球輸血歴、あるいは女性の場合には妊娠歴。

表 2.　血小板減少症に対して血小板輸血を受けた患者の人口統計学的特性

	投与群、例数（%）	
	被験群、52 例	対照群、51 例
性別、例数（%）		
男性	30 (58)	28 (55)
女性	22 (42)	23 (45)
年齢、平均 ± SD、歳	48.6 ± 14.1	51.1 ± 13.2
体重、平均 ± SD、kg	73.56 ± 15.30	74.25 ± 15.69
身長、平均 ± SD、cm	173.09 ± 8.70	171.47 ± 8.19
体表面積、平均 ± SD、m^2	1.88 ± 0.23	1.89 ± 0.22

統計学的に有意な群間差は認められなかった（$p > 0.05$）。

血小板輸血

第 1 サイクル中に施行された被験輸血 390 回中 311 回（80%）および対照輸血 286 回中 256 回（90%）には、治験実施計画書に従って調製された血小板製剤が使用された。平均血小板輸血回数（治験実施計画書に適合する輸血、治験実施計画書に適合しない輸血とも含めた場合）は、被験群 7.5 ± 5.8 回に対し、対照群 5.6 ± 5.5 回であった（$p = 0.09$）。治験実施計画書に適合する輸血の平均回数は、被験群 6.2 ± 4.2 回に対し、対照群 5.0 ± 4.8 回であった（$p = 0.22$）。治験実施計画書に適合する輸血 1 回あたりの平均血小板輸血投与量（10^{11}）は、被験群が対照群より少なかった（3.9 ± 1.0 vs 4.3 ± 1.2、$p < 0.001$）。治験実施計画書に適合する輸血の血小板輸血総投与量の平均には、群間差は認められなかった（被験群 22.3×10^{11} vs 対照群 21.2×10^{11}、$p = 0.74$）。被験血小板製剤の血小板数が対照

製剤より低くなった要因は、S-59 濃度測定のため追加のサンプル（10 mL）を採取する必要があったことと、処理セットに試作品を使用したため、血小板を移し替える際のキット内残留による損失（20 mL）があったことの 2 点であった。被験プールの輸血前保存期間の平均は対照プールと同等で（3.5 ± 1.1 日 vs 3.4 ± 1.2 日、$p = 0.28$）、輸血前保存期間の中央値は被験プール 4 日、対照プール 3 日であった。さらに、保存 5 日目に輸血された被験プールおよび対照プールの割合は、それぞれ 22%および 20%であった。被験血小板製剤および対照血小板製剤はすべて、ABO 型が適合していた。

表 3. 血小板減少症に対して血小板輸血を受けた患者の試験開始時の血液学的検査値および凝固検査値

	投与群			
	被験群、52		対照群、51例	
	例数*	値	例数*	値
ヘマトクリット、%、平均 ± SD	47	0.27 ± 0.05	50	0.26 ± 0.05
血小板数、平均 ± SD、× 10^9/L	52	19.1 ± 13.3	51	16.7 ± 13.1
ヘモグロビン値、平均 ± SD、g/L	51	94.4 ± 15.8	48	89.0 ± 19.1
フィブリノゲン値、平均 ± SD、g/L	47	4.673 ± 1.647	44	4.724 ± 1.203
白血球数、中央値†、× 10^9/L	52	0.350	51	0.220
プロトロンビン時間、平均 ± SD、秒	28	12.8 ± 4.1	26	12.1 ± 1.8
部分トロンボプラスチン時間、平均 ± SD、秒	50	35.0 ± 9.2	46	34.7 ± 9.2

統計学的に有意な群間差は認められなかった（$p > 0.05$）。
*ベースライン値が得られた患者数
†値が正規分布しなかったため、平均値でなく中央値を示す。

輸血 1 時間後の血小板増加数

1～8 回目の輸血 1 時間後の平均血小板増加数は、被験群 27.5 ± 13.5 × 10^9/L、対照群 35.8 ± 23.3 × 10^9/L であった。平均値の差 8.3 × 10^9/L［95%信頼区間（CI）、0.9～15.8 × 10^9/L］は統計学的に有意であった（$p = 0.03$）。両群の血小板増加数とも、報告されている治療域内であった[16,17]。輸血 1 時間後の血小板増加数を血小板投与量の差に関して補正したところ（CCI）、輸血 1 時間後の平均 CCI には統計学的に有意な投与群間差は認められず（13,100 ± 5,400 vs 14,900 ± 6,200、$p = 0.11$）、平均値の差は 1,800（95% CI、-400～4,100）であった。第 1 サイクルの全輸血を評価対象として時系列回帰分析を実施したところ、輸血 1 時間後の血小板数に関して、PCT 血小板濃厚液と非 PCT 血小板濃厚液の間に有意差は認められなかった（$p = 0.53$）。PCT がもたらした影響は、輸血 1 時間後の血小板数の 1.5 ×

10^9/L（95% CI、-3.1～6.1 × 10^9/L）減少と推定された。

輸血後血小板数に有意な影響（$p < 0.05$）を与えた共変量は、有意水準が高い順に、血小板輸血投与量（$p < 0.001$）、血小板濃厚液の輸血前保存期間（$p < 0.001$）、輸血前血小板数（$p < 0.001$）および患者の体重（$p < 0.001$）であった。これらの共変量は、PCT と血小板輸血投与量の交互作用項（$p = 0.73$）を含め、PCT と有意な交互作用を示さなかった。第 1 サイクルの全輸血を対象とした回帰分析では、血小板輸血投与量が等しい条件では、輸血 1 時間後の血小板増加数に関して、PCT 血小板と非 PCT 血小板の間に差がみられなかった（図 1）。PCT が輸血後血小板増加数に与える影響の大きさおよび傾向について、試験施設間に有意差は認められなかった（$p = 0.80$）。

輸血 24 時間後の血小板増加数および血小板輸血間隔

輸血 24 時間後の平均血小板増加数（1～8 回目の輸血）は、被験群（16.4 ± 9.5 × 10^9/L）が対照群（24.7 ± 17.6 × 10^9/L）と比較して少なかった（$p = 0.004$）。輸血 24 時間後の平均 CCI（1～8 回目の輸血）も、被験群（7,400 ± 5,500）が対照群（10,600 ± 7,100）と比較して少なかった（$p = 0.02$）。第 1 サイクルの全輸血を評価対象として時系列回帰分析を実施したところ、輸血 24 時間後の血小板数に関して、PCT 血小板濃厚液と非 PCT 血小板濃厚液の間に有意差は認められなかった（$p = 0.19$）。PCT がもたらした影響は、輸血 24 時間後の血小板数の 2.6 × 10^9/L（95% CI、-1.3～6.5 × 10^9/L）減少と推定された。輸血 24 時間後の血小板数に有意な影響を与えた共変量は、輸血 1 時間後の解析結果と同じで、これらの共変量はいずれも、PCT と有意な交互作用を示さなかった。PCT 処理と血小板輸血投与量の交互作用項も有意でなかった（$p = 0.31$）。第 1 サイクルの全輸血を対象とした回帰分析では、血小板輸血投与量が等しい条件では、輸血 24 時間後の血小板増加数に関して、PCT 血小板と非 PCT 血小板に差がみられなかった（図 2）。

試験施設のうち 2 施設は対照血小板製剤に 100%血漿を使用し、残り 2 施設は血漿･T-Sol 混合液を使用した。このため、対照群の輸血 1 時間後および 24 時間後の血小板増加数および CCI 反応を、2 種類の浮遊液で比較した。その結果、浮遊液による差は認められず、既報の血漿・T-Sol 混合液の使用経験と矛盾しなかった[18]。

初回血小板輸血期間（期間 1）の長さ、ならびに血小板輸血期間全体の長さに関して、被験群と対照群の間に統計学的有意差は認められなかった（表 4)。初回血小板輸血期間には全患者（103 例）とも輸血を受けたことから、初回血小板輸血期間中の平均血小板輸血間隔を算出したが、被験群と対照群の間に有意差はみられなかった（表 4)。

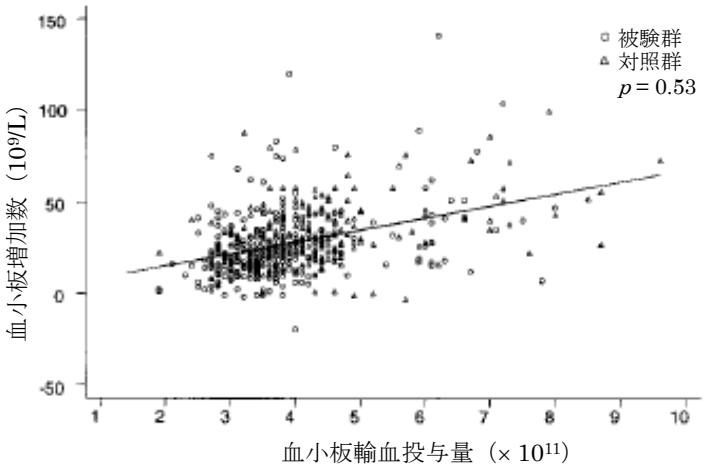

図 1.　被験および対照血小板輸血 1 時間後の血小板増加数に対する血小板輸血投与量の影響

第 1 サイクルに行った治験実施計画書に適合するすべての（被験および対照）輸血を評価対象として、輸血 1 時間後の血小板増加数と血小板輸血投与量の間の関係を示す。被験血小板製剤と対照血小板製剤の血小板輸血投与量が等しい条件では、輸血 1 時間後の血小板増加数は、血小板輸血投与量の範囲にわたり同等であった（$p = 0.53$)。被験血小板輸血と対照血小板輸血の回帰直線は、重なるものと判断される。

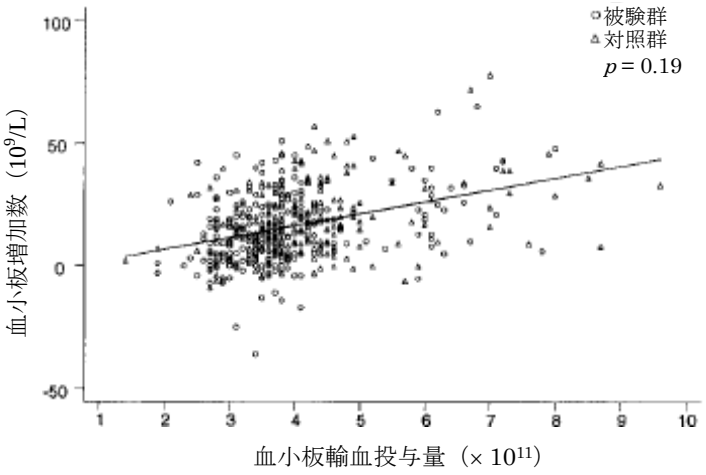

図 2.　被験および対照血小板輸血 24 時間後の血小板増加数に対する血小板輸血投与量の影響

第 1 サイクルに行った治験実施計画書に適合するすべての（被験および対照）輸血を評価対象として、輸血 24 時間後の血小板増加数と血小板輸血投与量の間の関係を示す。被験血小板製剤と対照血小板製剤の血小板輸血投与量が等しい条件では、輸血 24 時間後の血小板増加数は、血小板輸血投与量の範囲にわたり同等であった（p= 0. 19）。被験血小板輸血と対照血小板輸血の回帰直線は、重なるものと判断される。

第 2 サイクル登録患者の輸血後血小板数

第 2 サイクルに登録された患者は少数であったため（被験群 10 例および対照群 2 例）、輸血後血小板数データは時系列回帰分析のみ実施した。輸血 1 時間後の血小板数に関して、有意な投与群間差はみられなかった（p = 0.47）。PCT がもたらした影響は、輸血 1 時間後の血小板数の 4 × 10⁹/L 増加（95% CI、-7～14 × 10⁹/L）と推定された。PCT と血小板輸血投与量の交互作用項は有意でなかった（p = 0.74）。同様に、第 2 サイクルの輸血 24 時間後の血小板数に関しても、有意な投与群間差はみられなかった（p = 0.90）。PCT がもたらした影響は、輸血 24 時間後の血小板数の 1 × 10⁹/L 増加（95% CI、-10～11 × 10⁹/L）と推定された。第 2 サイクルでも、血小板輸血投与量が等しい条件では、輸血 1 時間後および 24 時間後の血小板増加数に関して、PCT 血小板と非 PCT 血小板に差はみられなかった。

出血、止血状態スコアおよび赤血球輸血

輸血前後の止血効果の評価に基づき、輸血前に出血が 1 回以上発生した患者の割合は、被験群 71%、対照群 63%であった（$p = 0.36$）。血小板輸血後の出血事象発生率は、両群とも輸血前より低下し、群間差もなかった（被験群 54%、対照群 49%：$p = 0.62$）。輸血前、輸血後を問わず、止血状態スコアに関して、被験群と対照群の間に有意差は認められず、止血状態スコアの平均は、両群とも輸血後に低下した（表 5）。輸血期間全体の止血状態を、報告された出血性有害事象に基づき評価したところ、出血性有害事象を 1 件以上発生した患者の割合は高く、群間差は認められなかった（表 6）。特に発生率が高かった出血性有害事象は、鼻出血（被験群 42% vs 対照群 41%）、歯肉出血（被験群 17% vs 対照群 12%）、注射部位出血（被験群 17% vs 対照群 6%）、紫斑（被験群 15% vs 対照群 14%）、点状出血（被験群 13% vs 対照群 16%）、血腫（被験群 13% vs 対照群 8%）であった。重度の出血性有害事象を発生した患者の割合は、各群 6%にすぎなかった（表 6）。出血は、輸血期間中に輸血された赤血球単位数の比較によっても間接的に評価した。第 1 サイクルの初回血小板輸血期間に輸血された平均赤血球単位数（被験群= 4.9 ± 4.2、対照群= 4.5 ± 5.4）に、群間差はみられなかった（$p = 0.68$）。さらに、第 1 サイクル（56 日間）中に輸血された平均赤血球単位数（被験群=9.3 ± 5.3、対照群= 8.2 ± 6.3）にも、有意な群間差はみられなかった（$p = 0.34$）。第 1 サイクルの初回血小板輸血期間における 1 日あたり平均赤血球単位数（被験群=0.41 ± 0.35、対照群= 0.56 ± 0.68）にも、有意な群間差はみられなかった（$p = 0.16$）。

血小板輸血不応状態、リンパ球傷害性抗体および S-59 が形成しうる新抗原に対する抗体

輸血 1 時間後の CCI が 2 回連続で 5,000 未満であった患者は、血小板輸血不応状態に分類した。不応状態と診断された全患者を対象として、リンパ球傷害性抗体（LCA）を評価した。本試験期間中に血小板輸血不応状態を 1 回以上来した患者は、7 例［被験群 4 例（8%）および対照群 3 例（6%）、$p = 0.72$］であった。この 7 例のうち、試験開始時に LCA 陽性であった患者数は、被験群 2 例および対照群 1 例であった。うち被験群 1 例は、試験開始時に LCA 陽性で、なおかつ第 1 サイクル全体を通して不応状態が持続した。その他の 2 例（被験群 1 例および対照群 1 例）は、第 1 サイクル中に LCA 陽性に転じた。各群 1 例は LCA 陰性に保たれた。S-59 に関連する血小板新抗原に対する抗体は、どの患者からも検出されなかった。

急性輸血反応およびその他の有害事象

全患者を対象として、血小板輸血後に輸血関連の症状および徴候を評価した。急性輸血反応に分類した輸血後の事象は、血小板輸血後 6 時間以内に発生した発熱、悪寒、悪心、皮疹、蕁麻疹、気管支痙攣、頻脈、低血圧、高血圧、ヘモグロビン尿、溶血反応およびバイタルサイン変化などであった。第 1 サイクルでは、急性輸血反応が発生した輸血回数の割合は、被験群 6%に対し、対照群 5%であった（$p \geq 0.61$）。

第 1 および第 2 サイクル中、評価対象患者集団の 5%以上に報告された有害事象は、計 147 種類であった。有害事象全体では、器官別大分類別の発生率に関して 2 投与群間に差は認められなかった。重篤な有害事象を報告した患者数は、被験群 14 例（27%）、対照群 13 例（25%）であった。血小板輸血に関連すると報告された有害事象に関して、統計学的に有意な群間差は認められなかった（$p = 0.20$）。本試験中の輸血に伴い、菌血症は認められなかった。被験群 4 例および対照群 5 例が本試験中に死亡した。本試験中の輸血に関連する死亡例はなかった。

表 4. 第 1 サイクルにおける血小板支持療法の全期間、血小板支持療法部分期間の日数、および血小板の輸血間隔

	投与群		
	被験群（52 例）	対照群（51 例）	*p*値
血小板支持療法全期間の日数、平均 ± SD、日	15.2 ± 11.2	12.7 ± 12.4	.29
第 1 サイクルにおける血小板指示療法部分期間の日数、平均 ± SD、日			
期間 1（被験者数）	11.7 ± 9.5 (52)	10.1 ± 11.7 (51)	≥.45
期間 2（被験者数）	7.5 ± 7.2 (22)	8.0 ± 7.9 (14)	
期間 3（被験者数）	15.0 (1)	5.0 ± 6.9 (3)	
最初の血小板支持療法期間（期間 1）における輸血間隔、平均 ± SD、日	3.0 ± 1.23	3.4 ± 1.21	.13

輸血期間（56 日間）中の ITT 集団に対するすべての輸血を記録した。血小板支持療法期間とは、初回の試験輸血から最終回の試験輸血までの日数と定義した。輸血間隔が 7 日以上あった場合は、非施行期間直前の輸血を、その輸血期間の最終回の血小板輸血とみなした。血小板輸血が再開された場合には、輸血期間（56 日間）中に実施した血小板輸血の合計日数を算出するため、期間 2 以降の実施日数を期間 1 の実施日数に加算した。そのため、1 サイクル（56 日間）中に血小板支持療法期間が複数回ある患者も存在する。

表 5. 第 1 サイクルにおける輸血前後の止血状態スコア*

	投与群		
	被験群（52 例）	対照群（51 例）	*p*値
輸血前スコア	0.43 ± 0.46	0.45 ± 0.57	.84
輸血後スコア	0.28 ± 0.39	0.30 ± 0.43	.81

出血のおそれがある 12 部位を盲検化された条件で評価し、0（出血なし）、1（少量の出血）または 2（多量の出血）で採点した。輸血前後の止血状態スコアは、各輸血前後に評価した個々の出血部位のグレードを合計することにより算出した。

*平均 ± SD

表 6. 第 1 サイクルに発生した出血性有害事象（器官別大分類別）

	被験群（52例）、例数（%）		対照群（51例）、例数（%）	
器官別大分類	全体	重度	全体	重度
合計	41 (79)	3 (6)	38 (79)	3 (6)
眼障害	7 (13)	1 (2)	0 (0)	0 (0)
眼出血	3 (6)	0 (0)	0 (0)	0 (0)
網膜出血	3 (6)	1 (2)	0 (0)	0 (0)
胃腸障害	18 (35)	0 (0)	14 (27)	1 (2)
歯肉出血	9 (17)	0 (0)	6 (12)	0 (0)
直腸出血	4 (8)	0 (0)	1 (2)	0 (0)
胃腸出血	3 (6)	0 (0)	5 (10)	1 (2)
全身障害および投与局所様態	11 (21)	0 (0)	3 (6)	0 (0)
注射部位出血	9 (17)	0 (0)	3 (6)	0 (0)
臨床検査	9 (17)	0 (0)	7 (14)	0 (0)
血尿	7 (13)	0 (0)	6 (12)	0 (0)
呼吸器、胸郭および縦隔障害	24 (46)	1 (2)	21 (41)	0 (0)
鼻出血	22 (42)	1 (2)	21 (41)	0 (0)
喀血	6 (12)	0 (0)	2 (4)	0 (0)
皮膚および皮下組織障害	19 (37)	0 (0)	15 (29)	0 (0)
紫斑	8 (15)	0 (0)	7 (14)	0 (0)
点状出血	7 (13)	0 (0)	8 (16)	0 (0)
斑状出血	3 (6)	0 (0)	0 (0)	0 (0)
外科および内科処置	3 (6)	0 (0)	1 (2)	0 (0)
術後出血	3 (6)	0 (0)	0 (0)	0 (0)
血管障害	7 (13)	0 (0)	4 (8)	0 (0)
血腫	7 (13)	0 (0)	4 (8)	0 (0)

発生率が 5%以上であった出血性有害事象の発生例数を、器官別大分類別および各器官別大分類内の基本語別に示す。器官別大分類に属する個別事象（基本語）の中には、発生率が 5%未満のものもあった。これらの事象（基本語）は表に示さなかったが、器官別大分類別の発生例数には含めた。一部の患者は、同じ器官別大分類に属する 2 種類以上の出血性有害事象を発生した。χ^2検定により発生率（%）を投与群間で比較し、統計学的有意差の有無を確認した。重度の出血性有害事象の割合は別個に示す。軽度および中等度の有害事象は、特別な治療的介入を必要としなかった。重度の有害事象は、治療的介入および患者の活動状態の変更を必要とした。いずれの出血性有害事象の発生率に関しても、統計学的に有意な投与群間差は認められなかった（$p > 0.05$）。

考察

対象とした患者集団の血小板輸血による支持療法期間の長さおよび人口統計学的特性から、本試験は、感染性因子不活化処理により調製した血小板成分の有効性を評価するうえで有意義と考えられる。本試験では、バフィーコート法により調製したランダムドナー由来のプール血小板を用いたが、これは欧州では常法とされる製造法である[19]。被験血小板製剤および対照血小板製剤はプール後、ろ過により白血球数を低減し、輸血まで最長 5 日間保存した。バフィーコート血小板と、多血小板血漿（PRP）法により調製した血小板では、輸血 1 時間後および 24 時間後の血小板増加数が同等であることが過去の研究により示されている[20]。アフェレーシスにより採取し 100%血漿に浮遊した血小板についても、同様の試験結果が報告されている[21]。また、対照血小板を 100%血漿に浮遊した場合と、血漿と血小板添加液（T-Sol）の 35% : 65%混合液に浮遊した場合の比較でも、血小板増加数および CCI が同等であるという結果が、我々のみならず他のグループによっても得られている[18]。PRP 血小板は 100%ドナー血漿に保存される単一ドナー由来製剤で、プール製剤のように血漿と血小板添加液の混合液には保存されない。したがって、PRP 血小板は、光増感剤により感染性因子不活化処理すると、臨床効果に変化が生じる可能性がある。我々は最近、アフェレーシスにより採取した血小板を添加液中で浮遊し、光増感剤により感染性因子不活化処理した単一ドナー由来血小板を用いた試験の予備的データを報告した[22]。血小板輸血を繰り返し受けている重度血小板減少症患者を対象とした試験では、単一ドナー由来 PCT 血小板製剤による出血の予防効果および治療効果は、従来の血小板製剤と同等であることが明らかになった。だが、単一ドナー由来 PCT 血小板製剤による支持療法受けた患者では、輸血 1 時間後および 24 時間後の平均血小板増加数が有意に低かった。したがって、異なる処理方法を用いると、輸血後の血小板増加数が変化する可能性がある。

本試験は、血小板増加数に関して PCT 血小板製剤と従来の血小板製剤との間に臨床的に意味のある差が存在する場合に、十分な検出力で検出できるよう計画した。輸血後の血小板増加数は、血小板輸血の有効性の代替指標として臨床現場で使用されている。血小板輸血に対する十分な反応を定義する基準は確立されていないが、輸血 1 時間後の CCI が 7,500 以上になった場合には十分な反応と一般に認識されている[23]。血小板輸血の効果に関する過去の研究では、血小板輸血の効果には、複数の共変量因子が影響を与えることが明らかになっている[24-26]。また、同種免疫が成立していない患者においても、輸血の繰り

返しに伴い、血小板増加数が減弱する[27]。そのため、輸血回数が様々な患者の平均血小板増加数を比較しても、頑健性が不十分となる可能性がある。このため、本試験では、大半の患者が 8 回以上の輸血を受けると予測されたことから、平均血小板増加数および CCI の解析対象を 1～8 回目の輸血に限定することにした。さらに、CCI は、血小板輸血投与量と患者の体格を組み込んだ比率指標であり、血小板輸血投与量と患者の体格はいずれも血小板増加反応に影響を与えると認識されている。このため、血小板輸血投与量、患者の体格などの因子（経時変数として使用した血小板輸血回数を含む）の影響を個別に評価するため、血小板増加数を従属変数とした線形回帰分析を採用した[28]。

治験実施計画書に適合する全輸血の 1 時間後および 24 時間後の血小板数の時系列回帰分析結果からは、輸血後血小板数に対する PCT の統計学的に有意な影響は明らかにならなかった。予想どおり、血小板輸血投与量、血小板成分の保存期間、輸血前血小板数および患者の体格は、輸血後血小板増加数、ひいては輸血後血小板数に対して高い有意性を示す共変量因子であった。被験血小板と対照血小板の輸血投与量には統計学的有意差がみられたが、両製剤の投与量とも、血小板減少症患者の支持療法としては十分であった。4 試験施設のうち 3 施設では、被験血小板プールと対照血小板プールは、等しい人数分のバフィーコートを用いて調製された。残る 1 試験施設では、標準操作手順書に従って、対照血小板プールは 4 人分のみのバフィーコートを用いて調製されたが、被験血小板プールは 5 人分のバフィーコートを用いて調製された。対照血小板、被験血小板を問わず、血小板輸血投与量は輸血時に決定された。そのため、血小板輸血投与量が血小板増加反応に与えた影響を、線形回帰分析により検討した。この分析の結果、被験血小板と対照血小板の輸血投与量が等しい条件では、輸血 1 時間後および 24 時間後の血小板増加数が同等であることが確認された。

PCT 処理による血小板損失率は約 10%であるが、被験血小板プールの平均輸血投与量は、3.0×10^{11}（血小板輸血による支持療法には十分な投与量と一般に認識されている輸血投与量）より十分に高値であった[29]。現在では、一体型の処理セットが使用されているため、血小板損失率は約 8%で、PCT 血小板製剤の調製に際して、使用するバフィーコートの人数分を増やす必要はないと予想される[30]。重要なことは、被験血小板濃厚液と対照血小板濃厚液に対する効果は、臨床現場で使用される血小板輸血投与量の範囲にわたり同等であ

り、被験群および対照群における輸血 1 時間後および 24 時間後の平均血小板増加数および平均 CCI は、同様の患者集団に関する既報の範囲内であった[16,17,26]。輸血間隔に対する血小板輸血投与量の影響を検討した他の最近の研究[17,29]との比較では、本試験の被験血小板の輸血間隔は、既報の範囲内であることが明らかになった。

輸血期間（56 日間）における治験実施計画書に適合する輸血の平均回数および血小板輸血総投与量は、被験群の方が多かった。しかし、この差の一因として、対照群の方が患者の試験中止率が高かったため（10 例 vs 5 例）、血小板減少症の日数が被験群で長くなったことが挙げられる。最も多かった中止理由は、追加療法として幹細胞移植プロトコールに登録されたため、試験的な血小板製剤の使用が禁止されたことであった。これらの患者に関しては、患者の血小板輸血群の割付に関して盲検下で主治医が本試験を中止させた。群間での分布がランダムでなかったのは、偶然と考えられた。本試験を中止しなかった患者（被験群 43 例と対照群 34 例）を対象として、初回血小板輸血期間中に施行された輸血回数を解析したところ、平均血小板輸血回数は、それぞれ 5.5 回および 4.1 回であった（p = 0.25）。2 回目の血小板輸血期間（期間 2）を必要とし、なおかつ第 1 サイクルを完了した患者（被験群 19 例と対照群 12 例）では、平均血小板輸血回数はそれぞれ 3.5 回および 4.4 回であった。

血小板輸血は、血小板減少症に伴う出血の予防および止血を目的として行われる。本試験では、止血効果の評価に、輸血前後の止血状態の評価、報告されたすべての出血性有害事象、全血小板輸血期間中に輸血された赤血球単位数を使用した。観察期間中、大多数の患者で出血性有害事象が 1 件以上認められたが、重度の出血性有害事象を来した患者は、被験群、対照群とも各 3 例にすぎなかった。輸血後期間中、脳出血により被験群 1 例および対照群 2 例が死亡した。本試験中に抗線維素溶解薬の投与を受けた患者は、対照群 5 例に対し被験群 0 例であった。抗線維素溶解薬はこの 5 例の出血回数を減少させた可能性があるが、この 5 例で出血回数の明らかな減少傾向は検出されなかった。これら 5 例は対照群の全患者を対象とした解析に組み入れた。最近の試験では、PCT 処理した単一ドナー血小板と従来の単一ドナー血小板は、血小板輸血を繰り返し必要とする重度血小板減少症における出血の予防効果および止血効果が同等であることが明らかになっている[22]。

本試験では、出血リスクの高い患者集団を対象として、血小板輸血による支持療法を評価した。本試験のデータは、S-59 を用いる感染性因子不活化装置で処理したプールバフィーコート由来血小板成分を保存 5 日以内に輸血した場合、血小板減少症患者における支持療法としての効果が、従来の血小板成剤と同等であるという結論を裏づけている。注目すべきことに、被験血小板製剤、対照血小板製剤を問わず、輸血前保存期間が 4 日以上であった製剤はかなりの割合にのぼった。本試験では、安全性プロファイルに関して、PCT 血小板と従来の血小板の間に差がみられなかったが、本試験の評価範囲は一部にすぎなかった。臨床試験では、小児または妊娠患者における PCT 血小板の安全性の明確な評価は実施できない。そのため、我々はこれらの問題に対処するため、妊娠および新生児動物を用いて前臨床安全性試験を実施した。これらの動物集団では、S-59 および PCT 血漿の輸血による毒性は検出されなかった [31]。さらに、p53 変異のヘテロ接合体マウスを用いたがん原性試験では、S-59 は臨床曝露量の 1,000 倍でがん原性を示さなかった [31]。

既報によれば、PCT 技術により血小板製剤中のウイルス、細菌および白血球が高い再現性で不活化されることが実証されている [5,8,9]。今回の臨床試験から、バフィーコート由来血小板製剤の現行の調製方法に追加可能な処理システムを用いて、プール血小板成分を PCT 処理し、最長 5 日間保存した場合、輸血関連感染症の抑制および残存する混入白血球の不活化が可能と考えられる。

Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial

Jeffrey McCullough, David H. Vesole, Richard J. Benjamin, Sherrill J. Slichter, Alvaro Pineda, Edward Snyder, Edward A. Stadtmauer, Ileana Lopez-Plaza, Steven Coutre, Ronald G. Strauss, Lawrence T. Goodnough, Joy L. Fridey, Thomas Raife, Ritchard Cable, Scott Murphy, Frank Howard IV, Kathryn Davis, Jin-Sying Lin, Peyton Metzel, Laurence Corash, Antonis Koutsoukos, Lily Lin, Donald H. Buchholz, and Maureen G. Conlan

We report a transfusion trial of platelets photochemically treated for pathogen inactivation using the synthetic psoralen amotosalen HCl. Patients with thrombocytopenia were randomly assigned to receive either photochemically treated (PCT) or conventional (control) platelets for up to 28 days. The primary end point was the proportion of patients with World Health Organization (WHO) grade 2 bleeding during the period of platelet support. A total of 645 patients (318 PCT and 327 control) were evaluated. The primary end point, the incidence of grade 2 bleeding (58.5% PCT versus 57.5% control), and the secondary end point, the incidence of grade 3 or 4 bleeding (4.1% PCT versus 6.1% control), were equivalent between the 2 groups ($P = .001$ by noninferiority). The mean 1-hour posttransfusion platelet corrected count increment (CCI) (11.1×10^3 PCT versus 16.0×10^3 control), average number of days to next platelet transfusion (1.9 PCT versus 2.4 control), and number of platelet transfusions (8.4 PCT versus 6.2 control) were different ($P < .001$). Transfusion reactions were fewer following PCT platelets (3.0% PCT versus 4.4% control; $P = .02$). The incidence of grade 2 bleeding was equivalent for PCT and conventional platelets, although posttransfusion platelet count increments and days to next transfusion were decreased for PCT compared with conventional platelets. (Blood. 2004;104: 1534-1541)

Introduction

More stringent donor selection and increased laboratory testing have been extremely effective in improving the safety of the US blood supply.[1-6] However, transmission of some infections still occurs because the present approach is limited to specific known pathogens, is not effective against bacterial contamination,[7-9] does not test for all pathogens,[10] fails to prevent transmission of cytomegalovirus (CMV) despite testing,[11] and tests for new pathogens, such as West Nile virus,[12] can only be implemented after the new agent is identified. With increasing globalization, previously localized transfusion-transmitted infections such as malaria, trypanosomiasis, or babesiosis are now becoming more widespread. Therefore, strategies have been developed to treat the blood components in a way that will inactivate viruses, bacteria, protozoa, and contaminating leukocytes but retain therapeutic efficacy of the components.[13-17]

Amotosalen HCl, formerly designated S-59, is a synthetic psoralen compound that intercalates into helical regions of DNA or RNA and on illumination with ultraviolet A (UVA) light reacts with pyrimidine bases to form internucleic and intranucleic acid strand cross-links. The photochemical treatment (PCT) inhibits replication of any DNA or RNA. This achieves reduction of a broad range of viruses, bacteria, and protozoa to levels below those likely to transmit infection (Table 1). Extensive toxicology, mutagenicity, carcinogenicity, phototoxicity, and pharmacologic studies established an adequate safety profile for PCT platelets.[24,25] In vitro platelet function of PCT platelets was preserved following up to 7 days of storage.[15,16] Recovery and survival of radiolabeled PCT platelets in healthy subjects were reduced compared with conventional untreated platelets but within acceptable therapeutic ranges.[26] PCT and conventional untreated platelets resulted in comparable correction of prolonged bleeding times in patients with thrombocytopenia.[27] A randomized, controlled, double-blind, parallel group phase 3 study in 103 patients with thrombocytopenia of PCT buffy coat platelets demonstrated that 1-hour platelet count increments

From the Department of Laboratory Medicine & Pathology, University of Minnesota, Minneapolis; the Blood and Marrow Transplant Program, Medical College of Wisconsin, Milwaukee; the Joint Program in Transfusion Medicine, Brigham & Women's Hospital, Boston, MA; Puget Sound Blood Center, Seattle, WA; the Department of Laboratory Medicine, Mayo Clinic, Rochester, MN; Yale University School of Medicine, Yale-New Haven Hospital, New Haven, CT; University of Pennsylvania Medical Center, Philadelphia; Institute for Transfusion Medicine, Pittsburgh, PA; Stanford Medical Center, Palo Alto, CA; DeGowin Blood Center, University of Iowa, Iowa City; Washington University School of Medicine, St Louis, MO; Blood Bank of San Bernadino County, San Bernadino, CA; Blood Center of Southeastern Wisconsin, Milwaukee; American Red Cross Blood Services, Farmington, CT; American Red Cross Blood Services, Penn-Jersey Region, Philadelphia, PA; Loma Linda University Cancer Institute, Loma Linda, CA; University of Washington, Seattle; Cerus Corp, Concord, CA; Quintiles Inc, Rockville, MD; and Baxter Healthcare Corp, Round Lake, IL.

Submitted January 8, 2004; accepted April 12, 2004. Prepublished online as *Blood* First Edition Paper, May 11, 2004; DOI 10.1182/blood-2003-12-4443.

J.-S.L., L.L., P.M., D.H.B., L.C., and M.G.C. are employees of Baxter or Cerus Corporations. J.M., S.J.S., and S.M. are members of the Cerus Medical Advisory Committee, for which $2500 per year is received. J.M., E.S., and S.M. are consultants for Baxter or served on the Fenwal Medical Advisory Committee, for which less than $10 000 was received in the past year. J.M., R.J.B., A.P., T.R., E.S., S.M., and I.L.-P. participated in other clinical trials sponsored by Cerus or Baxter; they received funds only to support the cost of those studies.

Reprints: Jeffrey McCullough, University of Minnesota, MMC 609, 420 Delaware St SE, Minneapolis, MN 55455; e-mail: mccul001@umn.edu.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

Table 1. Inactivation of pathogens in platelet concentrates after photochemical treatment with amotosalen and UVA light

Pathogen	Log-reduction in organisms
Enveloped viruses	
HIV (cell-free)	> 6.2
HIV (cell-associated)	> 6.1
CMV	> 5.9
Hepatitis B virus	> 5.5
Hepatitis C virus	> 4.5
Duck hepatitis B virus	> 6.2
Bovine viral diarrhea virus	> 6.0
Human T-cell leukemia virus type I/II	4.7/5.1
West Nile virus	> 6.0
Nonenveloped viruses	
Blue tongue	6.1-6.4
Parvovirus B19*	4.0-4.9
Gram-negative bacteria	
Escherhia coli	> 6.4
Serratia marcescens	> 6.7
Klebsiella pneumoniae	> 5.6
Pseudomonas aeruginosa	4.5
Salmonella choleraesuis	> 6.2
Yersinia enterocolitica	> 5.9
Enterobacter cloacae	5.9
Gram-positive bacteria	
Staphylococcus aureus	6.6
Staphylococcus epidermidis	> 6.6
Streptococcus pyogenes	> 6.8
Listeria monocytogenes	> 6.3
Corynebacterium minutissimum	> 6.3
Bacillus cereus	> 6.0
Gram-positive anaerobic bacteria	
Lactobacillus species	> 6.9
Propionibacterium acnes	> 6.7
Clostridium perfringens	> 7.0
Bifidobacterium adolescentis	> 6.5
Protozoa	
Trypanosoma cruzi	> 5.3
Plasmodium falciparum	> 7.0
Leishmania mexicana	> 5.2

Data are summarized from Lin,[14] Lin et al,[15,16,18] Van Voorhis et al,[19,20] Dupuis et al,[21] Savoor et al,[22] and Sawyer et al.[23]

*Preliminary data; inactivation was performed in 35% B19-infected plasma and 65% PAS III (platelet additive solution III) in the absence of platelets. Studies included a 15- or 30-minute rest between addition of amotosalen and UVA treatment.

were not different for PCT and conventional buffy coat platelets.[28] We now report on a prospective, randomized, controlled, double-blind, parallel group phase 3 study to evaluate the efficacy, as determined by the prevention and treatment of significant bleeding, and safety of PCT apheresis platelets compared with conventional platelets.

Patients, materials, and methods

Patients

Patients were eligible for enrollment if they had thrombocytopenia requiring platelet transfusion support and were at least 6 years of age. Patients were excluded from study participation if they had any factors that could potentially interfere with assessment of the study end points. These exclusion criteria included positive lymphocytotoxic antibody (> 20% panel reactive antibody at screening) or history of clinical refractoriness, history of immune or thrombotic thrombocytopenic purpura or hemolytic uremic syndrome, diagnosis of acute promyelocytic leukemia, recent surgery or psoralen ultraviolet A (PUVA) therapy, interleukin-11 therapy, or participation in another study with pathogen-inactivated blood products. Patients who met all inclusion and exclusion criteria were randomly assigned in a 1:1 ratio to receive all of their platelet transfusions with either PCT or control platelet concentrates for up to 28 days or until transfusion independence (7 days without platelet transfusion) prior to day 28. On completion of the transfusion period, patients entered a 7-day surveillance period to monitor for additional adverse events. The study was approved by each site's institutional review board (IRB), and all patients gave informed consent to participate.

All individuals involved in clinical care and assessment of patients were blinded to study treatment assignment. These individuals included the principal investigator, clinical study coordinators and nurses making hemostatic assessments, clinicians and nurses caring for the patient, and the study sponsor. Blood bank and transfusion service personnel responsible for randomization, collection, processing, and issue of study platelets were not blinded.

End points

The primary efficacy end point was the proportion of patients with grade 2 bleeding, as assessed by using expanded World Health Organization (WHO) criteria (Table 2),[29] on any day during the period of platelet support. Additional secondary efficacy end points included the proportion of patients with WHO grade 3 or 4 bleeding; number of days of WHO grade 2 bleeding; 1- and 24-hour platelet count increments (CIs) and corrected count increments (CCIs); number of days to next platelet transfusion; number of platelet transfusions; incidence of platelet refractoriness; and number of red blood cell (RBC) transfusions. Safety end points included number of platelet transfusion reactions, development of antibody to potential amotosalen neoantigens, and overall safety.

Platelet collection and photochemical treatment

Both PCT and control study platelet transfusions were collected on the Amicus Separator (Baxter Healthcare, Round Lake, IL), which includes process leukoreduction, to attain a targeted average platelet transfusion dose of 3.7×10^{11}. PCT platelets were suspended in 30% to 45% plasma and 70% to 55% platelet additive solution (Intersol; Baxter Healthcare, Deerfield, IL), whereas control platelets were suspended in 100% plasma. Photochemical treatment[15] was performed at each study site within 24 hours of platelet collection by adding 150 μM amotosalen, mixing, and exposing the platelets to 3 J/cm^2 UVA light in an illumination device for 3 to 5 minutes with constant gentle agitation. Following illumination, platelets were transferred to a plastic container with a compound adsorption device (CAD) to reduce the concentration of residual amotosalen and free photoproducts. After adsorption for 6 to 8 hours, PCT platelets were transferred to another container and were stored for up to 5 days according to blood bank standards.[30] All donors and platelet products underwent required blood bank testing.[30] PCT and control platelet concentrates were issued for transfusion in identical plastic containers with identical labeling. Because PCT platelets were manufactured solely for the purpose of the trial, there were occasional inventory shortages that resulted in transfusion of non-PCT platelets to patients randomly assigned to the PCT group ("off-protocol" transfusion) or transfusion of low-dose PCT products that would not otherwise have been transfused to prevent an off-protocol transfusion. Control platelet transfusions not collected on the Amicus Separator were also off-protocol transfusions.

Transfusion strategies

Platelet transfusions were given according to each institution's guidelines either prophylactically to prevent bleeding or therapeutically to treat existing bleeding or prepare for an invasive procedure. The most common threshold for prophylactic transfusions was 10×10^9/L. Each institution's policies determined platelet ABO type, use of irradiation, volume reduction, and HLA matching or cross-matching for donor selection. Patients received conventional red cell products; more than 98% of red cell units were leukocyte reduced and 99% were gamma irradiated in both treatment groups.

Table 2. Expanded WHO bleeding scale used for the hemostatic primary end point

Organ system	Bleeding grade	
	1	2
Mucocutaneous		
Epistaxis	< 1 h in duration	> 1 h in duration
Oropharyngeal	< 1 h in duration	> 1 h in duration
Petechiae/purpura	Localized petechiae of skin or oral mucosa; purpura < 1-inch diameter	Purpura > 1-inch diameter; generalized petechiae or purpura
Gastrointestinal		
Melena	NA	Melanotic stool with positive occult blood
Rectal bleeding/ hematochezia	Occult blood in stool; no visible blood	Visible blood in stool
Hematemesis	NA	Occult or visible blood in vomit or gastric contents
Genitourinary		
Hematuria	< 1+ (slight, trace, small) blood in urine	≥ 2+ (moderate)
Vaginal bleeding, abnormal	Spotting; < 2 saturated pads/d	> 2 saturated pads/d
Bronchopulmonary	NA	Hemoptysis; blood-tinged sputum; bloody bronchopulmonary lavage
Musculoskeletal and soft tissue	NA	Spontaneous hematoma; any joint bleed
Body cavity (pleural, peritoneal, pericardial, retroperitoneal)	NA	RBCs on microscopic examination of any body fluid
Central nervous system	NA	Retinal bleeding without visual impairment
Invasive sites	NA	Any bleeding around a catheter, venipuncture site, or other invasive or surgical site

Grade 3 bleeding requires RBC transfusion; grade 3 body cavity bleeding is grossly bloody body fluid; grade 3 central nervous system (CNS) bleeding is bleeding on computed tomography or magnetic resonance imaging scan without clinical consequence. Grade 4 bleeding is associated with hemodynamic instability (hypotension; > 30 mm Hg decrease in systolic or diastolic blood pressure) or fatal bleeding; grade 4 musculoskeletal bleeding is associated with a permanent debilitating joint change; grade 4 CNS bleeding is CNS bleeding with neurologic symptoms and signs, or retinal bleeding with visual impairment (field deficit). Expanded scale is based on WHO bleeding scale from Miller et al.[29] NA indicates not applicable.

Hemostatic assessments and laboratory evaluation

Hemostatic assessments of 8 potential bleeding sites were performed by trained observers blinded to the treatment assignment. At each assessment, each of the 8 potential bleeding sites was assigned a WHO bleeding grade (Table 2) ranging from 0 (no bleeding) to 4 (life-threatening bleeding). The first hemostatic assessment encompassed the 12 hours preceding the first study platelet transfusion. Subsequent hemostatic assessments were performed daily and for 3 days following the last study platelet transfusion. The overall bleeding grade for each assessment was the highest grade observed for any of the 8 sites assessed. If grade 2 bleeding was observed at any potential bleeding site on any assessment during the transfusion period, the patient met the primary end point. For example, a patient with a 2-inch ecchymosis on day 3 of the transfusion period but no other bleeding events during the transfusion period would have been classified as having experienced grade 2 bleeding and would have met the primary end point of the trial.

The daily platelet count obtained for routine care was used for the study pretransfusion platelet count. The 1-hour and 24-hour posttransfusion platelet counts were obtained 10 minutes to 4 hours and 10 to 24 hours, respectively, following each platelet transfusion. Lymphocytotoxic antibody (LCA) testing to determine study eligibility was performed locally, and patients whose serum reacted with more than 20% of panel cells (PRA) were excluded. Plasma samples for LCA and antibody to amotosalen neoantigen testing were drawn weekly; baseline and end-of-study samples were analyzed at central laboratories for LCA by using standard techniques[31] and for antibodies to potential amotosalen neoantigens by using a validated enzyme-linked immunosorbent assay (ELISA; Cerus, Concord, CA).[28] If the patient became platelet refractory, all samples from the patient were analyzed for LCA, antibody to amotosalen neoantigens, and platelet-specific alloantibodies[32,33] in central laboratories. The CCI, a measure of the response to platelet transfusion that takes into account patient body size as well as transfused platelet dose, was calculated as the difference between the platelet count after transfusion and the platelet count before transfusion, multiplied by the body surface area (in meters squared) and divided by the number of platelets transfused ($\times 10^{-11}$). A patient was considered clinically refractory if the 1-hour CCI was less than 5×10^3 following each of 2 consecutive platelet transfusions. Immunologic refractoriness was defined as clinical refractoriness (2 consecutive CCIs $< 5 \times 10^3$) in the presence of any of the following: LCA (> 20% PRA), platelet-specific alloantibodies, and/or antibody to amotosalen neoantigens.

Adverse events and transfusion reactions

Adverse events were collected from initiation of first study transfusion through the end of the 7-day surveillance period. Adverse event and transfusion reaction severity was assigned on the basis of the most severe symptom or sign present. Reactions to study platelet transfusions were assessed for the 6 hours following each transfusion.

Randomization and statistical methods

A sample size of 300 patients per group was estimated before the start of the study to provide more than 90% power to reject the null hypothesis of inferiority with respect to grade 2 bleeding at a significance level of 0.05. All patients who received at least one study platelet transfusion were included in the analyses. Randomization was stratified by study site.

The study was designed as a noninferiority trial. Differences between treatment groups for the primary end point (the proportion of patients with grade 2 bleeding) and one secondary end point (the proportion of patients with grade 3 or 4 bleeding) were analyzed using one-sided tests of noninferiority with prespecified noninferiority margins of 12.5% and 7%, respectively. All other secondary end points were analyzed for differences between treatment groups. For the primary end point, the test statistic was $(P_T - P_R - 0.125)/(\mathrm{Var}[P_T - P_R])^{1/2}$, where P_T is the observed proportion of patients with grade 2 bleeding in the PCT group, P_R is the observed proportion of patients with grade 2 bleeding in the control group, and $\mathrm{Var}(P_T - P_R)$ is the variance estimated by the maximum likelihood estimate.[34] The one-sided 95% confidence interval for the treatment difference in the proportion used the same estimated variance.

Analysis of variance with treatment and study site in the model was used for continuous variables. Fisher exact test was used for comparison of adverse events. Time to grade 2 bleeding was compared by using the log-rank test. Longitudinal regression analysis was used to adjust platelet count increment and transfusion interval for platelet dose.[35,36] Except for

the tests of noninferiority, all other statistical tests were 2-sided with a significance level of 0.05.

Results

Of the 671 patients randomly assigned, 645 received at least one study platelet transfusion (318 PCT; 327 control) and composed the intention-to-treat (ITT) population. The 26 patients not included in the ITT analyses did not require platelet transfusions before recovery from thrombocytopenia. There were no differences between the groups for sex, age, ethnic origin, diagnosis, or receipt of stem cell transplant (Table 3) or in baseline hematology, chemistry, and coagulation laboratory studies (data not shown).

The proportion of patients completing the transfusion period (89%) and the surveillance period (81%), the mean duration of platelet support (11.8 days PCT versus 10.6 days control), and the proportion of patients achieving and maintaining platelet transfusion independence prior to day 28 (66% PCT versus 70% control) were not different between treatment groups (Table 4).

The primary end point of the trial, the proportion of patients with grade 2 bleeding, was equivalent for the PCT group and control group, both overall, as well as for any of the 8 potential bleeding sites (Table 5). Grade 2 bleeding occurred during the transfusion period in 58.5% of patients in the PCT group compared with 57.5% of patients in the control group. The time to onset of grade 2 bleeding after beginning the study was not significantly different between PCT and control patients, either for the ITT population (Figure 1A, $P = .78$) or for those patients without grade 2 bleeding at study entry (Figure 1B, $P = .91$). Grade 2 bleeding occurred on a mean of 3.2 days in the PCT group as compared with 2.5 days in the control group ($P = .02$) and on a median of 1 day for each group.

The maximum grade of bleeding at any potential bleeding site was grade 2 for most patients. Grade 3 or 4 bleeding occurred in only 4.1% of patients in the PCT group and 6.1% in the control group. There were no statistically significant differences between the groups in grade 3 or 4 bleeding overall or for any of the 8 potential bleeding sites. The most common site of grade 3 or 4 bleeding was the neurologic system (3 of 318, 0.9% PCT versus 6 of 327, 1.8% control).

The 645 patients in this study received a total of 4719 platelet transfusions (2678 PCT; 2041 control) (Table 6). Most units of platelets transfused (91.5% PCT and 95.2% control) were prepared according to study methods ("on-protocol transfusions"). During the study transfusion period, exclusively on-protocol transfusions were received by 68% of patients in the PCT group and 85% of patients in the control group ($P < .01$). Of patients who received any off-protocol transfusions, most

Table 3. Patient characteristics

	PCT; n = 318	Control; n = 327
Sex		
% male	54	51
Age, y		
Mean	47	46
Range	7-85	6-75
% younger than 16 y	2	5
Ethnic origin, %		
White	91	91
African American	3	3
Hispanic	3	3
Other	3	3
Stem cell transplantation, %		
Bone marrow	20	22
Peripheral blood	54	55
Cord blood	2	3
Total	76	80
Source of stem cells, %		
Autologous	64	65
Allogeneic	36	35
Underlying diagnosis, %		
Acute leukemia	29	28
Chronic leukemia	11	11
Lymphoma	24	29
Myelodysplasia	3	2
Plasma cell dyscrasia	20	18
Nonhematopoietic solid tumor	8	8
Other	5	4
WHO grade 2 bleeding at study entry, %	15.7	16.5

All characteristics had $P > .05$, thus showing no differences.

Table 4. Patient participation

	Treatment group		
	PCT, n (%) n = 318	Control, n (%) n = 327	*P*
Completed transfusion period	280 (88)	294 (90)	.53
Reason for not completing transfusion period			
Patient decided to withdraw	8 (2.5)	4 (1.2)	.26
Physician withdrew patient	10 (3.1)	4 (1.2)	.11
Adverse event(s)	0 (0)	0 (0)	—
Lost to follow-up	1 (< 1)	1 (< 1)	—
Death	10 (3.1)	15 (4.6)	.42
Other	9 (2.8)	9 (2.8)	—
Total	38 (11.9)	33 (10.1)	.44
Mean days of platelet support	11.8	10.6	.08
Achieved and maintained platelet independence prior to day 28	210 (66)	230 (70)	.27
Completed surveillance period	248 (78)	273 (84)	.09

— indicates not applicable.

Table 5. Proportion of patients with grade 2 or higher bleeding

	PCT, n (%) n = 318	Control, n (%) n = 327	*P**
Any grade 2 bleeding	186 (58.5)	188 (57.5)	<.01†
Grade 2 bleeding by bleeding site			
Genitourinary	104 (32.7)	103 (31.5)	0.80
Mucocutaneous	82 (25.8)	65 (19.9)	0.08
Invasive sites	69 (21.7)	65 (19.9)	0.63
Gastrointestinal	60 (18.9)	63 (19.3)	0.92
Respiratory	35 (11.0)	28 (8.6)	0.35
Musculoskeletal	15 (4.7)	18 (5.5)	0.72
Body cavity	0 (0.0)	1 (0.3)	1.00
Neurologic	0 (0.0)	0 (0.0)	—
Any grade 3 or 4 bleeding	13 (4.1)	20 (6.1)	<.01‡

— indicates not applicable.

*Fisher exact test was used to calculate the *P* value for each of the 8 potential bleeding sites.

†The *P* value for the overall proportion of patients with grade 2 bleeding was < .01, based on a noninferiority test with a noninferiority margin of 0.125 (one-sided 95% confidence interval of difference: −1, 0.07). By using this method, a *P* value of < .05 indicates that PCT was not inferior to control.

‡The *P* value for any grade 3 or 4 bleeding was < .01, based on a noninferiority test with a noninferiority margin of .07 (one-sided 95% confidence interval of difference: −1, 0.013). By using this method, a *P* value of < .05 indicates that PCT was not inferior to control.

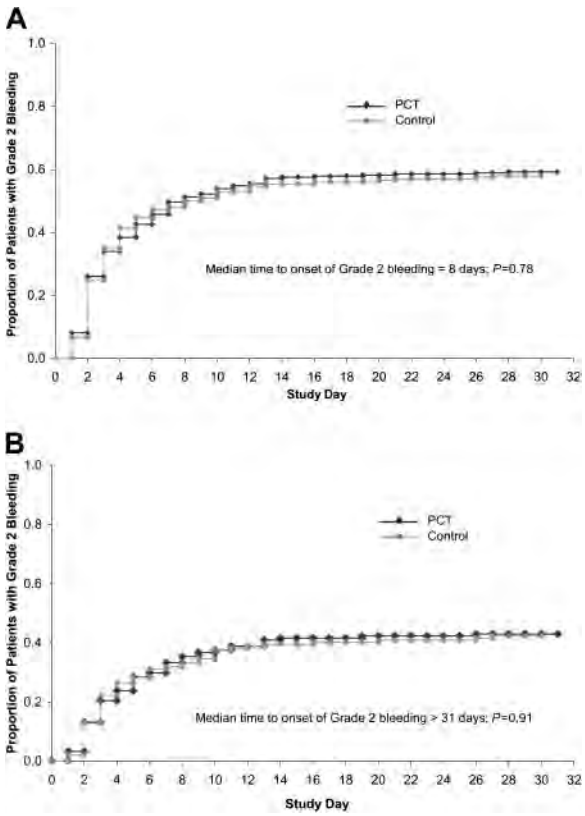

Figure 1. Time to onset of grade 2 bleeding. (A) Time to onset of grade 2 bleeding in ITT population (n = 645). Median time to onset of grade 2 bleeding was 8 days, log rank $P = .78$. (B) Time to onset of grade 2 bleeding in patients with no (grade 0) bleeding at baseline (n = 541). Median time to onset of bleeding more than 31 days, log-rank test $P = .91$.

(53% PCT and 59% control) received only one. The proportion of platelet transfusions that were HLA matched (1.5%), crossmatch compatible (0.2%), volume reduced (7.5%), or irradiated (99.8%) were comparable between the 2 groups. Slightly more PCT transfusions were ABO-matched (with patient pretransplantation blood type) than control transfusions (78.5% versus 75.4%, $P = .01$). Mean platelet storage duration prior to transfusion was 3.4 days for PCT as compared with 3.6 days for control platelets ($P < .01$).

Table 6. Platelet and RBC transfusions during the study

	PCT, n = 318	Control, n = 327	*P*
Platelet transfusions			
Total number	2678	2041	—
Mean number per patient	8.4	6.2	< .001
Mean number per day of platelet support*	0.74	0.65	< .001
Interval between transfusions, d	1.9	2.4	< .001
Platelet dose, $\times 10^{11}$			
Mean average dose	3.7	4.0	< .001
Percentage of platelet doses less than 3.0 $\times 10^{11}$	20	12	< .01
Mean total dose over entire transfusion period	29.4	24.1	.01
Duration of platelet storage, d	3.4	3.6	< .05
RBC transfusions			
Mean number per patient	4.8	4.3	.13
Mean number per day of platelet support*	0.31	0.30	.53

— indicates not applicable.

*Days of platelet support is defined as number of days from the first to the last study platelet transfusion.

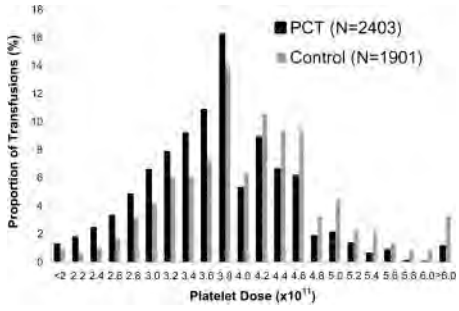

Figure 2. Distribution of transfused platelet doses. A greater proportion of doses were less than 3.0×10^{11} in the PCT group compared with the control group ($P < .01$).

Patients in the PCT group received more platelet transfusions overall (8.4 PCT versus 6.2 control; $P < .001$; Table 6) and more platelet transfusions per day of platelet support (0.74 PCT versus 0.65 control; $P < .001$). These differences may be partially explained by the lower mean dose of platelets per transfusion in the PCT group compared with the control group (3.7×10^{11} PCT versus 4.0×10^{11} control; $P < .001$) and the greater proportion of PCT platelet doses that contained less than 3.0×10^{11} platelets (20% PCT versus 12% control; $P < .01$; Figure 2). Sixty percent of patients in the PCT group received at least one platelet dose less than 3.0×10^{11} compared with 36% of patients in the control group ($P < .01$). However, by using longitudinal linear regression to adjust for platelet dose, when equal doses of PCT and control platelets were given, the 1-hour posttransfusion platelet count was estimated to be 10.4×10^9/L lower for PCT than for control platelets ($P < .001$), and the time to the next transfusion was shorter by 0.4 days for PCT than for control platelets ($P < .001$). Other factors that can affect platelet recovery,[37] such as splenomegaly, fever, sepsis, and amphotericin use were comparable between treatment groups. There was no difference between the groups in the mean number of red blood cell transfusions or the mean number of red blood cell transfusions per day of platelet support (Table 6).

Most transfusions were given for prophylaxis (93.5% PCT versus 90.1% control; $P < .01$); the others were considered to be therapeutic either to treat active bleeding or to prepare for an invasive procedure. Although mean pretransfusion platelet counts were similar for patients in both groups, the mean 1-hour posttransfusion platelet count was lower in the PCT group (36.5×10^9/L PCT versus 49.5×10^9/L control; $P < .001$), as were the mean 1-hour and 24-hour CI and CCI (Table 7).

Platelet clinical refractoriness occurred in 21.4% of PCT as compared with 7.0% of control patients ($P < .001$; Table 8).

Table 7. Mean platelet responses following platelet transfusions

	PCT; n = 318	Control; n = 327
Before transfusion		
Platelet count, $\times 10^9$/L	15.1	15.2
1 h after transfusion		
Platelet count, $\times 10^9$/L	36.5*	49.5
Count increment, $\times 10^9$/L	21.4*	34.1
Corrected count increment, $\times 10^3$	11.1*	16.0
24 h after transfusion		
Platelet count, $\times 10^9$/L	27.9*	36.1
Count increment, $\times 10^9$/L	13.2*	21.5
Corrected count increment, $\times 10^3$	6.7*	10.1

*$P < .001$ compared with control.

Table 8. Refractoriness to platelet transfusions

	PCT	Control	*P*
ITT population, n	318	327	—
Any refractory episode, %*	21.4	7.0	< .001
Any transfusion with CCI less than 5×10, %	27.4	12.7	< .001
Refractory subset of patients, n	68	23	—
Single episode of refractoriness, %	57	65	.63
Refractory to end of study, %	6	9	.64
Immunologic refractoriness†			
LCA and/or platelet alloantibody, %	22	44	.06
Lymphocytotoxic antibodies, %	15	39	.02
Platelet specific alloantibodies, %	12	10	1.00
Antibody to amotosalen neoantigens	0	0	—

— indicates not applicable.

*Episode is 2 consecutive platelet transfusions with 1-hour CCI $< 5 \times 10^3$.

†Immunologic refractoriness, defined as the presence of LCA (> 20% PRA), platelet alloantibodies, and/or antibody to potential amotosalen neoantigens in the presence of 2 consecutive 1-hour CCI less than 5×10^3.

One-hour CCIs less than 5×10^3 were observed with 27.4% of all PCT transfusions and 12.7% of all control platelet transfusions ($P < .001$) and 33.4% of PCT as compared with 12.3% of control platelet transfusions with platelet doses less than 3.0×10^{11} ($P < .001$). Most refractory episodes were transient, involving only a single episode of 2 consecutive 1-hour CCIs less than 5×10^3 (57% PCT versus 65% control). Only 6% of refractory patients in the PCT group and 9% of refractory patients in the control group remained refractory through study completion. Alloimmunization to HLA, platelet-specific antigens, or amotosalen neoantigens as the basis for platelet refractoriness occurred 4.7% of PCT patients as compared with 3.1% of control patients in the ITT population ($P = .31$) and in 22% of PCT patients as compared with 44% of control patients in the refractory subset of patients ($P = .06$). Among refractory patients, LCA was more common in the control group (39%) compared with the PCT group (15%; $P = .02$). Platelet alloantibodies occurred with similar frequency among refractory patients (12% PCT compared with 10% control; $P = 1.00$).

Although there were fewer transfusion reactions following transfusion of PCT platelet units (3.0% PCT versus 4.4% control transfusions; $P = .02$), there was no difference in the proportion of patients who experienced a reaction (16.0% PCT versus 19.3% control; $P = .30$). Reactions were primarily fever, chills, urticaria, or rash. Almost all patients experienced one or more adverse events (Table 9). Adverse events were coded to 898 MedDRA Preferred Terms.[39] The most common adverse events (reported in > 30% of patients in either treatment group), such as hematuria, diarrhea, hypokalemia, rigors, petechiae, epistaxis, fecal occult blood, contusion (bruising), and dermatitis, were consistent with those expected for the patient population enrolled in this study. As expected, with the large number of statistical comparisons performed, there were statistically significant differences between treatment groups for some types of adverse events, but these differences were not considered to be clinically relevant and will be reported in detail separately. Grade 3 or 4 adverse events, those considered by the investigator to be probably or possibly related to study platelet transfusion, and adverse events meeting US Food and Drug Administration (FDA) criteria for serious were not different between the PCT and control groups (Table 9). There were 28 deaths (3.5% PCT versus 5.2% control) during the study, mostly because of infectious or respiratory complications.

Discussion

Despite improvements in the safety of the US blood supply, the public wants transfusion risks to be as close to zero as possible, and political and health policy decisions reflect this goal. As new transfusion-transmitted infectious agents are identified, new tests for these agents may be implemented, but this approach will always have limitations. Inactivation of a broad spectrum of viruses, bacteria, and protozoa in blood products is a promising new strategy to improve blood safety.

The low prevalence of pathogens in blood components precludes a study of the prevention of transfusion-transmitted infection by PCT platelets. Therefore, we studied the effect of PCT on platelet transfusion hemostatic effectiveness rather than transfusion transmissible infections. The trial, the largest one of its kind, evaluated platelet hemostasis as the primary end point while also evaluating the quality and safety of PCT platelets. PCT and control platelets were hemostatically comparable overall and, for each of the 8 potential bleeding sites evaluated, established that PCT platelets were clinically effective. Patients who received PCT platelets had lower platelet count increments following transfusion, received more platelet transfusions, and had a shorter interval between transfusions compared with patients who received conventional apheresis platelets. The lower platelet count increment is partly explained by the lower mean platelet dose in the PCT group and the disproportionate number of transfusions containing doses less than 3.0×10^{11} (Figure 2). The greater proportion of low-dose platelets transfused to the PCT group may have resulted in the greater number of platelet transfusions in the PCT group.[41] Reasons for lower platelet doses in the PCT group primarily reflected clinical trial requirements. These reasons included a clinical prototype of the device was used with a nonintegrated processing set and a prototype CAD; processing loss for PCT platelets was acknowledged; samples taken for amotosalen assay came from PCT but not control; to avoid off-protocol transfusions, low doses of PCT platelets were transfused when a higher dose unit was not available; and because PCT units were produced solely for the purpose of the clinical trial, control units were more readily available, resulting in higher platelet doses. During routine use, it is expected that doses of PCT platelets will be comparable to control platelets. Following completion of this trial, an integrated PCT processing set with an improved CAD was developed and evaluated in a small supplemental trial in Europe. That trial in 43 patients demonstrated no increase in the number of platelet transfusions

Table 9. Adverse events during the study

	PCT, %; n = 318	Control, %; n = 327	*P*
Any adverse event*	99.7	98.2	.12
Grade III or IV adverse event	78.9	78.6	.92
Serious adverse event†	27.0	24.8	.53
Treatment-related adverse event‡	26.4	29.4	.43
Death§	3.5	5.2	.34

*Adverse events were graded I to IV using the National Cancer Institute Common Toxicity Criteria (NCI-CTC)[38] and coded to Preferred Term by using Medical Directory for Regulatory Affairs (MedDRA).[39]

†Serious adverse events were defined by using Food and Drug Administration (FDA) criteria.[40]

‡Treatment-related adverse events were reported as possibly or probably related to the study platelet transfusions by the blinded investigator at each site.

§One patient in each group died of hemorrhage; both deaths involved pulmonary alveolar hemorrhage thought to result from toxicity of the myeloablative preparative regimen.

required to manage patients transfused with PCT platelets for up to 28 days[42]; those results will require confirmation in a larger study.

Another factor accounting for the reduced platelet responses with PCT platelets was a decrease in platelet viability; ie, at equal platelet doses, there was a significant reduction in both platelet increment and days to next transfusion comparing PCT with control platelets. An effect of the PCT process on platelet viability was suggested in previous studies in healthy research subjects and patients.[26,27] As a consequence of the lower platelet count increments in the PCT group, clinical platelet refractoriness occurred more frequently in patients receiving PCT platelets; however, it tended to be transient, persisting to the end of the study in only 6% of PCT and 9% of control refractory patients. Alloimmune platelet refractoriness and the need for HLA-matched platelets were uncommon and were similar in both groups. Among platelet refractory patients, the incidence of LCA was lower in the PCT group, but platelet-specific alloantibodies were similar. Despite the lower platelet count increments, the shorter intervals between platelet transfusions, and the resultant greater number of PCT platelets transfused, the PCT platelets were hemostatically equivalent to the control platelets; therefore, differences in these secondary end points appear to have little effect on product efficacy and patient benefit.

Overall, no unusual toxicities or adverse events were associated with the transfusion of PCT platelets. A companion safety analysis will be reported separately. Although the proportion of patients who experienced a transfusion reaction was similar in the 2 groups, fewer PCT platelet transfusions were associated with a reaction. This could be due to leukocyte inactivation, resulting in less cytokine production during storage of PCT platelets or the reduced volume of plasma in the PCT units.[43] Other adverse events, including hemorrhagic adverse events and death, were not different between the 2 groups of patients.

Photochemically treated platelets were clinically effective in maintaining hemostasis, appear to be associated with an acceptable safety profile, and offer the potential to further reduce the infectious risks of blood transfusion, including those associated with emerging transfusion-transmitted infections.

Acknowledgments

We thank the many nurses, technologists, and study coordinators who worked on this project at each study site and to the following: *Cerus Corporation:* Steven Anderson, MT; Tanya Baculik, MD; Marlene Bartram, RN, MT; Ruth Dye, RN; Anne Elliott, RN; and Lecia Shaffer. *Baxter Healthcare Corporation:* Janis Drerup, Julie Gavigan, Jaime Houghton, Alamdar Rizvi, and Lindsey Wood, PhD. *Data Safety Monitoring Board:* Margot Kruskall, MD, Chair; Marcella Contreras, MD; Paul Holland, MD; and Janet Wittes, PhD.

References

1. Klein HG. Will blood transfusion ever be safe enough? JAMA. 2000;284:238-240.
2. AuBuchon JP, Birkmeyer JD, Busch MP. Safety of the blood supply in the United States: opportunities and controversies. Ann Intern Med. 1997; 127:904-909.
3. Dodd RY, Notari EP, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion. 2002;42:975-979.
4. Glynn SA, Kleinman SH, Wright DJ, Busch MP. International application of the incidence rate and window-period model. National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study. Transfusion. 2002;42:966-973.
5. Busch M, Chamberland M, Epstein J, Kleinman S, Khabbaz R, Nemo G. Oversight and monitoring of blood safety in the United States. Vox Sang. 1999;77:67-76.
6. Stramer SL, Caglioti S, Strong DM. NAT of the United States and Canadian blood supply. Transfusion. 2000;40:1165-1168.
7. Klein HG, Dodd RY, Ness PM, Fratantoni JA, Nemo GJ. Current status of microbial contamination of blood components: summary of a conference. Transfusion. 1997;37:95-101.
8. Roth VR, Kuehnert MJ, Haley NR, et al. Evaluation of a surveillance system for bacterial contamination of blood and blood products in the United States. Transfusion. 2001;41:1486-1492.
9. Perez P, Salmi R, Follea G, et al. Determinants of transfusion-associated bacterial contamination: result of the French bacthem case-control study. Transfusion. 2001;41:862-872.
10. Leiby DA, Herron RM, Read EJ, Lenes BA, Stumpf RJ. Trypanosoma cruzi in Los Angeles and Miami blood donors: impact of evolving donor demographics on seroprevalence and implications for transfusion transmission. Transfusion. 2002;42:549-555.
11. Nichols WG, Price TH, Gooley T, Corey L, Boeckh M. Transfusion-transmitted cytomegalovirus infection after receipt of leukoreduced blood products. Blood. 2003;101:4195-4200.
12. Biggerstaff BJ, Petersen LR. Estimated risk of West Nile Virus transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion. 2002;42:1019-1026.
13. Wollowitz S. Fundamentals of the psoralen-based Helinx technology for inactivation of infectious pathogens and leukocytes in platelets and plasma. Semin Hematol. 2001;38:4-11.
14. Lin L. Psoralen photochemical treatment of platelets. Sci Med. 1994;5:2-11.
15. Lin L, Cook DN, Wiesehahn, et al. Photochemical inactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. Transfusion. 1997;37:423-435.
16. Lin L, Alfonso R, Behrman B, et al. Photochemical treatment of platelet concentrates with a novel psoralen and UVA to enhance the safety of platelet transfusion. Infusion Ther Transfusion Med. 1998;25:39-48.
17. Grass, JA, Hei DJ. Metchette K, et al. Inactivation of leukocytes in platelet concentrates by photochemical treatment with psoralen plus UVA. Blood. 1998;91:2180-2188.
18. Lin L, Corten L, Murthy KK, Corash L, Alter HJ. Photochemical inactivation of hepatitis B (HBV) and hepatitis C (HCV) virus in human platelet concentrates as assessed by a chimpanzee infectivity model [abstract]. Blood. 1998;92:502a.
19. Van Voorhis WC, Barrett LK, Eastman T, Alfonso R, Dupuis K. Trypanosoma cruzi inactivation in human platelet concentrates and plasma by a psoralen (amotosalen HCl) and long-wavelength UV. Antimicrob Agents Chemother. 2003;47:475-479.
20. Van Voorhis W, Barrett L, Eastman R, et al. Helinx inactivates the malaria, Chagas' disease and leishmaniasis protozoa in human platelet concentrates [abstract]. Blood. 2002;100:708a.
21. Dupuis K, Alfonso R, Savoor A, et al. Helinx technology, used in the INTERCEPT Blood System, inactivates high titers of emerging insect-borne pathogens in platelets and red blood cells [abstract]. Presented at the VIII European Congress of the International Society of Blood Transfusion. July 2003. Istanbul, Turkey.
22. Savoor A, Mababangloob R, Kinsey J, Dupuis K, Lin L. The INTERCEPT Blood System for platelets inactivates anaerobic bacteria [abstract]. Transfusion. 2002;42:92S.
23. Sawyer L, Dupuis K, Alfonso R, et al. The INTERCEPT Blood System for platelets inactivates high titers of a variety of blood-borne pathogens in platelets [abstract]. Hematol J. 2003;4(suppl 2): 228.
24. Ciaravino V. Preclinical safety of a nucleic acid—targeted Helinx compound: a clinical perspective. Semin Hematol. 2001;38:12-19.
25. Ciaravino V, McCullough T, Dayan AD. Pharmacokinetic and toxicology assessment of INTERCEPT (S-59 and UVA treated) platelets. Hum Exp Toxicol. 2001;20:533-550.
26. Corash L, Behrman B, Rheinschmidt M, et al. Post-transfusion viability and tolerability of photochemically treated platelet concentrates [abstract]. Blood. 1997;90:267a.
27. Slichter SJ, Corash L, Grabowski M, et al. Viability and hemostatic function of photochemically-treated platelets in thrombocytopenic patients [abstract]. Blood. 1999;94:376a.
28. van Rhenen D, Gulliksson H, Cazenave JP, et al. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood. 2003;101:2426-2433.
29. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer. 1981;47:207-214.
30. Standards for blood banks and transfusion services. 22nd ed. Bethesda, MD: American Association of Blood Banks; 2003.
31. Fuller TC, Phelan DL, Gebel HM, Rodey GE. Antigenic specificity of antibody reactive in the antiglobulin-augmented lymphocytotoxicity test. Transplantation. 1982;34:24-29.

32. Furihata K, Nugent DJ, Bissonette A, Aster RH, Kunicki TJ. On the association of the platelet-specific alloantigen, Pena with glycoprotein IIIa. Evidence for heterogeneity of glycoprotein IIIa. J Clin Invest. 1987;80:1624-1630.
33. Moore SB, Degoey SR. Serum platelet antibody testing: evaluation of solid-phase enzyme immunoassay and comparison with indirect immunofluorescence. Am J Clin Path. 1998;109:190-195.
34. Farrington CP, Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. Stat Med. 1990;9: 1447-1454.
35. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika. 1986;73:13-22.
36. Davis KB, Slichter SJ, Corash L. Corrected count increment and percent platelet recovery as measures of posttransfusion platelet response: problems and a solution. Transfusion. 1999;39:586-592.
37. Bishop JF, Matthews K, McGrath K, Yuen K, Wolf MM, Szer J. Factors influencing 20-hour increments after platelet transfusion. Transfusion. 1991;31:392-396.
38. Trotti A, Byhardt R, Stetz J, et al for the Radiation Therapy Oncology Group. Common Toxicity Criteria, version 2.0: an improved reference for grading the acute effects of cancer treatment: impact on radiotherapy. Int J Radiat Oncol Biol Phys. 2000;47:13-47.
39. MedDRA Introductory Guide. Version 3.3. Reston, VA: Northrop Grumman Information Technology; December 2002.
40. Office of the Federal Register. 21CFR Part 21 §312.32(a): IND Safety Reports; Definitions. Washington, DC: US Government Printing Office; April 1, 2002.
41. Murphy S, Snyder E, Cable R, et al. Transfusion of INTERCEPT platelets vs. reference platelets at doses $= 3.0 \times 10^{11}$ results in comparable hemostasis and platelet and RBC transfusion requirements. Results of the SPRINT trial [abstract]. Blood. 2003;102:815a.
42. Kluter H, Chapuis B, Cazenave J-P, et al. Apheresis platelets treated with the INTERCEPT Blood System for pathogen inactivation provide platelet count increments and hemostasis comparable to conventional platelets [abstract]. Vox Sang. 2002; 83:110.
43. Hei DJ, Grass J, Lin L, et al. Elimination of cytokine production in stored platelet concentrate aliquots by photochemical treatment with psoralen plus ultraviolet A light. Transfusion. 1999;39:239-248.

光増感剤を用いて感染性因子不活化処理した血小板の治療効果および安全性：SPRINT 試験（仮訳）

Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial

本稿は、合成ソラレン誘導体である光増感剤アモトサレン塩酸塩により感染性因子不活化処理をした血小板の輸血試験の報告である。血小板減少症患者を光増感剤処理（PCT）血小板輸血群または従来の（対照）血小板輸血群にランダムに割り付け、輸血を最長 28 日間行った。主要エンドポイントは、血小板輸血による支持療法期間中に世界保健機関（WHO）グレード 2 の出血を来した患者の割合とした。計 645 例（PCT 群 318 例、対照群 327 例)を評価した。主要エンドポイントとしたグレード 2 の出血発生率(PCT 群 58.5% vs. 対照群 57.5%）および副次エンドポイントとしたグレード 3 または 4 の出血発生率（PCT 群 4.1% vs. 対照群 6.1%）に群間差は認められなかった（$P = 0.001$、非劣性に関する検定)。輸血 1 時間後の平均補正血小板増加数（CCI）（PCT 群 11.1 $\times 10^3$ vs. 対照群 16.0 $\times 10^3$)、平均輸血間隔（PCT 群 1.9 日 vs. 対照群 2.4 日)、血小板輸血回数（PCT 群 8.4 回 vs. 対照群 6.2 回）には、群間差が認められた（$P < 0.001$)。輸血副作用の発生率は、PCT 血小板輸血後の方が低かった（PCT 群 3.0% vs. 対照群 4.4%、$P = 0.02$)。PCT 血小板群では、従来の血小板群と比較して、輸血後の血小板増加数が少なく、輸血間隔が短かったが、グレード 2 の出血発生率は等しかった。(Blood. 2004;104: 1534-1541)

緒言

ドナー選択の厳格化および臨床検査項目の増加が大きく奏効し、米国における輸血用血液の安全性は改善している[1-6]。しかし、現行のアプローチが有効なのは特定の既知の感染性因子のみであること、細菌汚染には有効でないこと[7-9]、すべての感染性因子を検査対象としていないこと[10]、検査を行ってもサイトメガロウイルス（CMV）の伝播を予防できないこと[11]、また、新たな感染性因子（ウエストナイルウイルス[12]など）の検査は感染性因子自体の同定後でなければ導入できないことから、一部の感染症の伝播は依然として発生し続けている。また、グローバル化の進展に伴い、それまで局地的に発生していた輸血感染症（マラリア、トリパノソーマ症、バベシア症など）の発生地域が拡大しつつある。そのため、ウイルス、細菌、原虫および混入白血球を不活化しつつ、血液成分の治療効果を

損なわない手段による血液成分の処理方法が開発されている[13-17]。

アモトサレン塩酸塩（開発名 S-59）は合成ソラレン化合物で、DNA または RNA のラセン領域にインターカレートし、紫外線 A 照射によりピリミジン塩基と反応することにより、核酸鎖間および核酸鎖内に架橋を形成する。光増感剤による処理（PCT）は、すべての DNA または RNA の複製を阻害することにより、多種類のウイルス、細菌および原虫を感染可能な量未満にまで減少させる（表 1）。PCT 血小板の安全性プロファイルは、毒性、変異原性、がん原性、光毒性および薬理試験など広範な分野の試験により安全なものであることが確認されている[24,25]。PCT 血小板では、*in vitro* における血小板機能が最長 7 日間の保存時まで保たれた[15,16]。健常被験者においてアイソトープでラベルした PCT 血小板の回収率および寿命は、従来の未処理血小板を下回ったものの、許容できる治療域内であった[26]。血小板減少症患者における出血時間延長の是正効果は、PCT 血小板と従来の未処理血小板で同等であった[27]。血小板減少症患者 103 例を対象として PCT バフィーコート血小板輸血を検討したランダム化、比較、二重盲検、並行群間第 III 相試験では、輸血 1 時間後の血小板増加数に関して、PCT バフィーコート血小板群と従来のバフィーコート血小板群の間に差はみられなかった[28]。我々は、PCT アフェレーシス血小板の輸血効果（重大な出血の予防および治療効果により評価）および安全性を、従来の血小板と比較評価する前向き、ランダム化、比較、二重盲検、並行群間第 III 相試験を実施したので報告する。

表 1.　光増感剤アモトサレンと紫外線 A 処理による血小板濃厚液中の感染性因子不活化

感染性因子	感染性因子の対数減少値
エンベロープウイルス	
HIV（細胞外）	> 6.2
HIV（細胞内）	> 6.1
CMV	> 5.9
B 型肝炎ウイルス	> 5.5
C 型肝炎ウイルス	> 4.5
アヒル B 型肝炎ウイルス	> 6.2
ウシウイルス性下痢ウイルス	> 6.0
ヒト T 細胞白血病ウイルス I/II 型	4.7/5.1
ウエストナイルウイルス	> 6.0
ノンエンベロープウイルス	
ブルータングウイルス	6.1-6.4
パルボウイルス B19*	4.0-4.9
グラム陰性菌	
Escherhia coli	> 6.4
Serratia marcescens	> 6.7
Klebsiella pneumoniae	> 5.6
Pseudomonas aeruginosa	4.5
Salmonella choleraesuis	> 6.2
Yersinia enterocolitica	> 5.9
Enterobacter cloacae	5.9
グラム陽性菌	
Staphylococcus aureus	6.6
Staphylococcus epidermidis	> 6.6
Streptococcus pyogenes	> 6.8
Listeria monocytogenes	> 6.3
Corynebacterium minutissimum	> 6.3
Bacillus cereus	> 6.0
グラム陽性嫌気性菌	
Lactobacillus species	> 6.9
Propionibacterium acnes	> 6.7
Clostridium perfringens	> 7.0
Bifidobacterium adolescentis	> 6.5
原虫	
Trypanosoma cruzi	> 5.3
Plasmodium falciparum	> 7.0
Leishmania Mexicana	> 5.2

データは、Lin[14]、Lin ら[15,16,18]、Van Voorhis ら[19,20]、Dupuis ら[21]、Savoor ら[22]、Sawyer ら[23]をまとめたものである。

*予備的データ：不活化処理は、血小板不含の B19 感染血漿と PAS III（platelet additive solution III）を 35%と 65%の比率で混合したものを用いて実施した。アモトサレン添加から紫外線 A 照射までの間に、15 または 30 分間の静置時間を設けた。

患者、材料および方法

患者

血小板輸血による支持療法を必要とする 6 歳以上の血小板減少症患者を登録適格とした。試験エンドポイントの評価を妨げうる要因を 1 つ以上有する患者は、試験から除外した。これらの除外基準には、1）リンパ球傷害性抗体が陽性（スクリーニング時のパネル反応抗体検査の結果が 20%超）か臨床的不応状態の既往がある場合、2）免疫性または血栓性血小板減少性紫斑病、あるいは溶血性尿毒症症候群の既往がある場合、3）急性前骨髄球性白血病と診断されている場合、4）手術またはソラレン紫外線 A（PUVA）療法を最近受けた場合、5）インターロイキン-11 療法を受けている場合、6）感染性因子不活化処理をした血液製剤に関する他の試験に登録された場合とした。組み入れ基準および除外基準をすべて満たした患者は、PCT 血小板群と対照血小板群に 1：1 の比率でランダムに割り付け、割り付けた血小板濃厚液を用いて、すべての血小板輸血を施行した。輸血期間は 28 日間とし、第 28 日より前に輸血から離脱した場合（血小板輸血を 7 日以上施行しなかった場合）には離脱日までとした。輸血期間の完了時に 7 日間の調査期間を開始し、有害事象をさらにモニターした。本試験は、各施設の治験審査委員会（IRB）が承認し、全患者から参加に関するインフォームド･コンセントを取得した。

患者の臨床ケアおよび評価に携わった担当者は全員、試験の投与割付について盲検化した。盲検化の対象者は、治験責任医師、治験コーディネーター、止血状態を評価した看護師、患者のケアを担当した臨床医および看護師、および治験依頼者であった。治験薬（血小板）のランダム化、採取、処理および供給を担当した血液銀行および輸血業務担当者は、盲検化しなかった。

エンドポイント

主要有効性エンドポイントは、血小板輸血による支持療法期間中にグレード 2 の出血を来した患者の割合とし、評価には世界保健機関（WHO）基準の増補版（表 2）[29] を用いた。副次有効性エンドポイントは、WHO グレード 3 または 4 の出血を来した患者の割合、WHO グレード 2 の出血日数、輸血 1 および 24 時間後の血小板増加数（CI）および補正血小板増加数（CCI）、血小板輸血間隔（日）、血小板輸血回数、血小板輸血不応状態の発生率、赤血球輸血回数とした。安全性エンドポイントは、血小板輸血副作用の発生件数、アモトサレン関連新規抗原、全般的な安全性などとした。

血小板の採取と光増感剤による処理

被験輸血に用いる PCT 血小板、対照血小板とも、白血球除去機能を装備した Amicus Separator（Baxter Healthcare、イリノイ州ラウンドレーク）を用いて、平均血小板輸血投与量の目標値を 3.7×10^{11} 個として採取した。PCT 血小板は、血漿と血小板添加液（Intersol、Baxter Healthcare、イリノイ州ディアフィールド）の混合液（30～45%：55～70%の比率）に浮遊した一方、対照血小板は 100%血漿に浮遊した。光増感剤による処理[15]は、血小板採取後 24 時間以内に各試験施設で実施した。処理手順は以下の通りである。血小板に 150 μM アモトサレンを添加し混合後、照射装置に入れ、一定速度で静かに撹拌しながら紫外線 A を 3 J/cm^2 で 3～5 分間照射した。照射後、血小板を別のプラスチック製バッグに移し、Compund Adsorption Device（CAD）を用いて残留アモトサレンおよび光化学反応による分解生成物の濃度を低下させた。6～8 時間の吸着処理後、PCT 血小板を最終保存バッグに移し、血液銀行の基準に従って最長 5 日間保存した[30]。すべてのドナーおよび血小板製剤には、血液銀行が規定する検査を実施した[30]。PCT 血小板濃厚液と対照血小板濃厚液は、同じ種類のプラスチック製バッグに入れ、同じラベルを貼付して供給した。PCT 血小板は本治験のためのみに製造したため、在庫が不足する場合があった。そのため、PCT 血小板群に PCT 未処理血小板が輸血されたり（「治験実施計画書に適合しない」輸血（〝off-protocol〞 transfusion））、治験実施計画書に適合しない輸血を回避するため、通常では使用されないような少ない投与量の PCT 製剤が輸血される場合があった。Amicus Separator で採取されなかった対照血小板の輸血も、治験実施計画書に適合しない輸血とした。

輸血方法

血小板は、各施設のガイドラインに従って、予防的（出血を予防するため）または治療的（既存の出血を治療するため、あるいは侵襲的手技の前処置として）に輸血した。予防的輸血に最もよく使用された閾値は 10×10^9/L であった。血小板の ABO 型、放射線照射の使用、製剤容量の低減、HLA 適合性または交差適合性を示すドナーを選ぶか否かは、各施設の方針に委ねた。患者への赤血球輸血には、通常の赤血球製剤を用いた。両投与群に輸血された赤血球製剤の 98%以上は白血球除去血であり、99%はγ線照射血であった。

表 2. 主要エンドポイント（止血状態）の評価に用いた WHO 出血スケール　増補版

	出血グレード	
器官系	**1**	**2**
粘膜・皮膚出血		
鼻出血	出血時間が 1 時間未満	出血時間が 1 時間超
口腔咽頭出血	出血時間が 1 時間未満	出血時間が 1 時間超
点状出血／紫斑	皮膚または口腔粘膜局所の点状出血、直径 2.5 cm 未満の紫斑	直径 2.5 cm を超える紫斑、全身性の点状出血または紫斑
胃腸出血		
下血	NA	潜血陽性の黒色便
直腸出血／血便	便潜血陽性だが、血便なし	血便
吐血	NA	嘔吐物または胃内容物に潜血または血液混入
泌尿・生殖器出血		
血尿	< 1+（軽度、微量、少量）血尿	≥ 2+（中等度）血尿
膣からの異常出血	少量の出血：1 日あたり交換パッド数 < 2	1 日あたり交換パッド数 > 2
気管支肺出血	NA	喀血、血痰、気管支肺洗浄液の血液混入
筋骨格系・軟組織出血	NA	特発性血腫、関節出血（関節を問わず）
体腔（胸腔、腹腔、心膜、後腹膜）出血	NA	体液の顕微鏡検査にて赤血球検出
中枢神経系出血	NA	視力障害を伴わない網膜出血
侵襲部位出血	NA	カテーテル、静脈穿刺部位などの侵襲または手術部位周囲の出血

グレード 3 の出血は赤血球輸血を必要とするもので、グレード 3 の体腔出血は体液に肉眼で確認できる血液混入がみられるものとする。グレード 3 の中枢神経系（CNS）出血は、コンピュータ断層撮影（CT）または磁気共鳴画像（MRI）スキャンで検出されるものの臨床的に重要でない出血とする。グレード 4 の出血は、血行動態不安定（低血圧：収縮期または拡張期血圧の 30 mm Hg を超える低下）を伴うか、致死性の出血とする。グレード 4 の筋骨格系出血は、関節に永続的な破壊性変化をもたらすもの、グレード 4 の CNS 出血は、神経症状または徴候を伴う CNS 出血、あるいは視力障害（視野欠損）を伴う網膜出血とする。拡大版のスケールは、Miller らが報告した WHO 出血スケール[29]に基づく。NA は「該当なし」を意味する。

止血状態の評価および臨床検査

出血のおそれがある 8 部位の止血状態は、訓練を受けた担当者が投与割付を盲検化された条件で評価した。各評価時には、出血のおそれがある 8 部位それぞれに、0（出血なし）～4（生命を脅かす出血）の WHO 出血グレード（表 2）を付与した。止血状態の初回評価は、初回血小板輸血前 12 時間以内に行った。その後の止血状態の評価は、最終血小板輸血の 3 日後まで毎日行った。各評価時の総合出血グレードは、8 評価部位で認められたグ

レードの最高値とした。輸血期間中のいずれかの評価時にグレード 2 の出血が 8 評価部位のいずれかに認められた場合、当該患者は主要エンドポイントに達したことになる。例えば、輸血期間の第 3 日に直径 5 cm の斑状出血を認めた場合でも、輸血期間中に他のいかなる出血事象もみられなかった患者については、グレード 2 の出血の発生例に分類し、本試験の主要エンドポイントに達したことと判定される。

ルーチンケアのために日常的に測定した血小板数を、被験輸血前の血小板数として用いた。輸血 1 および 24 時間後の血小板数は、各血小板輸血のそれぞれ 10 分～4 時間後および 10～24 時間後に測定した。本試験への適格性を確認するためのリンパ球傷害性抗体（LCA）検査は、各施設で実施し、（PRA 検査にて）血清が 20%超のパネル細胞と反応した患者は除外した。LCA 検査およびアモトサレンに関連する新規抗原性（アモトサレン関連新規抗体産生）検査用の血漿検体は週 1 回採取した。試験開始時および試験終了時の検体は、主要な検査室で分析した。LCA 検査には標準的な手法 [31] を使用し、アモトサレンに関連する新規抗原性（アモトサレン関連新規抗体産生）検査には妥当性が確認されている酵素結合免疫吸着法（ELISA：Cerus、カリフォルニア州コンコード）[28] を使用した。患者が血小板輸血不応状態になった場合には、中央検査室にて、当該患者の全検体を LCA 検査、アモトサレンに関連する新規抗原性（アモトサレン関連新規抗体産生）検査、ならびに血小板特異的同種抗体の検査 [32,33] に供した。CCI（患者の体格および血小板輸血投与量を反映させた血小板輸血効果の指標）は、輸血後血小板数と輸血前血小板数の差に体表面積（m^2）を乗じた後、輸血された血小板数で除することにより算出した。2 回連続した血小板輸血において輸血 1 時間後の CCI が 5×10^3 個を下回った患者は、臨床的不応状態と判断した。免疫学的不応状態とは、臨床的不応状態（CCI が 2 回連続で 5×10^3 個を下回った場合）で、次のいずれかが認められた場合と定義した：LCA（PRA 検査における反応率が 20%を超えた場合）、血小板同種抗体、アモトサレン関連新規抗原（アモトサレン関連新規抗体産生）。

有害事象および輸血副作用

有害事象の収集は、初回被験輸血開始時から 7 日間の調査期間終了時まで行った。有害事象および輸血副作用の重症度は、各患者に認められた最も重度の症状または徴候について判定した。治験薬による血小板輸血に対する反応は、各輸血後 6 時間にわたり評価した。

ランダム化および統計手法

グレード 2 の出血に関して PCT 血小板群が対照群より劣性であるという帰無仮説を、検出力が 90%を超え、有意水準 0.05 で棄却するために必要な症例数は、試験開始前には 1 群 300 例と推定された。治験薬による血小板輸血を 1 回以上受けた全患者を解析対象例とした。ランダム化は試験施設により層別化した。

本試験は非劣性試験として計画した。主要エンドポイント（グレード 2 の出血を来した患者の割合）および副次エンドポイントの 1 つ（グレード 3 または 4 の出血を来した患者の割合）に関する投与群間差は、事前に規定した非劣性限界値（それぞれ 12.5%および 7%）を用いた非劣性検定（片側検定）により解析した。その他の副次エンドポイントはすべて、投与群間差を解析した。主要エンドポイントについては、検定統計量は$(P_T - P_R - 0.125)/(\mathrm{Var}[P_T - P_R])^{1/2}$とした［ただし、$P_T$は PCT 血小板群におけるグレード 2 の出血を来した患者の割合、P_Rは対照群におけるグレード 2 の出血を来した患者の割合、$\mathrm{Var}(P_T - P_R)$は最尤（さいゆう）推定法により推定した分散である］[34]。患者の割合に関する投与群間差の片側 95%信頼区間の算出にも、同じ分散推定値を用いた。

連続変数の解析には、投与および試験施設を組み込んだモデルの分散分析を用いた。有害事象の比較には、Fisher の直接法を用いた。グレード 2 の出血が発生するまでの期間は、ログランク検定により比較した。血小板輸血投与量に関する血小板増加数および輸血間隔の補正には、継時的回帰分析を用いた[35,36]。非劣性検定を除くすべての統計検定とも両側検定とし、有意水準は 0.05 とした。

表 3.　患者の特性

	PCT 群、318 例	対照群、327 例
性別		
男性の比率、%	54	51
年齢、歳		
平均	47	46
範囲	7-85	6-75
16 歳未満の比率、%	2	5
民族的要因、%		
白人	91	91
アフリカ系米国人	3	3
ヒスパニック系	3	3
その他	3	3
幹細胞移植、%		
骨髄	20	22
末梢血	54	55
臍帯血	2	3
合計	76	80
幹細胞の由来、%		
自家	64	65
同種	36	35
診断された基礎疾患、%		
急性白血病	29	28
慢性白血病	11	11
リンパ腫	24	29
骨髄異形成	3	2
形質細胞疾患	20	18
非造血組織の固形腫瘍	8	8
その他	5	4
試験登録時にWHOグレード2の出血を来していた患者の割合、%	15.7	16.5

いずれの特性に関しても $P > 0.05$ であったことから、群間差は認められなかった。

表 4.　試験の進展状況

	投与群		
	PCT 群、例数 (%) 318 例	対照群、例数 (%) 327 例	*P*値
輸血期間完了例	280 (88)	294 (90)	.53
輸血期間が完了されなかった理由			
患者による中止決定	8 (2.5)	4 (1.2)	.26
医師による中止	10 (3.1)	4 (1.2)	.11
有害事象	0 (0)	0 (0)	—
追跡不能	1 (< 1)	1 (< 1)	—
死亡	10 (3.1)	15 (4.6)	.42
その他	9 (2.8)	9 (2.8)	—
合計	38 (11.9)	33 (10.1)	.44
血小板輸血による支持療法の平均日数	11.8	10.6	.08
第 28 日より前に血小板輸血からの離脱が達成・維持された患者	210 (66)	230 (70)	.27
調査期間の完了例	248 (78)	273 (84)	.09

— 該当なしを意味する。

表 5.　グレード 2 以上の出血を来した患者の割合

	PCT 群、例数 (%) 318 例	対照群、例数 (%) 327 例	*P*値*
グレード2のすべての出血	186 (58.5)	188 (57.5)	<.01†
出血部位別のグレード2の出血			
泌尿・生殖器出血	104 (32.7)	103 (31.5)	0.80
粘膜・皮膚出血	82 (25.8)	65 (19.9)	0.08
侵襲部位出血	69 (21.7)	65 (19.9)	0.63
胃腸出血	60 (18.9)	63 (19.3)	0.92
呼吸器出血	35 (11.0)	28 (8.6)	0.35
筋骨格系出血	15 (4.7)	18 (5.5)	0.72
体腔出血	0 (0.0)	1 (0.3)	1.00
神経系出血	0 (0.0)	0 (0.0)	—
グレード3または4のすべての出血	13 (4.1)	20 (6.1)	<.01‡

— 該当なしを意味する。
*出血のおそれがある 8 部位別の *P*値は、Fisher の直接法を用いて算出した。
†グレード 2 の出血を来した全患者の割合に関して、非劣性限界値 0.125 として非劣性検定を行ったところ、*P* < 0.01 であった（群間差の片側 95%信頼区間：-1、0.07）。この手法により求めた *P*値が 0.05 未満であることは、PCT が対照と比較して非劣性であることを意味する。
‡グレード 3 または 4 の出血を来した全患者の割合に関して、非劣性限界値 0.07 として非劣性検定を行ったところ、*P* < 0.01 であった（群間差の片側 95%信頼区間：-1、0.013）。この手法により求めた *P*値が 0.05 未満であることは、PCT が対照と比較して非劣性であることを意味する。

結果

ランダム割付例 671 例のうち、治験薬による血小板輸血を 1 回以上受け、intention-to-treat（ITT）集団を構成した患者は 645 例（PCT 群 318 例、対照群 327 例）

であった。ITT 解析の対象外とされた 26 例は、血小板減少症の回復前に血小板輸血を必要としなかった患者であった。性別、年齢、民族的要因、診断、幹細胞移植施行の有無（表 3)、あるいは試験開始時の血液学的検査値、臨床生化学的検査値および凝固検査値（データは示さず）には、群間差は認められなかった。

輸血期間および調査期間を完了した患者の割合（患者全体でそれぞれ 89%および 81%)、血小板輸血による支持療法の平均期間（PCT 群 11.8 日 vs. 対照群 10.6 日)、第 28 日より前に血小板輸血からの離脱が達成･維持された患者の割合（PCT 群 66% vs. 対照群 70%）には、投与群間差は認められなかった（表 4)。

本試験の主要エンドポイント（グレード 2 の出血を来した患者の割合）には、出血のおそれがある 8 部位の合計割合、部位別割合を問わず、PCT 群と対照群の間に差はみられなかった（表 5)。輸血期間中にグレード 2 の出血を来した患者の割合は、PCT 群 58.5%に対し対照群 57.5%であった。試験開始からグレード 2 の出血が発生するまでの期間に関しては、ITT 集団を解析対象とした場合にも（図 1A、P = 0.78)、試験登録時にグレード 2 の出血を来していなかった患者を解析対象とした場合にも（図 1B、P= 0.91)、PCT 群と対照群の間に有意差はみられなかった。グレード 2 の出血が発生した平均日数は、PCT 群 3.2 日に対し対照群 2.5 日で（P= 0.02)、中央値は両群とも 1 日であった。

出血のおそれがある部位に発生した出血の最高グレードは、ほとんどの患者がグレード 2 であった。グレード 3 または 4 の出血を来した患者の割合は、PCT 群 4.1%、対照群 6.1%にすぎなかった。グレード 3 または 4 の出血を来した患者の割合に関しては、出血のおそれがある 8 部位の合計割合、部位別割合を問わず、統計学的に有意な群間差は認められなかった。グレード 3 または 4 の出血が最も高頻度に発生した部位は神経系であった［PCT 群 318 例中 3 例（0.9%）vs. 対照群 327 例中 6 例（1.8%)］。

本試験期間中、患者 645 例に対し計 4,719 回の血小板輸血が施行された（PCT 群 2,678 回、対照群 2,041 回）（表 6)。輸血された血小板単位の大半（PCT 群 91.5%、対照群 95.2%）は、本試験の方法に従って調製された（「治験実施計画書に適合する輸血」（〝on-protocol transfusion〞))。本試験の輸血期間中に治験実施計画書に適合する輸血のみを受けた患者

の割合は、PCT 群 68%、対照群 85%であった（$P < 0.01$）。治験実施計画書に適合しない輸血を受けた患者の大半（PCT 群 53%、対照群 59%）において、実施計画書に適合しない輸血回数は 1 回のみであった。輸血された血小板の比率は、HLA 適合血小板 1.5%、交差適合血小板 0.2%、既定量以下の血小板 7.5%、放射線照射血小板は 99.8%であり、この比率に群間差はみられなかった。（患者の移植前の血液型と）ABO 型が適合する輸血を受けた患者の割合は、PCT 群が対照群よりわずかに高かった（78.5% vs. 75.4%、$P = 0.01$）。輸血時の血小板の平均保存期間は、PCT 群 3.4 日に対し、対照群 3.6 日であった（$P < 0.01$）。

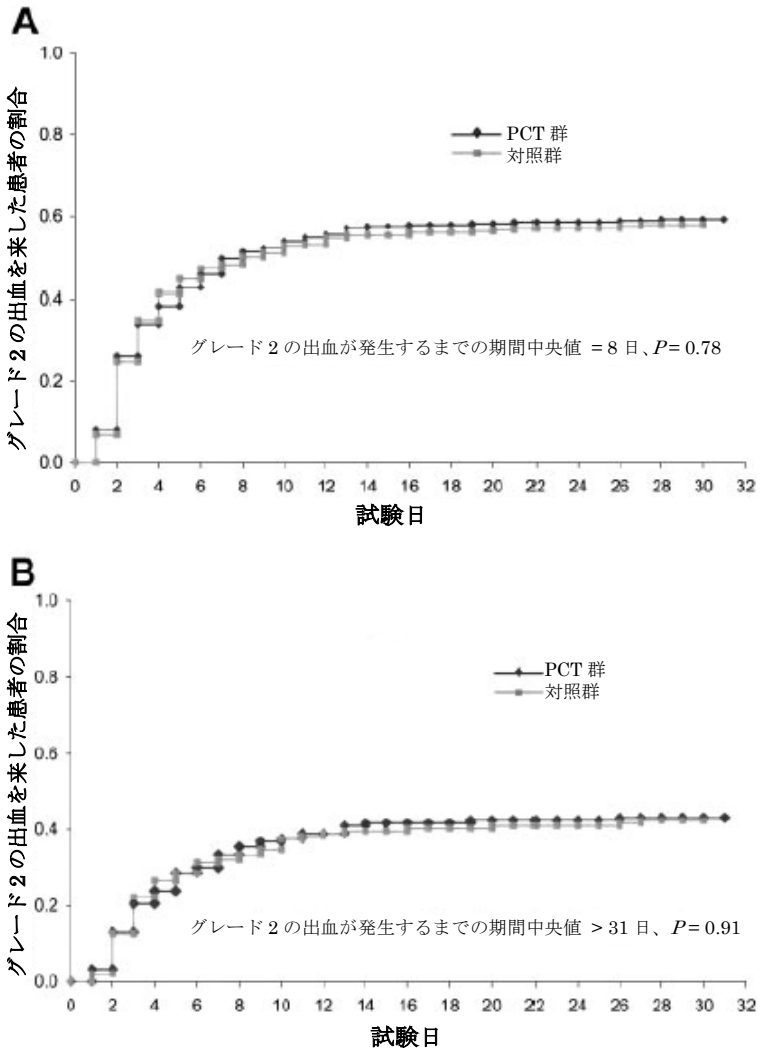

図 1.　グレード 2 の出血が出現するまでの期間

（A）ITT 集団（645 例）におけるグレード 2 の出血が出現するまでの期間。グレード 2 の出血が出現するまでの期間中央値は 8 日であった（$P = 0.78$、ログランク検定）。（B）試験開始時に出血を来していなかった（グレード 0）患者（541 例）におけるグレード 2 の出血

が発生するまでの期間。グレード 2 の出血が出現するまでの期間中央値は 31 日超であった（*P* = 0.91、ログランク検定）。

表 6.　試験期間中の血小板輸血および赤血球輸血

	PCT 群、318 例	対照群、327 例	*P* 値
血小板輸血			
合計回数	2678	2041	—
1 例あたり平均回数	8.4	6.2	< .001
血小板輸血による支持療法期間1日あたりの平均回数*	0.74	0.65	< .001
輸血間隔、日	1.9	2.4	< .001
血小板輸血投与量、× 10^{11}			
1回あたり平均血小板輸血投与量の群平均	3.7	4.0	< .001
血小板輸血投与量が 3.0 × 10^{11} 個未満であった輸血の割合、%	20	12	< .01
全輸血期間にわたる血小板輸血総投与量の群平均	29.4	24.1	.01
血小板保存期間、日	3.4	3.6	< .05
赤血球輸血			
1 例あたり平均回数	4.8	4.3	.13
血小板輸血による支持療法期間 1 日あたりの平均回数*	0.31	0.30	.53

— 該当なしを意味する。
*血小板輸血による支持療法期間とは、治験薬による血小板輸血の初回施行日から最終施行日までの日数と定義する。

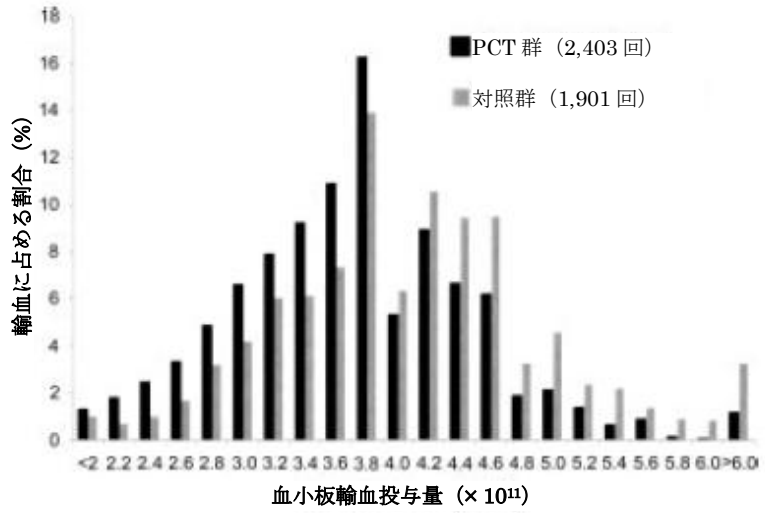

図 2.　血小板輸血投与量の分布。血小板輸血投与量が 3.0 × 10^{11} 個未満であった輸血の割合は、PCT 群が対照群と比較して高かった（*P* < 0.01）。

PCT 群は、血小板輸血の合計回数（PCT 群 8.4 回 vs. 対照群 6.2 回、$P < 0.001$、表 6）および血小板輸血による支持療法期間1日あたりの血小板輸血回数（PCT 群 0.74 回 vs. 対照群 0.65 回、$P < 0.001$）が、対照群と比較して多かった。これらの群間差は、PCT 群の輸血 1 回あたり平均血小板輸血投与量が対照群より少なかったこと（PCT 群 3.7×10^{11} 個 vs. 対照群 4.0×10^{11} 個、$P < 0.001$）、また、血小板輸血投与量が 3.0×10^{11} 個未満であった輸血の割合が対照群より高かったこと（PCT 群 20% vs. 対照群 12%、$P < 0.01$、図 2）により、ある程度説明がつく。血小板輸血投与量 3.0×10^{11} 個未満の輸血を 1 回以上受けた患者の割合は、PCT 群 60%に対し、対照群 36%であった（$P < 0.01$）。だが、継時的線形回帰モデルを用いて血小板輸血投与量を補正したところ、PCT 群と対照群の血小板輸血投与量が等しい条件でも、PCT 群では、輸血 1 時間後の血小板数は対照群より 10.4×10^9 個/L 少ないと推定され（$P < 0.001$）、輸血間隔は対照群より 0.4 日短かった（$P < 0.001$）。血小板回収率に影響を及ぼしうるその他の要因（脾腫、発熱、敗血症、アムホテリシン使用など）[37] には、投与群間差はみられなかった。赤血球輸血の平均回数および血小板輸血による支持療法期間 1 日あたりの平均赤血球輸血回数には、群間差はみられなかった（表 6）。

大半の輸血は予防的輸血であり（PCT 群 93.5% vs. 対照群 90.1%、$P < 0.01$）、その他の輸血は出血の治療または侵襲的手技の前処置としての治療的輸血とみなされた。輸血前の平均血小板数には群間差はみられなかったが、輸血 1 時間後の平均血小板数は PCT 群の方が少なく（PCT 群 36.5×10^9/L vs. 対照群 49.5×10^9/L、$P < 0.001$）、輸血 1 時間後および 24 時間後の平均 CI および平均 CCI も PCT 群の方が少なかった（表 7）。

表 7.　血小板輸血後の血小板輸血効果の平均値

	PCT 群、318 例	対照群、327 例
輸血前		
血小板数、× 10^9/L	15.1	15.2
輸血1時間後		
血小板数、× 10^9/L	36.5*	49.5
血小板増加数、× 10^9/L	21.4*	34.1
補正血小板増加数、× 10^3	11.1*	16.0
輸血24時間後		
血小板数、× 10^9/L	27.9*	36.1
血小板増加数、× 10^9/L	13.2*	21.5
補正血小板増加数、× 10^3	6.7*	10.1

*P < 0.001、対照群との比較

臨床的な血小板輸血不応状態を来した患者の割合は、PCT 群 21.4%に対し対照群 7.0%であった（P < 0.001、表 8)。輸血 1 時間後の CCI が 5 × 10^3 個を下回った輸血が全輸血回数に占める割合は、PCT 群および対照群でそれぞれ 27.4%および 12.7%であり（P < 0.001)、血小板輸血投与量が 3.0 × 10^{11} 個未満であった輸血が全輸血回数に占める割合は、PCT 群 33.4%に対し対照群 12.3%であった（P< 0.001)。大半の不応エピソードは一過性で、輸血 1 時間後の CCI が 5 × 10^3 個を下回った輸血の連続回数は 2 回にとどまった(PCT 群 57% vs. 対照群 65%)。試験完了時まで不応状態が持続した患者の割合は、PCT 群 6%、対照群 9%にすぎなかった。血小板輸血不応状態の基準とした、HLA 抗体、血小板抗原、あるいはアモトサレンに関連する新規抗原性が認められた患者の割合は、ITT 集団では PCT 群 4.7%に対し対照群 3.1%（P= 0.31)、不応状態を来した患者では PCT 群 22%に対し対照群 44%であった（P = 0.06)。不応状態を来した患者のうち、LCA が検出された患者の割合は、対照群（39%）が PCT 群（15%）より高く（P = 0.02)、血小板に対する同種抗体が産生された患者の割合には、群間差はみられなかった (PCT 群 12%に対し対照群 10%、P= 1.00)。

表 8.　血小板輸血不応状態

	PCT 群	対照群	*P*値
ITT集団、例数	318	327	—
不応状態を来した患者の割合、%*	21.4	7.0	< .001
CCI が 5×10^3 個を下回った輸血の割合、%	27.4	12.7	< .001
不応状態を来した患者、例数	68	23	—
不応エピソードが1回のみ発生した患者の割合、%	57	65	.63
試験終了時まで不応状態であった患者の割合、%	6	9	.64
免疫学的不応状態†			
LCAおよび／または血小板に対する同種抗体が産生された患者の割合、%	22	44	.06
リンパ球傷害性抗体が産生された患者の割合、%	15	39	.02
血小板同種抗体が産生された患者の割合、%	12	10	1.00
アモトサレンに関連する新規抗原性（アモトサレン関連新規抗体産生）が発現した患者の割合	0	0	—

— 該当なしを意味する。
*不応エピソードとは、連続 2 回の輸血で輸血 1 時間後の CCI が 5×10^3 個を下回った場合と定義する。
† 免疫学的不応状態とは、連続 2 回の輸血で輸血 1 時間後の CCI が 5×10^3 個を下回り、なおかつ LCA（PRA 検査における反応率が 20%超であった場合）、血小板に対する同種抗体、および／またはアモトサレンに関連する新規抗原性（アモトサレン関連新規抗体産生）が認められた場合と定義する。

輸血副作用が発生した血小板輸血の割合は、PCT 群が対照群と比較して低かったが（PCT 群 3.0% vs. 対照群 4.4%、$P = 0.02$）、輸血副作用を来した患者の割合には差はみられなかった（PCT 群 16.0% vs. 対照群 19.3%、$P = 0.30$）。輸血副作用の大半は、発熱、悪寒、蕁麻疹または発疹であった。ほぼすべての患者に 1 件以上の有害事象が発生した（表 9）。有害事象は 898 個の MedDRA 基本語にコード化された[39]。特に高頻度に発生した有害事象（1 群以上で 30%を超える患者に報告された事象）は、血尿、下痢、低カリウム血症、悪寒、点状出血、鼻出血、便潜血、挫傷（打撲傷）、皮膚炎などで、本試験に登録した患者集団で予測される有害事象に一致した。多数の統計比較を実施したところ、予想どおり、ある種の有害事象に関して統計学的に有意な群間差が認められたが、これらの差は臨床的に重要とは判断されなかった。詳細は別報に示す予定である。グレード 3 または 4 の有害事象のうち、治験責任医師が治験薬の血小板輸血と「関連性が強い」または「関連性が示唆される」と判定した事象、ならびに米国食品医薬品局（FDA）の重篤度に関する基

準に適合する有害事象に関しては、PCT 群と対照群の間に差はみられなかった（表 9)。試験期間中に 28 例が死亡した（PCT 群 3.5% vs. 対照群 5.2%)。死因の大半は感染あるいは呼吸器疾患の併発であった。

表 9. 試験期間中に発生した有害事象

	PCT 群、% 318 例	対照群、% 327 例	*P* 値
全有害事象*	99.7	98.2	.12
グレード III または IV の有害事象	78.9	78.6	.92
重篤な有害事象†	27.0	24.8	.53
投与に関連する有害事象‡	26.4	29.4	.43
死亡§	3.5	5.2	.34

*有害事象は、米国国立癌研究所の共通毒性基準（NCI-CTC）[38] を用いて I～IV にグレード化し、医薬規制用語集（MedDRA）[39] を用いて基本語にコード化した。

† 重篤な有害事象は、米国食品医薬品局（FDA）の基準 [40] により定義した。

‡ 投与に関連する有害事象は、各施設の治験責任医師が盲検下にて治験薬による血小板輸血に「関連性が示唆される」または「関連性が強い」と報告した事象である。

§ 各群 1 例が出血により死亡した。両死亡例とも肺胞出血を来しており、移植前処置に用いた骨髄除去療法の毒性が原因と考えられた。

考察

米国における輸血用血液の安全性は向上している。だが、輸血に伴うリスクを可能な限りゼロに近づけることが社会的に求められており、政治判断および健康政策の決定にはこの目標が反映されている。輸血により伝播する感染性因子が新たに同定される度に、これらの因子に対する検査法を新たに導入するという方策もあるが、このアプローチには必ず限界がある。血液製剤に含まれる多種類のウイルス、細菌および原虫を対象とする不活化技術は、血液の安全性を改善する新たな方法として有望である。

感染性因子が血液成分製剤に混在する可能性は低いため、PCT 血小板による輸血感染症の予防効果を確認することはできない。そのため、我々は PCT の輸血感染症に対する予防効果でなく、血小板輸血の止血効果に対する影響について検討した。本試験は、この種の試験としては最も大規模な試験であり、血小板の止血効果を主要エンドポイントとして評価すると同時に、PCT 血小板の品質および安全性も評価を行った。PCT 血小板と対照血小板の止血効果は、出血のおそれがある 8 部位全体、部位別を問わず同等であったことから、PCT 血小板が臨床的に有効であることが確認された。PCT 血小板群では、従来の

アフェレーシス血小板群と比較して、輸血後の血小板増加数が少なく、血小板輸血回数が多かったほか、輸血間隔も短縮された。輸血後の血小板増加数が相対的に少なかったことは、PCT 群の平均血小板輸血投与量が対照群より少なかったこと、また、血小板輸血投与量が 3.0×10^{11} 個未満であった輸血回数が相対的に多かったことにより、一応の説明がつく（図 2)。PCT 群の血小板輸血回数が対照群より多かった原因は、血小板輸血投与量が少なかった血小板製剤の輸血の割合が対照群より高かったためと考えられる [41]。PCT 群に対する血小板輸血投与量が対照群より少なかった理由には、主に臨床試験の条件が関係していた。これらの理由には、処理セットとプロトタイプの CAD が一体化されていない試作モデルが使用されたこと、PCT 処理による血小板減少が認められていること、アモトサレン分析用のサンプルを PCT 血小板から採取したが対照血小板からは採取しなかったこと、治験実施計画書に適合しない輸血を避けるため、高単位の血小板を使用できない場合には少ない投与量の PCT 血小板が輸血されたこと、PCT 血小板は本臨床試験のためのみに製造したことから、対照血小板の方が入手しやすく、結果として血小板輸血投与量が相対的に多かったことなどが挙げられる。ルーチンの輸血に使用される際には、PCT 血小板の輸血投与量は対照血小板と同等になると予想される。本試験完了後、PCT 処理セットと改良型 CAD の一体モデルが開発され、欧州における小規模な補足的試験で評価された。患者 43 例を対象とした同試験では、患者管理に必要な血小板輸血回数は輸血開始 28 日目まで PCT 血小板群で相対的に多くはないことが明らかになったが [42]、この結果はより大規模な試験で確認する必要があると考えられる。

PCT 血小板輸血後の血小板の応答が相対的に小さかった説明となるもう一つの要因は、血小板生存率の低下であった。すなわち、血小板輸血量が等しい場合でも、PCT 群では、対照群と比較して血小板増加数が有意に少なく、輸血間隔が有意に短かった。PCT 処理が血小板生存率に影響を与えることは、健常被験者および患者を対象とした過去の試験において示唆されている [26,27]。PCT 群では、血小板増加数が相対的に少なかった結果として、臨床的な血小板輸血不応状態の発生率が相対的に高かった。しかし、一過性を示す傾向にあり、不応状態が試験終了時まで持続した患者の割合は、PCT 群 6%、対照群 9%にすぎなかった。同種免疫による血小板輸血不応状態が発生した場合に、HLA 適合ドナー由来の血小板輸血が必要となることはまれであり、その頻度に群間差はみられなかった。血小板輸血不応状態の発生患者における LCA 出現率は、PCT 群の方が低かったが、血小板特異

同種抗体の出現率に群間差はみられなかった。PCT 群では、対照群と比較して血小板増加数が少なく、血小板輸血間隔が短く、結果として血小板輸血回数が多かったものの、止血効果は対照群と等しかった。そのため、これらの副次エンドポイントの差は、血小板製剤の輸血効果および患者の治療効果にほとんど影響を及ぼさないと考えられる。

全体として PCT 血小板輸血に伴い、従来の血小板輸血とは異なる毒性または有害事象は認められなかった。本試験の安全性解析は別報に示す予定である。輸血副作用を来した患者の割合に群間差はみられなかったが、輸血副作用を来した輸血回数は、PCT 血小板の方が少なかった。この原因は、PCT 血小板では、白血球不活化により PCT 血小板保存期間中のサイトカイン産生量が少なかったこと、あるいは血小板再浮遊に使用した血小板単位数あたりの血漿容量が少なかったことにあると考えられた[43]。その他の有害事象（出血性の有害事象および死亡を含む）には、群間差はみられなかった。

光増感剤によって処理された血小板は、臨床的な止血維持効果を有し、安全性プロファイルが許容できると考えられるともに、輸血による感染リスク（新興輸血感染症の関連リスクを含む）をさらに低減する可能性がある。

Clinical effectiveness of leucoreduced, pooled donor platelet concentrates, stored in plasma or additive solution with and without pathogen reduction

Jean-Louis H. Kerkhoffs,[1,2] Wim L. J. van Putten,[3] Viera M. J. Novotny,[4] Peter A.W. Te Boekhorst,[5] Martin R. Schipperus,[2] Jaap Jan Zwaginga,[6] Lizzy C. M. van Pampus,[4] Georgine E. de Greef,[5] Marleen Luten,[3] Peter C. Huijgens,[7] Anneke Brand[1,6] and Dick J. van Rhenen[1] on behalf of the Dutch – Belgian HOVON cooperative group

[1]Sanquin Blood Bank, Southwest Region, Rotterdam, [2]Haga Teaching Hospital, The Hague, [3]Erasmus University Medical Centre, HOVON Data Centre, Rotterdam, [4]UMC St Radboud, Nijmegen, [5]Erasmus University Medical Centre, Rotterdam, [6]Leiden University Medical Centre, Leiden, and [7]VU Medical Centre, Amsterdam, The Netherlands

Received 26 January 2010; accepted for publication 6 April 2010

Correspondence: Jean-Louis H. Kerkhoffs, Sanquin Southwest Region, Plesmanlaan 1a, 2333 BZ Leiden, The Netherlands.
E-mail: j.kerkhoffs@sanquin.nl

http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=861 http://www.controlled-trials.com/ISRCTN88278819/HOVON+82

Summary

Pathogen reduction (PR) of platelet products increases costs and available clinical studies are equivocal with respect to clinical and haemostatic effectiveness. We conducted a multicentre, open-label, randomized, non-inferiority trial comparing the clinical effectiveness of buffy-coat derived leucoreduced platelet concentrates (PC) stored for up to 7 d in plasma with platelets stored in platelet additive solution III (PASIII) without and with treatment with amotosalen-HCl/ultraviolet-A (UVA) photochemical pathogen reduction (PR-PASIII). Primary endpoint of the study was 1-h corrected count increment (CCI). Secondary endpoints were 24-h CCI, bleeding, transfusion requirement of red cells and PC, platelet transfusion interval and adverse transfusion reactions. Compared to plasma-PC, in the intention to treat analysis of 278 evaluable patients the mean difference for the 1-h CCI of PR-PASIII-PC and PASIII-PC was −31% ($P < 0{\cdot}0001$) and −9% (P = n.s.), respectively. Twenty-seven patients (32%) had bleeding events in the PR-PASIII arm, as compared to 19 (19%) in the plasma arm and 14 (15%) in the PASIII arm ($P = 0{\cdot}034$). Despite the potential advantages of pathogen (and leucocyte) inactivation of amotosalen-HCl/UVA-treated platelet products, their clinical efficacy is inferior to platelets stored in plasma, warranting a critical reappraisal of employing this technique for clinical use.

Keywords: platelet, buffy-coat, amotosalen/UVA pathogen reduction, efficacy.

For the generally accepted indications for treatment and prevention of bleeding, millions of platelet products are transfused annually, warranting vigilance towards emerging logistical problems and safety issues (Slichter, 2007; Stroncek & Rebulla, 2007). Donor counselling and screening, including molecular techniques, have reduced the risk of transmission of hepatitis B, hepatitis C, human immunodeficiency virus, human T-cell lymphotropic virus (HTLV) type I and –II. However, despite the bacterial culture of platelet products, a risk of 1 in 25 000 platelet transfusions for transfusion-related sepsis still remains (Goodnough *et al*, 1999; Kuehnert *et al*, 2001; Dodd *et al*, 2002; Blajchman *et al*, 2005; Schrezenmeier *et al*, 2007). The availability of platelets and reduction of costs due to reduced outdating would benefit from extending the storage time of platelet products, which is hampered mainly by the risk of bacterial growth beyond 5 d of storage (Lee *et al*, 2003). Pathogen reduction (PR) has been shown to be very effective for the inactivation of several viruses and bacteria (Lin *et al*, 2004, 2005). Moreover, PR might also comprise a solution for emerging pathogens, cytomegalovirus and an alternative for γ-irradiation for the prevention of graft-*versus*-host-disease (Grass *et al*, 1999; Lin, 2001). Several countries have considered implementing PR as a standard for all platelet products, but concerns still exist with regard to clinical efficacy and potential long-term toxicity as well as uncertainty as to whether PR- platelet products can be stored for longer than 5 d (Council of Europe expert committee in blood transfusion study group on pathogen inactivation of labile blood products, 2001; Simonsen *et al*, 2006). Although PR platelet products using amotosalen-HCl and ultraviolet-A (UVA) fulfil standard

First published online 9 May 2010
doi:10.1111/j.1365-2141.2010.08227.x

release criteria up to 7 d of storage, this treatment results in considerable metabolic deterioration, increased platelet activation during storage and inconsistent findings by *in vitro* haemostatic assessment (Van Rhenen *et al*, 2000; Janetzko *et al*, 2004; Jansen *et al*, 2004; Picker *et al*, 2004; Apelseth *et al*, 2007; Lozano *et al*, 2007; van der Meer *et al*, 2009). Nevertheless, transfusion in thrombocytopenic patients corrected prolonged bleeding times (Slichter *et al*, 2006). Radiolabeled, autologous amotosalen-HCl/UVA-treated platelets stored for 5 d showed a significant lower recovery and reduction in survival time as compared to platelets stored in platelet additive solution III (PASIII) (Snyder *et al*, 2004). Three randomized controlled trials have been performed using amotosalen-HCl/UVA-treated platelet concentrates (PC) (van Rhenen *et al*, 2003; McCullough *et al*, 2004; Janetzko *et al*, 2005). In the S-59 Platelet Recovery in Thrombocytopenia (SPRINT) trial (645 patients), that used aphaeresis PC stored in plasma as control, significantly lower post transfusion platelet increments were found, combined with a reduced transfusion interval and an increased rate of transfusion failure (McCullough *et al*, 2004). The S-59 Platelet Recovery in Thrombocytopenia in Europe (EuroSPRITE) trial (103 patients) reported no significant differences with regard to transfusion efficacy, however the control arm of this study used buffy-coat derived platelets stored in plasma as well as in additive solution (PASII) for approximately half of the transfusions (van Rhenen *et al*, 2003). In a previous randomized clinical trial (RCT) we showed that PASII PC have a 20% lower corrected count increment (CCI) as compared to plasma PC, which might mask a relevant difference (Kerkhoffs *et al*, 2006). A third small trial with 43 patients showed a borderline significant reduction in transfusion efficacy (Janetzko *et al*, 2005). None of these trials reported inferior haemostatic efficacy. Before implementing PR platelet products, extension of the storage time to 7 d while maintaining clinical efficacy is an important aspect to compensate for the additional costs of the procedure. We performed a multicentre open-label, randomized clinical trial to study the clinical efficacy, in terms of transfusion response, of pooled, random donor PC stored for up to 7 d in platelet additive solution (Intersol, Fenwal, Inc., Lake Zurich, IL, USA) without additional PR (PASIII) and with amotosalen-HCl/UVA photochemical PR (PR-PAS-III, Intercept Blood System, Cerus Corporation, Concord, CA, USA), compared to platelets stored in plasma.

Methods

Study design

The study was designed as a prospective, randomized open-label non-inferiority trial in haemato-oncological patients with thrombocytopenia or expected to be thrombocytopenic caused by myelosuppression. Patients were recruited from the haematology wards of eight Dutch hospitals. The study protocol and consent forms were approved both by a central ethics committee as well as local institutional review boards. The study was conducted according to the International Conference on Harmonization/World Health Organization (WHO) Good Clinical Practice (ICH-GCP) guidelines and the declaration of Helsinki. During the study all centres were audited and trial conduct was monitored by an independent organization. All adult patients (aged >18 years) with a haemato-oncological disease were eligible for inclusion if they were expected to receive 2 or more platelet transfusions. Exclusion criteria were immunological refractoriness to random platelet transfusions due to human leucocyte antigen (HLA)- and/or human platelet antigen (HPA)-antibodies or clinical relevant auto-antibodies, pregnancy (or lactating) and previous inclusion in this study. After informed consent eligible patients were registered and randomized, stratified by centre, before platelet transfusions were started in a 1:1:1 ratio to receive per protocol up to a maximum of five platelet transfusions with Plasma-PC, PASIII-PC or PR-PASIII-PC in a period of maximal 42 d. Off protocol platelet transfusions were allowed during the study period in case of non-availability of the correct component. Apart from normal completion, reasons to go off study were refusal to continue by the patient or treating physician, intercurrent death and immunological refractoriness.

Platelet products, transfusions and monitoring

All products were produced by the Sanquin Blood Bank. PCs were prepared from five pooled whole-blood buffy-coats (BC) with the same ABO-blood group using standard procedures and with regard to pathogen reduction using manufacturer's instructions (van Rhenen *et al*, 2003; Kerkhoffs *et al*, 2006). Samples were obtained prior to storage to measure platelet content. Samples of all products were cultured for 7 d using the BacT/Alert culturing system (BioMerieux, Boxtel, the Netherlands). All products were stored with gentle agitation at 20–24°C for up to 7 d. The PCs were γ-irradiated if requested by the hospital.

Indications for platelet transfusions were divided into platelet count-based prophylaxis, intervention-related prophylaxis and treatment of bleeding. Generally accepted guidelines were used for the indication of platelet transfusions. The requirement for, and timing of platelet transfusion(s) was determined by the treating physician. In summary, in stable, non-bleeding patients a platelet transfusion was advised to maintain the platelet count $\geq 10 \times 10^9$/l and $\geq 40 \times 10^9$/l when these patients received anti-coagulant therapy or treatment with anti-thymocyte globulin. A transfusion trigger platelet count of 40×10^9/l was recommended in endoscopic evaluation of the gastrointestinal or respiratory tract, when no biopsies were performed, diagnostic pleural or peritoneal puncture with a thin needle, lumbar puncture, extraction of a central venous catheter and minor surgical interventions. A trigger platelet count of 60×10^9/l was recommended in case of bleeding, endoscopic evaluation with biopsies, dental

extractions, placement of a central venous catheter and major surgical interventions, with the exception of neurosurgery and cardiac surgery. In case of cerebral bleeding, diffuse alveolar haemorrhage, neurosurgery and cardiac surgery, a trigger of 100×10^9/l was recommended. A pretransfusion platelet count was preferably measured just before transfusion up till a maximum of 6 h before transfusion. A 1-h post-transfusion platelet count was measured between 10 and 120 min after transfusion and a 24-h post-transfusion platelet count was measured between 16 and 28 h after transfusion. The CCI was calculated as follows: $CCI_{1/24\ h} = [(\text{post-transfusion platelet count}_{1/24\ h} - \text{pre-transfusion platelet count } (\times 10^9/l)) \times \text{body surface area } (m^2)]/\text{platelet dose} \times 10^{11}$. Transfusions given shortly after one another without platelet counts between the transfusions were considered to be multi-dose transfusions and analysed as a single transfusion. If available, ABO-identical PC were used, although minor- and major incompatible PC were not excluded. Platelet transfusion failure was defined as a 1-h CCI below 7·5 and/or and 24-h CCI below 4·5 (Kerkhoffs *et al*, 2006). Immunological refractoriness was defined as the occurrence of transfusion failure of two consecutive ABO-matched random platelet transfusions combined with the existence of HLA- and/or HPA-alloantibodies.

Study endpoints

The primary endpoint was the 1-h CCI. Secondary endpoints were 24-h CCI, bleeding, the transfusion requirement of red cells and PCs, platelet transfusion interval and adverse transfusion reactions. The following characteristics were recorded at entry: gender, age, blood group, haematological disease and treatment phase, WHO performance status, existence of enlarged spleen, transfusion history, treatment with anti-coagulation, medical history, medication, bleeding and presence of active infection. The following characteristics were recorded at each transfusion: the reason of the transfusion (trigger platelet count, bleeding or intervention), the blood group of the PC, presence of fever, presence of infection (graded according to the Common Toxicity Criteria for Adverse Events, CTCAE, Version 3; http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf), presence of mucosal damage, and use of acetaminophen, steroids or antihistamines. Patients were evaluated daily by trained personnel to observe, describe and grade bleeding complications at eight defined sites according to the CTCAE under supervision of the local investigator (http://ctep.info.nih.gov/reporting/ctc.html). Briefly, grade 1 or minor bleeding comprised petechiae, minimal or microscopic bleeding not requiring interventions. Grade 2 bleeding was defined as gross, symptomatic bleeding for which minimal intervention (i.e. aspiration, cauterization, irrigation of the urinary tract) was indicated. Grade 3 was severe bleeding requiring red cell transfusions and/or major interventions. Generalized petechiae/purpura as well as retinal bleeding with visual impairment was also classified as grade 3. Catastrophic bleeding defined grade 4, as did central nervous system (CNS) bleeding causing neurological deficit or disability. Lethal bleeding was classified as grade 5. All major bleeding complications were reviewed centrally. Infections were scored in case of positive cultures or if a focus was likely as shown by clinical or radiological examination. In addition to haematological parameters, prothrombin time, activated partial thromboplastin time and fibrinogen levels were measured regularly. Some centres performed routine periodic serological testing of HLA- and/or HPA-alloantibodies, whereas other centres performed these tests only on indication.

Reporting of serious adverse events and Data Safety Monitoring Board

Serious adverse events (SAE) for the purpose of this study were defined as any untoward medical occurrence that resulted in death, a life-threatening event or any other medical condition that might jeopardize the patient or required intervention to prevent more serious sequelae. SAE reporting was mandatory within 24 h of the initial observation. An independent Data Safety Monitoring Board (DSMB) was installed before the start of the study. An interim analysis was planned after 300 transfusions. All serious adverse events (SAEs) were reviewed by the DSMB. Two criteria for early stopping of an experimental arm were defined: (i) A negative 24-h CCI (decrement) not caused by immunological factors in more than 20% of the transfusions, (ii) Statistically significant more bleeding complications (CTCAE $\geq$ 2) compared to the Plasma arm.

Power calculation and statistical analysis

The study was designed as a one-sided, non-inferiority study comparing the 1-h CCI of the transfusions in the PR-PASIII and PASIII arms with the Plasma arm. Inferiority of an experimental arm was defined as a 20% lower mean 1-h CCI compared to the Plasma arm. A mean 1-h CCI of 15·6 and a standard deviation of 6·0 were used, based on a previous study

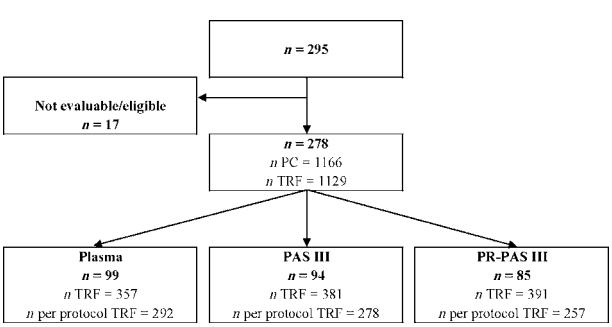

Fig 1. The study randomization scheme, together with evaluable patients, transfusions and endpoints. *n*, number of patients, *n* PC, number of single platelet concentrates, *n* TRF, number of PC transfusion events (includes pooled transfusions). Of the 17 non-evaluable patients, four were non-eligible due to anti-HLA antibodies and 13 patients did not receive any platelet transfusions, without differences between study groups.

(Kerkhoffs *et al*, 2006). For a power of 90% and an alpha of 0·025 (multiple testing) 100 patients per arm were required. In case of multi-dose transfusions, the sum of the platelet content of the PC was used. If one of the PC products differed from the allocated arm, the multi-dose transfusion was considered as not according to protocol. The mean of the storage times of the PC in a multi-dose transfusion was used as the storage time. The 1- and 24-h counts after the infusion of the last PC of a multi-dose transfusion were used for analysis. To account for the hierarchical structure of the data with a variable number of transfusions per patient, the data were analysed using mixed regression models with random effects for patient and transfusion number. Besides the CCIs, 1- and 24-h post-transfusion counts were used as endpoints in regression models with as additional covariates besides arm, platelet dose, pretransfusion counts and body surface area of the patient (Davis *et al*, 1999). The data were analysed by intention to treat (ITT) as well as per protocol (PP). To assess safety, the incidence of bleeding complications and adverse reactions were analysed through tabulation. Pearson's chi-square test was used to compare categorical patient characteristics by arm and the Kruskal-Wallis test to compare ordinal or continuous characteristics by arm. The relationship between storage time and the post-transfusion counts and CCIs was assessed by adding this factor as covariate to the regression models. The association between the patient and transfusion characteristics mentioned above was assessed by adding each of these variables separately as covariate to the regression models. All

Table I. Characteristics of patients and transfusions.

	Plasma	PAS III	PR-PAS III
No. of patients	99	94	85
Male/Female	52/47	53/41	47/38
Age, years ± SD	54 ± 12	55 ± 12	53 ± 12
Body surface area, m^2 ± SD	1·93 ± 0·22	1·94 ± 0·19	1·96 ± 0·25
Enlarged spleen, *N* (%)*	10 (10)	5 (5)	6 (7)
Diagnosis, *N* (%)			
AML/MDS	42 (42)	52 (55)	44 (52)
ALL	9 (9)	4 (4)	3 (4)
Lymphoma	22 (22)	14 (15)	18 (21)
Multiple myeloma	22 (22)	21 (22)	17 (20)
Other	4 (4)	3 (3)	3 (4)
Therapy, *N* (%)			
Remission induction	47 (47)	46 (49)	39 (46)
Consolidation	5 (5)	6 (6)	3 (4)
Autologous transplantation	32 (32)	31 (33)	33 (39)
Allogeneic transplantation	12 (12)	5 (5)	6 (7)
Other	3 (3)	6 (6)	4 (5)
Transfusion history, *N* (%)			
RBC concentrates	55 (56)	59 (63)	43 (51)
PCs	48 (48)	61 (65)	41 (48)
No. of PC transfusion events	357	381	391
Product type according to protocol (%)	292 (82)	278 (73)†	257 (66)†
Multi-dose transfusion (%)	14 (4)	12 (3)	11 (3)
PC transfusion indication, *N* (%)			
Prophylactic, trigger based	304 (85)	334 (88)	327 (84)
Intervention	38 (11)	25 (7)	44 (11)
Treatment of bleeding complication	11 (3)	19 (5)	16 (4)
Unknown	4 (1)	3 (1)	4 (1)
Platelet product content, mean × 10^{11} ± SD	3·9 ± 1·0	3·6 ± 0·8†	3·4 ± 0·8†
Storage time, mean days ± SD	4·0 ± 1·8	3·8 ± 1·8	4·0 ± 1·6
Pre transfusion platelet count × 10^9/l ± SD	18 ± 13	17 ± 13	16 ± 11‡

Major ABO-incompatibility occurred in only six PC transfusions.

AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukaemia; RBC, red blood cell; PC, platelet concentrate; SD, standard deviation.

*Number (%) of evaluable patients and transfusions.

†$P < 0·001$ as compared to plasma.

‡$P = 0·04$ as compared to plasma.

Table II. Transfusion response parameters: ITT and according to protocol (PP).

No. of patients	Plasma 99	PAS III 94	PR-PAS III 85
ITT analysis			
CCI-1 h, mean ± SD	17·1 ± 7·3	15·3 ± 6·5	11·4 ± 5·3¶
Mean diff (97·5% CI)*		−9% (−22%; 4%)	−31% (−43%; −18%)
CCI-24 h, mean ± SD	12·8 ± 7·8	11·6 ± 7·6	7·9 ± 5·3¶
Mean difference (97·5% CI)*		−7% (−26%; 12%)	−34% (−52%; −17%)
PP analysis			
CCI-1 h, mean ± SD	17·1 ± 7·3	15·3 ± 6·7	10·6 ± 5·0¶
Mean diff (97·5% CI)*		−10% (−23%; 4%)	−36% (−49%; −24%)
CCI-24 h, mean ± SD	12·5 ± 7·7	11·7 ± 7·6	6·8 ± 5·9¶
Mean difference (97·5%CI)*		−4% (−24%; 16%)	−42% (−61%; −23%)
Other response parameters (ITT)			
CI-1 h, mean × 10^9/l ± SD	34 ± 15	29 ± 13	20 ± 10§
CI-24 h, mean × 10^9/l ± SD	25 ± 15	21 ± 13	14 ± 10‡
PC transfusions/patient, mean ± SD	4 ± 2	4 ± 3	5 ± 3†
TRF interval (h), mean ± SD	81 ± 47	77 ± 44	61 ± 47‡
Transfusion failure (ITT)			
No. of evaluable CCI-1 h	314	340	350
CCI-1 h < 7·5 (%)**	48 (15)	66 (19)	97 (28) ¶
No. of evaluable CCI-24 h	319	343	351
CCI-24 h < 4·5 (%)**	72 (23)	94 (27)	125 (36)‡

The mean corrected count increment (CCI) and count increment (CI) values were calculated as the mean of the average CCI/CI of all transfusions per patient.

ITT, intention-to-treat; PP, per protocol; TRF, transfusion.

*Mean difference with 97·5% confidence interval of PAS III and PR-PAS III compared to Plasma derived from mixed model regression analyses.

†$P < 0·05$, ‡$P < 0·01$, §$P < 0·001$, ¶$P < 0·0001$ as compared to plasma.

**Percentage of evaluable CCIs.

statistical analyses were performed using STATA. P values <0·05 were considered significant.

Results

Patients and platelet transfusions

Patients were included in the study beginning March 2007. The inclusion of patients in the PR-PASIII group was halted after 92 patients in January 2009 on advice of the DSMB because of lower CCI's ($P < 0·0001$) and more bleeds ($P = 0·045$) compared to the plasma group. Inclusion of patients in the plasma and PASIII group ended in May 2009 and overall 295 patients were randomized. There were 17 non-evaluable patients, resulting in a total of 278 evaluable patients and 1129 transfusion events (Fig 1). There were no significant differences in the patient characteristics of the study groups (Table I). A total of 302 transfusion events (27%) were not according to the allocated study arm, more frequent in both study arms. Eighty-five percentage of the off-protocol PC were platelets stored in PASII, 15% were platelets stored in plasma. The study products had a lower platelet content, with a mean difference of 6% and 11% for PASIII-PC and PR-PASIII-PC as compared to plasma PC, respectively (Table I, $P < 0·001$).

Platelet transfusion efficacy

All efficacy analyses were done ITT as well as PP. The 1-h CCI and 24-h CCI were evaluable in 1004 (88·9%) and 1013 (89·7%) of the transfusion events, respectively. The single reason for a non-evaluable CCI-1/24 h was failure to perform a platelet count after transfusion and, with respect to these missing evaluations, there were no significant differences between the study groups or between the PP and off-protocol transfusion events. All transfusion efficacy parameters showed inferiority of PR-PASIII-PC transfusions. There were no significant differences in transfusion responses between PAS-III-PC and Plasma-PC (Table II). The proportion PC stored for 6 and 7 d was equally distributed across the arms, being 24%, 21% and 26% of transfused PC in the plasma arm, the PASIII arm and the PRPASIII arm, respectively. Both the 1- and 24-h CCI decreased with longer storage time in all study groups. However both CCIs were significantly less in PR-PASIII-PC at each day of storage as compared to plasma PC (Fig 2A, B). The 1- and 24-h CCIs of PASIII-PC were not significantly different to those of plasma PC up to 7 d of storage. Linear regression analysis of 1- and 24-h platelet count showed a platelet dose-independent effect of PR (Fig 2C, D, Table III). A number of product- and patient-related covariates were tested for an association with CCIs, adjusted for arm

(Tables IV). Storage time, enlarged spleen and fever were highly significantly associated with lower CCIs, while the use of steroids as premedication was associated with a higher 1-h CCI and transfusion for a bleeding indication was associated with a lower 24-h CCI.

Bleeding and other clinical complications

A total of 67 new bleeding episodes (CTCAE grade 1–3) were observed in 60 patients during the on-study period from the start of the first transfusion, with significantly more ($P = 0{\cdot}034$) and higher grade ($P = 0{\cdot}044$) bleeding in the PR-PASIII group (Tables V). Distribution of bleeding sites was not different between the study groups. Fourteen of the bleeding patients were on anticoagulant therapy at the time of bleeding, without differences between the groups. We did not observe lethal bleeding complications in the on protocol period; however, one patient in the PR-PASIII arm deceased due to intracranial bleeding after going off-protocol. We did not find an association between platelet dose, storage time or γ-irradiation and the occurrence of bleeding (all grades). There were no differences between the groups with regard to number of RBC transfusions received. The mean number of RBC transfusions in the plasma group was 4 ± 3 as compared to 5 ± 3 and 4 ± 3 in the PASIII and PR-PASIII group, respectively. Twenty-eight mostly mild transfusion reactions occurred in 25 patients, without significant differences between groups (Tables V). Incidences of infections and SAE's were equally distributed among the groups. Three SAE's were possibly related to PC transfusion, one in each group. In the plasma group, one patient developed a severe, generalized skin reaction, a possible case of transfusion-related acute lung injury was reported in the PASIII arm and, in the PR-PASIII arm, one patient developed acute glottis oedema that was treated successfully with antihistamines and steroids.

Discussion

In a non-selected population of thrombocytopenic haematology patients we studied the transfusion efficacy of PR-PASIII-PCs and PASIII-PCs in terms of increments, transfusion failures, PC consumption and transfusion interval as well as bleeding occurrence and adverse transfusion reactions, compared to plasma-PC. In accordance with the SPRINT trial but in contrast to the EuroSPRITE trial, we observed inferiority of transfusions with PR-PASIII-PC with regard to all transfusion efficacy-related endpoints (van Rhenen *et al*, 2003; McCullough *et al*, 2004). Moreover more patients in the PR-PASIII-PC arm experienced bleeding complications. As reported previously, both study products contained less platelets due to loss of platelets during the production process (McCullough *et al*, 2004; Kerkhoffs *et al*, 2006; Murphy *et al*, 2006; Pineda *et al*, 2006). As CCI might

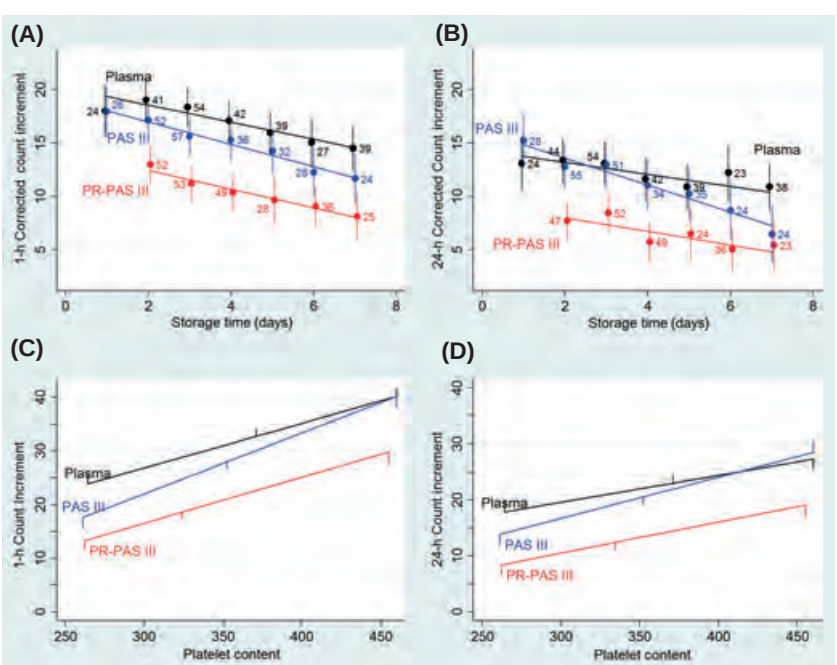

Fig 2. Fitted lines from linear regression analyses, restricted to per protocol transfusions. Black, blue and red represent Plasma, PAS III and PR-PAS III groups, respectively. (A, B) 1- and 24-h CCI as function of storage time for the three treatment groups. Point estimates with 95% confidence intervals and number of transfusions are indicated. The lines are the fitted lines assuming a linear relationship between CCI and storage time for each group. (C, D) Fitted 1- and 24-h increments as linear functions of storage time for the three treatment groups for a patient with body surface area of 1·93 m^2, pre-transfusion platelet count of 12×10^9/l and storage time of 4 d. Standard error bars are indicated.

Table III. Linear regression analysis 1- and 24-h platelet counts.

	1-h platelet count		24-h platelet count	
	Beta*	*P*-value	Beta	*P-value*
PASIII	−2·29	0·377	1·79	0·507
PR-PASIII	−9·63	0·001	−8·95	0·003
Storage time (d)	−1·55	<0·001	−1·24	<0·001
Body surface area (m^2)	−15·4	<0·001	−10·1	0·002
Transfusion sequence number	−0·38	0·047	−0·08	0·686
Platelet product content ($\times 10^9$)	0·09	<0·001	0·06	<0·001
Precount ($\times 10^9$/l	0·96	<0·001	0·96	<0·001

*Beta: regression coefficient. Multivariate linear regression analyses with patient as random factor and 1-h platelet count (Columns 2 and 3) and the 24-h platelet count (Columns 4 and 5) as dependent variables. The factors included in the models are shown in the Column 1. The estimated regression coefficients are shown in the Beta columns. The regression coefficients measure the strength of the effect per unit change of the corresponding factor; e.g. the 1-h platelet count decreases an average of $1{\cdot}55 \times 10^9$/l with each additional day of storage, while an increase of the content of the platelet product with 1×10^9 results in an average increase of $0{\cdot}09 \times 10^9$/l of the 1-h platelet count. The regression coefficients for PAS III and PR-PAS III indicate the average difference in the post-transfusion counts as compared to Plasma.

Table IV. Relation between covariates and the CCI-1 and CCI-24 adjusted for arm.

	1-h CCI		24-h CCI	
	Beta* (SE)	*P*-value	Beta (SE)	*P*-value
Storage time (d)	−0·9 (0·1)	<0·00001	−0·9 (0·1)	<0·00001
Spleen enlargement	−5·7 (1·4)	<0·00001	−6·5 (1·5)	<0·00001
Fever	−1·7 (0·4)	<0·00001	−1·5 (0·4)	0·0003
Steroids	2·6 (1·1)	0·02	1·0 (1·3)	0·43
Indication bleeding	1·1 (1·0)	0·29	−2·5 (1·1)	0·02
Indication Intervention	−0·6 (0·8)	0·39	−0·4 (0·8)	0·64
Age (years)	0·2 (0·3)	0·49	0·0 (0·3)	0·97
Sex	1·1 (0·8)	0·17	0·3 (0·8)	0·76
Prior platelet TRF	−1·0 (0·8)	0·22	−1·0 (0·8)	0·24
Prior RBC TRF	−0·9 (0·8)	0·25	−0·7 (0·8)	0·42
Prior TRF reactions	−2·4 (1·5)	0·12	−0·3 (1·7)	0·84
Infection	−0·5 (0·5)	0·33	−0·5 (0·5)	0·27
Mucosal damage	−0·1 (0·5)	0·82	0·1 (0·5)	0·82
ABO mismatch	0·2 (0·4)	0·68	0·4 (0·4)	0·33
Anti-histamines	−1·6 (1·3)	0·21	−1·8 (1·3)	0·16
Anti-coagulation	−1·3 (1·3)	0·31	−2·1 (1·4)	0·14
Acetaminophen	1·1 (1·3)	0·39	−1·3 (1·3)	0·31

Univariate random effects regression analysis adjusted for arm.
All covariates, with the exception of storage time and patient age, are no/yes covariates.
SE, standard error; TRF, transfusion; RBC, red blood cell concentrate.
*Beta: regression coefficient.

not adequately correct for dose differences between arms, linear regression analysis of the post-transfusion platelet counts were performed using the covariates of treatment arm,

Table V. Bleeding, transfusion reactions, infections and serious adverse reactions.

No. of patients	Plasma 99	PAS III 94	PR-PAS III 85
Bleeding after first PC transfusion			
No of patients (%)	19 (19)	14 (15)	27 (32)*
No of episodes	19	16	32
Maximum grade (%)			
Grade 1	12 (12)	10 (11)	16 (19)
Grade 2	6 (6)	4 (4)	6 (7)
Grade 3	1 (1)	–	5 (6)
Patients with transfusion reactions, *N* (%)	11 (11)	8 (9)	6 (7)
No. of transfusion reactions	13	8	7
Severity of events			
No or minor morbidity	11	7	6
Moderate morbidity	1	–	1
Serious morbidity	1	1	–
Patients with infectious complications, *N* (%)	40 (40)	39 (41)	42 (49)
Maximum grade (%)			
Grade 1 (%)	1	–	–
Grade 2 (%)	3	5	6
Grade 3 (%)	30	29	28
Grade 4 (%)	6	4	8
Grade 5 (%)	–	1	–
Immunological refractoriness, *N* (%)	2 (2)	–	2 (2)
SAEs, *N*	7	3	5
SAE related to PC transfusion	1	1	1
Death, *N*	3†	1	3

Except for the number of bleeding episodes, the numbers in the table reflect numbers (percentage) of patients. For the grades of bleeding and infections the maximum grade is used in case of more than one bleeding episode or more than one infection.
*$P = 0{\cdot}034$ as compared to plasma.
†One patient died in the plasma arm 24 d after the last transfusion (the fifth) without serious adverse event (SAE) report. The cause of death was reported on the off study form as related to the treatment of the underlying disease, with fever presumably due to sepsis.

platelet content and storage time, which also showed an independent effect of PR-PASIII PC (Davis *et al*, 1999). Using the linear regression analysis we estimated that a PR-PASIII-PC would need to contain an average of 200×10^9 platelets extra (i.e. approximately 3 BCs) to achieve a comparable count increment. The relationship between storage time for both CCIs showed a constant difference at each incremental day of storage, suggesting the decreased viability of a fixed number of platelets and normal disappearance of surviving platelets after treatment with this PR technique. To the same extent as plasma PC, PASIII PC showed a decrease in transfusion efficacy up to 7 d of storage and no difference in bleeding complications. Our results with regard to lower increments are in agreement with the SPRINT study. The

discordance with the EuroSPRITE as well as with a large phase IV trial may be due to the usage of PC stored in PASII in approximately half of the reference group attenuating the results of the reference groups in these other studies (van Rhenen *et al*, 2003; Osselaer *et al*, 2009).

Patients in the PR-PASIII group experienced more bleeds and more grade ≥2 bleeding compared with both the other arms. The EuroSPRITE and the other smaller European RCT reported no differences between the study arms with regard to bleeding complications (van Rhenen *et al*, 2003; Janetzko *et al*, 2005). However, in the extended safety report of the SPRINT trial the frequency of grade 2–4 bleeding appeared significantly higher in the PR-arm, 43% as compared to 34% in the control arm ($P = 0{\cdot}02$) (Snyder *et al*, 2005). It is unlikely that the difference in bleeding complications could be solely explained by a lower platelet dose resulting in lower post transfusion platelet peak levels. Estimating that approximately one-third of platelets were non-viable in PR-PC, the platelet dose was still comparable with the low to medium dose applied in a recently presented platelet dose trial, which showed that bleeding complications did not differ between low, medium or high dose levels of platelets transfused (Slichter *et al*, 2010). Possibly, damage of platelet mitochondrial nucleic acids by PR may not only result in loss of viability of a proportion of platelets, but may also impair haemostatic capacity (Keuren *et al*, 2006; Apelseth *et al*, 2007). We did not find significant differences in transfusion reactions, as observed in larger trials using PR-PASIII PC (Osselaer *et al*, 2008a, b).

This study has some shortcomings

The number of off-protocol transfusions in the PR-PASIII arm could be regarded as an important limitation of our study. However, performances of both an ITT as well as a PP analysis lead to similar conclusions. The open label aspect of our study was not expected to influence platelet counts, the primary endpoint of our study, although we cannot completely exclude bias with regard to evaluation of bleeding.

In conclusion, although there are clear advantages and arguments in favour of PR techniques to increase transfusion safety, our results warrant their reappraisal prior to routine implementation. The process of PR using amotosalen-HCl/UVA probably leads to decreased platelet viability and perhaps compromises haemostatic function, the primary goal of platelet transfusions in high risk patients. A comprehensive survey on the nature and consequences of amotosalen-HCl/UVA-induced platelet damage is needed to understand how this damage can be compensated for in routine transfusion practice.

Acknowledgements

We would like to thank all the physicians, nurses, technologists, data managers and study coordinators at each study site, as well as all the blood bank personnel who produced all the products. We also thank Cerus Corporation for support in the production of the PR-PASIII PCs.

References

Apelseth, T.O., Bruserud, O., Wentzel-Larsen, T., Bakken, A.M., Bjorsvik, S. & Hervig, T. (2007) *In vitro* evaluation of metabolic changes and residual platelet responsiveness in photochemical treated and gamma-irradiated single-donor platelet concentrates during long-term storage. *Transfusion*, **47,** 653–665.

Blajchman, M.A., Beckers, E.A., Dickmeiss, E., Lin, L., Moore, G. & Muylle, L. (2005) Bacterial detection of platelets: current problems and possible resolutions. *Transfusion Medicine Reviews*, **19,** 259–272.

Council of Europe expert committee in blood transfusion study group on pathogen inactivation of labile blood products. (2001) pathogen inactivation of labile blood products. *Transfusion Medicine*, **11,** 149–175.

Davis, K.B., Slichter, S.J. & Corash, L. (1999) Corrected count increment and percent platelet recovery as measures of posttransfusion platelet response: problems and a solution. *Transfusion*, **39,** 586–592.

Dodd, R.Y., Notari, E.P. & Stramer, S.L. (2002) Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion*, **42,** 975–979.

Goodnough, L.T., Brecher, M.E., Kanter, M.H. & AuBuchon, J.P. (1999) Transfusion medicine: blood transfusion. *New England Journal of Medicine*, **340,** 438–447.

Grass, J.A., Wafa, T., Reames, A., Wages, D., Corash, L., Ferrara, J.L. & Lin, L. (1999) Prevention of Transfusion-Associated Graft-versus-Host Disease by photochemical treatment. *Blood*, **93,** 3140–3147.

Janetzko, K., Lin, L., Eichler, H., Mayaudon, V., Flament, J. & Kluter, H. (2004) Implementation of the INTERCEPT Blood System for platelets into routine blood bank manufacturing procedures: evaluation of aphaeresis platelets. *Vox Sanguinis*, **86,** 239–245.

Janetzko, K., Cazenave, J.P., Klüter, H., Kientz, D., Michel, M., Beris, P., Lioure, B., Hastka, J., Marblie, S., Mayaudon, V., Lin, L., Lin, J.S., Conlan, M.G. & Flament, J. (2005) Therapeutic efficacy and safety of photochemically treated aphaeresis platelets processed with an optimized integrated set. *Transfusion*, **45,** 1443–1452.

Jansen, G.A., van Vliet, H.H., Vermeij, H., Beckers, E.A., Leebeek, F.W., Sonneveld, P. & van Rhenen, D.J. (2004) Functional characteristics of photochemically treated platelets. *Transfusion*, **44,** 313–319.

Kerkhoffs, J.L., Eikenboom, J.C., Schipperus, M.S., van Wordragen-Vlaswinkel, R.J., Brand, R., Harvey, M.S., de Vries, R.R., Barge, R., van Rhenen, D.J. & Brand, A. (2006) A multicenter randomized Study of the efficacy of transfusions with platelet stored in platelet additive solution II versus plasma. *Blood*, **108,** 3210–3215.

Keuren, J.F., Cauwenberghs, S., Heeremans, J., de Kort, W., Heemskerk, J.W. & Curvers, J. (2006) Platelet ADP response deteriorates in synthetic storage media. *Transfusion*, **46,** 204–212.

Kuehnert, M., Roth, V. & Haley, N. (2001) Transfusion-transmitted bacterial infection in the United States, 1998 – 2000. *Transfusion*, **41,** 1493–1499.

Lee, C.K., Ho, P.L., Lee, K.Y., Cheng, W.W., Chan, N.K., Tsoi, W.C. & Lin, C.K. (2003) Estimation of bacterial risk in extending the shelf life of PLT concentrates from 5 to 7 days. *Transfusion*, **43,** 1047–1052.

Lin, L. (2001) Inactivation of cytomegalovirus in platelet concentrates using Helinx technology. *Seminars in haematology*, **38,** 27–33.

Lin, L., Dikeman, R., Molini, B., Lukehart, S.A., Lane, R., Dupuis, K., Metzel, P. & Corash, L. (2004) Photochemical treatment of platelet concentrates with amotosalen and long-wavelength ultraviolet light inactivates a broad spectrum of pathogenic bacteria. *Transfusion*, **44,** 1496–1504.

Lin, L., Hanson, C.V., Alter, H.J., Jauvin, V., Bernard, K.A., Murthy, K.K., Metzel, P. & Corash, L. (2005) Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelenght ultraviolet light. *Transfusion*, **45,** 580–590.

Lozano, M., Galan, A., Mazzara, R., Corash, L. & Escolar, G. (2007) Leukoreduced buffy-coat derived platelet concentrates photochemically treated with amotosalen HCl and ultraviolet A light stored up 7 days: assessment of hemostatic function under flow conditions. *Transfusion*, **47,** 666–671.

McCullough, J., Vesole, D.H., Benjamin, R.J., Slichter, S.J., Pineda, A., Snyder, E., Stadtmauer, E.A., Lopez-Plaza, I., Coutre, S., Strauss, R.G., Goodnough, L.T., Fridey, J.L., Raife, T., Cable, R., Murphy, S., Howard, F. IV, Davis, K., Lin, J.S., Metzel, P., Corash, L., Koutsoukos, A., Lin, L., Buchholz, D.H. & Conlan, M.G. (2004) Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. *Blood*, **104,** 1534–1541.

van der Meer, P.F., Kerkhoffs, J.L., Curvers, J., Scharenberg, J., de Korte, D., Brand, A. & de Wildt-Eggen, J.(2009) *In vitro* comparison of platelet storage in plasma and in four platelet additive solutions, and the effect of pathogen reduction: a proposal for an *in vitro* rating system. *Vox Sanguinis*, DOI: 10.1111/j.1423-0410.2009.01283.x.

Murphy, S., Snyder, E., Cable, R., Slichter, S.J., Strauss, R.G., McCullough, J., Lin, J.S., Corash, L. & Conlan, M.G. (2006) Platelet dose consistency and its effect on the number of platelet transfusions for support of thrombocytopenia: an analysis of the SPRINT trial of platelets photochemically treated with amotosalen HCl and ultraviolet A light. *Transfusion*, **46,** 24–33.

Osselaer, J.C., Messe, N., Hervig, T., Bueno, J., Castro, E., Espinosa, A., Accorsi, P., Junge, K., Jacquet, M., Flament, J. & Corash, L. (2008a) A prospective observational cohort safety study of 5106 platelet transfusions with components prepared with photochemical pathogen inactivation treatment. *Transfusion*, **48,** 1061–1071.

Osselaer, J.C., Cazenave, J.P., Lambermont, M., Garraud, O., Hidajat, M., Barbolla, L., Tardivel, R., Defoin, L., Waller, C., Mendel, I., Raidot, J.P., Kandel, G., De Meuter, R., Fabrigli, P., Dehenau, D., Arroyo, J.L., Padrón, F., Gouezec, H., Corral, M., Jacquet, M., Sundin, D., Lin, L. & Corash, L. (2008b) An active haemovigilance programme characterizing the safety profile of 7437 platelet transfusions prepared with amotosalen photochemical treatment. *Vox Sanguinis*, **94,** 315–323.

Osselaer, J.C., Doyen, C., Defoin, L., Debry, C., Goffaux, M., Messe, N., Van Hooydonk, M., Bosly, A., Lin, J.S., Lin, L. & Corash, L. (2009) Universal adoption of pathogen inactivation of platelet components: impact on platelet and red blood cell component use. *Transfusion*, **49,** 1412–1422.

Picker, S.M., Speer, R. & Gathof, B.S. (2004) Functional characteristics of buff-coat PLTs photochemically treated with amotosalen-HCl for pathogen inactivation. *Transfusion*, **44,** 320–329.

Pineda, A., McCullough, J., Benjamin, R.J., Cable, R., Strauss, R.G., Burgstaler, E., Porter, S., Lin, L., Metzel, P. & Conlan, M.G. (2006) Pathogen inactivation of platelets with a photochemical treatment with amotosalen HCl and ultraviolet light: process used in the SPRINT trial. *Transfusion*, **46,** 562–571.

van Rhenen, D., Gulliksson, H., Cazenave, J.P., Pamphilon, D., Ljungman, P., Klüter, 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. (2003) Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euro SPRITE trial. *Blood*, **101,** 2426–2433.

Schrezenmeier, H., Walther-Wenke, G., Müller, T.H., Weinauer, F., Younis, A., Holland-Letz, T., Geis, G., Asmus, J., Bauerfeind, U., Burkhart, J., Deitenbeck, R., Förstemann, E., Gebauer, W., Höchsmann, B., Karakassopoulos, A., Liebscher, U.M., Sänger, W., Schmidt, M., Schunter, F., Sireis, W. & Seifried, E. (2007) Bacterial contamination of platelet concentrates: results of a prospective multicenter study comparing pooled whole blood-derived platelets and apheresis platelets. *Transfusion*, **47,** 644–652.

Simonsen, A.C., Johansson, P.I., Conlan, M.G., Jacquet, M., Lin, J.S., Junge, K., Lin, L., Sørensen, H., Borregaard, N. & Flament, J. (2006) Transfusion of 7-day old amotosalen photochemically treated buffy-coat platelets to patients with thrombocytopenia: a pilot study. *Transfusion*, **46,** 424–433.

Slichter, S.J. (2007) Platelet transfusion therapy. *Hematology/Oncology Clinics of North America*, **21,** 697–729.

Slichter, S.J., Raife, T.J., Davis, K., Rheinschmidt, M., Buchholz, D.H., Corash, L. & Conlan, M.G. (2006) Platelets photochemically treated with amotosalen HCl and ultraviolet A light correct prolonged bleeding times in patients with thrombocytopenia. *Transfusion*, **46,** 731–740.

Slichter, S.J., Kaufman, R.M., Assmann, S.F., McCullough, J., Triulzi, D.J., Strauss, R.G., Gernsheimer, T.B., Ness, P.M., Brecher, M.E., Josephson, C.D., Konkle, B.A., Woodson, R.D., Ortel, T.L., Hillyer, C.D., Skerret, D.L., McCrae, K.R., Sloan, S.R., Uhl, L., George, J.N., Aquino, V.M., Manno, C.S., McFarland, J.G., Hess, J.R., Leissinger, C. & Granger, S. (2010) Dose of Prophylactic Platelet Transfusions and Prevention of Hemorrhage. *New England Journal of Medicine*, **362,** 600–613.

Snyder, E., Raife, T., Lin, L., Cimino, G., Metzel, P., Rheinschmidt, M., Baril, L., Davis, K., Buchholz, D.H., Corash, L. & Conlan, M.G. (2004) Recovery and life span of ^{111}Indium-radiolabeled platelets treated with pathogen inactivation with amotosalen HCl (S-59) and ultraviolet light. *Transfusion*, **44,** 1732–1740.

Snyder, E., McCullough, J., Slichter, S.J., Strauss, R.G., Lopez-Plaza, I., Lin, J.S., Corash, L. & Conlan, M.G. (2005) Clinical safety of platelets photochemical treated with amotosalen HCl and ultraviolet A light for pathogen inactivation: the SPRINT trial. *Transfusion*, **45,** 1864–1875.

Stroncek, D.F. & Rebulla, P. (2007) Platelet transfusions. *Lancet*, **370,** 427–438.

Van Rhenen, D.J., Vermeij, J., Mayaudon, V., Hind, C., Lin, L. & Corash, L. (2000) Functional characteristics of S-59 photochemically treated platelet concentrates derived from buffy coats. *Vox Sanguinis*, **79,** 206–214.

プール白血球除去濃厚血小板を血漿保存または低減化処理および未処理の状態で血小板用添加液に保存した場合の臨床効果（仮訳）

Clinical effectiveness of leucoreduced, pooled donor platelet concentrates, stored in plasma or additive solution with and without pathogen reduction

要約

血小板製剤の感染性因子低減化（PR）技術はコストを増加させるが、これまでの臨床試験では、臨床効果および止血効果について明確な結果が得られていない。我々は、多施設共同、非盲検、ランダム化、非劣性試験を実施し、最長 7 日間保存した 3 種類のバフィーコート由来白血球除去血小板濃厚液（PC）、すなわち、血漿保存 PC、platelet additive solution III（PASIII）保存 PC、光増感剤であるアモトサレン塩酸塩存在下での紫外線 A（UVA）照射による PR 処理済み PASIII（PR-PASIII）保存 PC の臨床効果を比較した。本試験の主要エンドポイントは、血小板輸血 1 時間後の補正血小板増加数（1-h CCI）とした。副次エンドポイントは、血小板輸血 24 時間後の CCI（24-h CCI）、出血、赤血球および PC 輸血の必要量、PC 輸血間隔および輸血副作用とした。評価可能患者 278 例を対象とした intention to treat 解析（包括解析）では、PR-PASIII-PC 群および PASIII-PC 群における 1-h CCI 平均値の血漿 PC 群との差は、それぞれ-31%（$P < 0.0001$）および-9%（P = n.s.）であった。出血事象発生例数は、PR-PASIII-PC 群が 27 例（32%）であったのに対し、血漿 PC 群 19 例（19%）、PASIII-PC 群 14 例（15%）であった（$P = 0.034$）。アモトサレン塩酸塩存在下での UVA 照射による血小板製剤の感染性因子（および白血球）低減化処理には利点があると考えられるものの、その臨床効果は血漿保存血小板と比較して劣ることから、この技術の臨床使用については批判的再評価が必要である。

キーワード: 血小板、バフィーコート、アモトサレン／UVA 照射による感染性因子低減化、有効性

出血の治療および予防が必要と一般に認められている状態に対する血小板製剤の輸血回

数は、年間数百万回にのぼることから、輸血の実施および安全上、新たな問題の発生に警戒を怠ってはならない（Slichter, 2007; Stroncek & Rebulla, 2007）。ドナーのカウンセリングおよびスクリーニング（分子的手法を含む）により、B 型肝炎、C 型肝炎、ヒト免疫不全ウイルス、ヒト T リンパ球向性ウイルス（HTLV）I 型および II 型の伝播リスクは低下している。しかし、血小板製剤の細菌培養検査が実施されているにもかかわらず、血小板輸血による輸血関連敗血症の発生リスクは、依然として 25,000 回に 1 回の頻度で存在する（Goodnough et al, 1999; Kuehnert et al, 2001; Dodd et al, 2002; Blajchman et al, 2005; Schrezenmeier et al, 2007）。期限切れ本数削減による血小板の利用可能性向上およびコスト低減には、血小板製剤の保存期間延長が有効と考えられるが、保存期間が 5 日を超えると細菌増殖のリスクがあること（Lee et al, 2003）が主な原因となり実現していない。感染性因子低減化（PR）は、数種類のウイルスおよび細菌の不活化にきわめて有効であることが示されている（Lin et al, 2004, 2005）。さらに、PR は新興感染性因子や、サイトメガロウイルスに対する解決策であるほか、移植片対宿主病（GVHD）の予防策としてγ線照射の代替法に使用できると考えられる（Grass et al, 1999; Lin, 2001）。一部の国では、すべての血小板製剤に対する標準的処理法として PR 導入を検討しているが、その臨床有効性、長期毒性の可能性や、PR 処理した血小板製剤の保存期間を 5 日より長く設定することの是非については、依然として懸念がある（Council of Europe expert committee in blood transfusion study group on pathogen inactivation of labile blood products, 2001; Simonsen et al, 2006）。アモトサレン塩酸塩存在下での紫外線 A（UVA）照射により PR 処理した血小板製剤は、保存期間 7 日までは標準的な出荷基準を満たすが、この処理により、保存中に大幅な代謝抑制や血小板の活性化亢進が生じるほか、*in vitro* における止血評価で一貫した成績が得られていない（Van Rhenen et al, 2000; Janetzko et al, 2004; Jansen et al, 2004; Picker et al, 2004; Apelseth et al, 2007; Lozano et al, 2007; van der Meer et al, 2009）。しかし、血小板減少症患者への輸血において、出血時間延長の改善を認めた（Slichter et al, 2006）。アモトサレン塩酸塩存在下で UVA 照射し、アイソトープでラベルした自己血小板を 5 日間保存後に輸血したところ、platelet additive solution III（PASIII）保存血小板と比較して、血小板回収率が有意に低く、血小板寿命が短いことが示された（Snyder et al, 2004）。アモトサレン塩酸塩存在下で UVA 照射した血小板濃厚液（PC）を用いたランダム化対照試験は、これまでに 3 件実施されている（van Rhenen et al, 2003; McCullough et al, 2004; Janetzko et al, 2005）。血漿保存したアフェ

レーシス PC を対照とした S-59 Platelet Recovery in Thrombocytopenia（SPRINT）試験（645 例）では、輸血後の血小板増加数が有意に減少したほか、輸血間隔が短縮し、輸血無効例の割合が上昇した（McCullough et al, 2004）。S-59 Platelet Recovery in Thrombocytopenia in Europe（EuroSPRITE）試験（103 例）では、輸血効果に関する有意差は報告されなかったが、同試験の対照群には、血漿保存したバフィーコート由来血小板以外に、platelet additive solution II（PASII）保存した血小板が輸血回数の約半分で使用された（van Rhenen et al, 2003）。我々の過去のランダム化臨床試験（RCT）においてPASII 保存 PC を輸血した後の補正血小板増加数（CCI）は血漿保存 PC より 20%少ないことが示されていることから、EuroSPRITE 試験では重要な差がマスクされていた可能性がある（Kerkhoffs et al, 2006）。3 件目の小規模試験（43 例）では、輸血効果低下の有意性は境界域にとどまることが示された（Janetzko et al, 2005）。これらの試験のうち、止血効果の劣性が報告された試験はなかった。PR 処理血小板製剤を導入する上で、臨床効果を保ちつつ保存期間を 7 日まで延長させることは、PR 処理に要する追加コストを埋め合わせる重要な側面である。我々は、多施設共同、非盲検、ランダム化臨床試験を実施し、輸血効果の点からプールランダムドナー由来 PC について、PR 非処理の場合（PASIII、Intersol, Fenwal, Inc.、イリノイ州レークズリク、米国）と、光増感剤であるアモトサレン塩酸塩存在下で UVA 照射により PR 処理し（PR-PASIII、Intercept Blood System、Cerus Corporation、カリフォルニア州コンコード、米国）、血小板添加液に最長 7 日間保存した場合の臨床効果を血漿保存 PC と比較した。

方法

試験デザイン

本試験のデザインは、骨髄抑制により血小板減少症を来しているか、来すと予想される血液腫瘍患者を対象とした前向き、ランダム化、非盲検、非劣性試験であった。患者は、オランダ国内の 8 病院の血液科病棟から登録した。治験実施計画書および同意書は、中央倫理委員会のほか、施設内審査委員会によっても承認された。本試験は、日米 EU 医薬品規制調和国際会議（International Conference on Harmonization）／世界保健機関（WHO）の GCP（ICH-GCP）ガイドラインおよびヘルシンキ宣言に従って実施した。試験期間中、第三者機関が全施設を監査するとともに、治験実施をモニターした。血液腫瘍疾患を有する成人患者（18 歳超）のうち、予想される血小板輸血回数が 2 回以上であった全患者を組

入れ適格とした。除外基準は、ヒト白血球抗原（HLA）および／またはヒト血小板抗原（HPA）に対する抗体あるいは臨床的な重要な自己抗体を有するためランダム血小板輸血に対して免疫学的不応状態である場合、妊娠（または授乳）中である場合、本試験への登録歴がある場合とした。インフォームドコンセントの取得後、適格例を登録した。血小板輸血を開始する前に血漿 PC 群、PASIII-PC 群、PR-PASIII-PC 群に 1：1：1 の比率で施設による層別ランダム化を行い、治験実施計画書に従って、最長 42 日間に最高 5 回の血小板輸血を行った。試験期間中に血小板数が是正されなかった場合には、治験実施計画書に適合しない血小板輸血を実施してもよいものとした。通常の試験完了以外で試験中止に至った理由は、患者または担当医による継続拒否、治験中の死亡および免疫学的不応状態であった。

血小板製剤、輸血およびモニタリング

血小板製剤はすべて、Sanquin Blood Bank で製造された。PC は同じ ABO 血液型の 5 バッグのプール全血バフィーコート（BC）から標準的手順を用いて調製し、感染性因子低減化は製造業者の説明書を用いて実施した（van Rhenen et al, 2003; Kerkhoffs et al, 2006）。保存前にサンプルを採取し、製剤中の血小板数を測定した。すべての血小板製剤のサンプルは、BacT/Alert 培養システム（BioMerieux、ボクステル、オランダ）を用いて 7 日間培養した。全血小板製剤とも、緩徐に撹拌しながら 20～24ºC で最長 7 日間保存した。病院の要請があった場合には、PC にγ線を照射した。

血小板輸血の適応は、血小板数に基づく予防的投与、介入による予防的投与、出血の治療に分けられた。血小板輸血の適応に関しては、一般に受け入れられているガイドラインを使用した。血小板輸血の必要性および実施時期は、担当医が判断した。要約すると、出血がない安定状態の患者には血小板数を 10×10^9/L 以上に維持するため、また、これらの患者のうち抗凝固療法または抗胸腺細胞グロブリン治療を受けている者には血小板数を 40×10^9/L 以上に維持するために血小板輸血を行うよう助言した。輸血の血小板トリガー値は、消化管または気道の内視鏡検査（生検非実施時）、細い針を用いた胸膜または腹膜の診断的穿刺、腰椎穿刺、中心静脈カテーテルの抜去および小手術を施行する場合には、40×10^9/L とすることを推奨した。出血を来している場合、内視鏡検査（生検実施時）、抜歯、中心静脈カテーテルの留置、大手術（脳神経外科手術および心臓手術を除く）を施行する

場合には、60 × 10^9/L とすることを推奨した。脳出血、びまん性肺胞出血を来しているか、脳神経外科手術および心臓手術を施行する場合には、100 × 10^9/L とすることを推奨した。輸血前血小板数は、輸血直前（輸血前 6 時間以内）の測定が望ましいとした。輸血 1 時間後の血小板数は輸血 10～120 分後に測定し、輸血 24 時間後の血小板数は輸血 16～28 時間後に測定した。CCI は次式により算出した：$CCI_{1/24\,h}$ = [(輸血後血小板数 $_{1/24\,h}$ − 輸血前血小板数（× 10^9/L）) × 体表面積（m^2）]/血小板投与量 × 10^{11}。短期間に連続して輸血を行い、輸血と輸血の間で血小板数を測定しなかった場合は、分割輸血とみなし、1 回の輸血として解析した。可能な限り ABO 型が一致した PC を使用したが、ABO メジャーおよびマイナーミスマッチの PC が輸血された場合もデータから排除しなかった。血小板輸血無効の定義は、1-h CCI が 7.5 未満、24-h CCI が 4.5 未満の場合とした（Kerkhoffs et al, 2006）。免疫学的不応状態の定義は、ABO 型が一致したランダム血小板輸血が 2 回連続無効で、なおかつ HLA および／または HPA に対するアロ抗体が存在する場合とした。

試験のエンドポイント

プライマリーエンドポイントは、1-h CCI とした。セカンダリーエンドポイントは、24-h CCI、出血、赤血球および PC 輸血の必要量、PC 輸血間隔および輸血副作用とした。登録時には次の特性を記録した：性別、年齢、血液型、血液腫瘍と治療段階、WHO のパフォーマンスステータス、脾腫の有無、輸血歴、抗凝固薬による治療、病歴、使用薬剤、出血、感染症罹患の有無。各輸血時には次の特性を記録した：輸血の理由（血小板トリガー値、出血、介入のいずれか）、PC の血液型、発熱の有無、感染の有無（Common Toxicity Criteria for AdverseEvent 、CTCAE 、Version 3 、http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3. pdf）に従ってグレード分類）、粘膜障害の有無、アセトアミノフェン、ステロイドまたは抗ヒスタミン薬の使用。患者の評価は、訓練を受けた担当者が治験担当医師の監督下で毎日行い、規定された 8 部位における出血性合併症を CTCAE に従って観察・記録・グレード分類した（http://ctep.info.nih.gov/reporting/ctc.html）。グレード 1 または軽度の出血は、介入を要さない点状出血、ごく少量の出血または顕微鏡的出血とした。グレード 2 の出血は、最小限の介入（吸引、焼灼、尿道洗浄）を要する症候性の肉眼的出血と定義した。グレード 3 は、赤血球輸血および／または重大な介入を要する重度出血と定義した。全身性の点状出血／紫斑、ならびに視力障害を伴う網膜出血もグレード 3 に分類した。重篤な出血、

ならびに神経脱落症状または身体機能障害を引き起こす中枢神経系（CNS）出血は、グレード 4 の出血と定義した。致死性の出血はグレード 5 に分類した。すべての重大な出血性症状は改めて精査した。感染事例は、培養検査陽性の場合、あるいは感染巣が存在する可能性が高いことが診察またはレントゲン検査により認められた場合にスコア化した。血液学的パラメータのほか、プロトロンビン時間、活性化部分トロンボプラスチン時間およびフィブリノゲン値を定期的に測定した。HLA および／または HPA に対するアロ抗体は、ルーチンの血清学的検査として定期的に検査した施設もあれば、必要時にのみ検査した施設もあった。

重篤な有害事象の報告とデータ安全性モニタリング委員会

本試験の目的上、重篤な有害事象（SAE）とは、死亡、生命を脅かす事象、あるいは患者を危険にさらす可能性がある他の医学的状態に至るか、より重篤な後遺症を予防するため介入を必要とするあらゆる好ましくない医療上の出来事と定義した。SAE 報告は、最初に観察されてから 24 時間以内に行うことを義務づけた。本試験開始前に、中立的立場のデータ安全性モニタリング委員会（DSMB）を設置した。中間解析は、輸血を 300 回終了した後に行う計画であった。重篤な有害事象（SAE）はすべて、DSMB が再調査した。被験群の早期中止基準は次の 2 つとした：(i) 輸血回数の 20%超で 24-h CCI が負の値（減少）を示し、その原因が免疫学的因子でなかった場合、(ii) 出血性合併症（CTCAE≧2）の発生件数が、血漿 PC 群と比較して統計学的に有意に多かった場合。

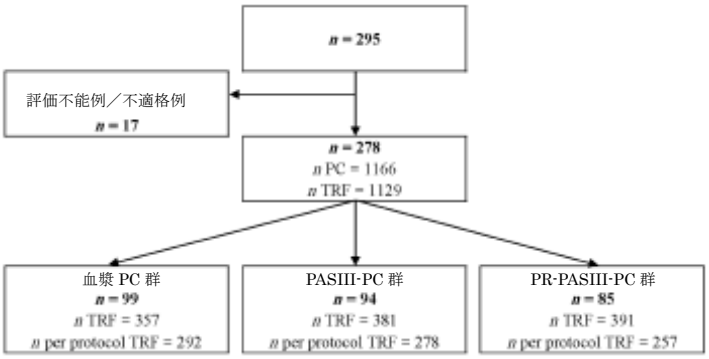

図 1. 本試験におけるランダム割付 評価可能例、輸血およびエンドポイントの概要。n、患者数、n PC、血小板濃厚液ののべ輸血回数、n TRF、PC 輸血回数（分割輸血を 1 回とし

て算出)。評価不能例 17 例のうち、4 例は抗 HLA 抗体を有していたため不適格例であり、13 例は血小板輸血を 1 回も受けなかった。被験群間に差は認められなかった。

表 I. 患者および輸血の特性

	血漿 PC 群	PASIII-PC 群	PR-PASIII-PC 群
患者数	99 例	94 例	85 例
男/女	52/47	53/41	47/38
年齢、歳 ± SD	54 ± 12	55 ± 12	53 ± 12
体表面積、m^2 ± SD	1.93 ± 0.22	1.94 ± 0.19	1.96 ± 0.25
脾腫、N（%）*	10 (10)	5 (5)	6 (7)
診断、N（%）			
AML/MDS	42 (42)	52 (55)	44 (52)
ALL	9 (9)	4 (4)	3 (4)
リンパ腫	22 (22)	14 (15)	18 (21)
多発性骨髄腫	22 (22)	21 (22)	17 (20)
その他	4 (4)	3 (3)	3 (4)
治療、N（%）			
寛解導入療法	47 (47)	46 (49)	39 (46)
地固め療法	5 (5)	6 (6)	3 (4)
自家移植	32 (32)	31 (33)	33 (39)
同種移植	12 (12)	5 (5)	6 (7)
その他	3 (3)	6 (6)	4 (5)
輸血歴、N（%）			
赤血球濃厚液	55 (56)	59 (63)	43 (51)
PC	48 (48)	61 (65)	41 (48)
PC 輸血回数	357	381	391
治験実施計画書に適合する種類の製剤（%）	292 (82)	278(73)†	257 (66)†
分割輸血（%）	14 (4)	12 (3)	11 (3)
PC 輸血の適応、N（%）			
トリガー値に基づく予防的投与	304 (85)	334 (88)	327 (84)
介入	38 (11)	25 (7)	44 (11)
出血性合併症の治療	11 (3)	19 (5)	16 (4)
不明	4 (1)	3 (1)	4 (1)
血小板製剤中の血小板数、平均 × 10^{11}± SD	3.9 ± 1.0	3.6 ±0.8†	3.4 ± 0.8†
保存期間、平均日数 ± SD	4.0 ± 1.8	3.8 ± 1.8	4.0 ± 1.6
輸血前の血小板数 × 10^9/L ± SD	18 ± 13	17 ± 13	16 ± 11‡

重大な ABO 不適合は、6 回の PC 輸血で発生したにとどまった。
AML、急性骨髄性白血病、MDS、骨髄異形成症候群、ALL、急性リンパ芽球性白血病、PC、血小板濃厚液、SD、標準偏差
*評価可能例の輸血後の発生件数（%）
†P < 0.001、血漿 PC 群との比較
‡P = 0.04、血漿 PC 群との比較

表 II. 輸血効果のパラメータ：ITT 解析および治験実施計画書に適合する対象集団（PP）の解析

患者数	血漿 PC 群 99 例	PASIII-PC 群 94 例	PR-PASIII-PC 群 85 例
ITT 解析			
CCI-1 h、平均 ± SD	17.1 ± 7.3	15.3 ± 6.5	11.4 ± 5.3¶
平均値の差（97.5% CI）*		-9% (-22%; 4%)	-31% (-43%; -18%)
CCI-24 h、平均 ± SD	12.8 ± 7.8	11.6 ± 7.6	7.9 ± 5.3¶
平均値の差（97.5% CI）*		-7% (-26%; 2%)	-34% (-52%; -17%)
PP 解析			
CCI-1 h、平均 ± SD	17.1 ± 7.3	15.3 ± 6.7	10.6 ± 5.0¶
平均値の差（97.5% CI）*		-10% (-23%; 4%)	-36% (-49%; -24%)
CCI-24 h、平均 ± SD	12.5 ± 7.7	11.7 ± 7.6	6.8 ± 5.9¶
平均値の差（97.5% CI）*		-4% (-24%; 16%)	-42% (-61%; -23%)
その他のパラメータ（ITT）			
CI-1 h、平均 × 10^9/L ± SD	34 ± 15	29 ± 13	20 ± 10§
CI-24 h、平均 × 10^9/L ± SD	25 ± 15	21 ± 13	14 ± 10‡
1 例あたり PC 輸血回数、平均 ± SD	4 ± 2	4 ± 3	5 ± 3‡
輸血間隔（時間）、平均 ± SD	81 ± 47	77 ± 44	61 ± 47‡
輸血無効（ITT）			
CCI-1 h の評価可能例数	314	340	350
CCI-1 h が 7.5 未満の例数（%）**	48 (15)	66 (19)	97 (28)¶
CCI-24 h の評価可能例数	319	343	351
CCI-24 h が 4.5 未満の例数（%）**	72 (23)	94 (27)	125 (36)‡

補正血小板増加数（CCI）および血小板増加数（CI）の平均値は、各患者の全輸血における CCI/CI 平均値の群平均として算出した。
ITT、intention-to-treat、PP、per protocol
* PASIII-PC 群および PR-PASIII-PC 群の平均値の血漿 PC 群との差と、その 97.5%信頼区間は、混合モデルを用いた回帰分析により求めた。
†P < 0.05、‡P < 0.01、§P < 0.001、¶P < 0.0001（血漿 PC 群との比較）
**評価可能 CCI 値に占める割合（%）

検出力の算出および統計解析

本試験は片側非劣性試験として計画し、PR-PASIII-PC 群および PASIII-PC 群における輸血時の 1-h CCI を血漿 PC 群と比較した。被験群が血漿 PC 群より劣性であることの定

義は、1-h CCI 平均値が血漿 PC 群より 20%以上低い場合とした。過去の試験（Kerkhoffs et al, 2006）に基づき、1-h CCI 平均値には 15.6、標準偏差には 6.0 を用いた。検出力 90%、有意水準（α）0.025（多重検定）を確保するために必要な患者数は、1 群 100 例であった。分割輸血された場合には、使用された PC の合計血小板数を解析に使用した。分割輸血のうち 1 回でも、割り付けられた種類とは異なる PC 製剤を使用した場合には、治験実施計画書からの逸脱とみなした。分割輸血の場合には、PC の平均保存期間を保存期間として用い、最終回の PC 輸血 1 時間後および 24 時間後の血小板数を解析に用いた。本試験のデータが階層構造を有し、患者あたりの輸血回数が異なることを解析に含めるため、患者および輸血回数を変量効果として組み込んだ混合回帰モデルを用いてデータを解析した。CCI のほか、輸血 1 時間後および 24 時間後の血小板数を回帰モデルのエンドポイントとして用い、その他の共変量として、群のほか、血小板投与量、輸血前血小板数および患者の体表面積を組み込んだ（Davis et al, 1999）。データの intention to treat（ITT）解析および per protocol（PP）解析を行った。安全性を評価するため、出血性症状および副作用の発生率を集計し解析した。患者の特性のうち、カテゴリー変数の群間比較には Pearson のカイ 2 乗検定を使用し、順序変数または連続変数の群間比較には Kruskal-Wallis 検定を用いた。保存期間と輸血後血小板数および CCI の関係は、回帰モデルに同因子を共変量として組み込むことにより評価した。上記の患者特性と輸血特性の関係は、回帰モデルにこれらの各変数を共変量として組み込むことにより評価した。すべての統計解析は STATA を用いて実施した。P 値が 0.05 未満の場合に有意と判定した。

結果

患者および血小板輸血

本試験への患者登録は 2007 年 3 月に開始した。PR-PASIII-PC 群への患者登録は、同群の CCI が血漿 PC 群より低く（$P < 0.0001$）、出血発生率が高かったことから（$P = 0.045$）、2009 年 1 月までに 92 例を登録した後、DSMB の助言により中止した。血漿 PC 群および PASIII-PC 群への患者登録は 2009 年 5 月に終了し、計 295 例をランダム化した。うち 17 例は評価不能例であったため、評価可能例は計 278 例、輸血回数は 1,129 回であった（図 1）。被験群の患者特性に、有意差は認められなかった（表 I）。割り付けられた被験群に適合しない種類の PC の輸血回数は計 302 回（27%）で、その頻度は両被験群が血漿 PC 群より高かった。治験実施計画書に適合しない使用 PC のうち、85%は PASII 保存 PC、15%

は血漿保存 PC であった。被験製剤中の血小板数は血漿保存 PC と比較して少なく、PASIII-PC および PR-PASIII-PC の平均値の血漿保存 PC との差は、それぞれ 6%および 11%であった（表 I、$P < 0.001$）。

血小板輸血の有効性

すべての有効性解析を ITT 法および PP 法にて実施した。1-h CCI および 24-h CCI が評価可能であった輸血回数は、それぞれ 1,004 回（88.9%）および 1,013 回（89.7%）であった。CCI-1/24 h が評価不能となった理由は、輸血後血小板数の測定失敗のみであり、このような欠測回数に関して、被験群間、あるいは PP 輸血と治験実施計画書に適合しない輸血の間に有意差はみられなかった。輸血効果のパラメータはすべて、PR-PASIII-PC が劣性であることを示した。PASIII-PC 群と血漿 PC 群の間では、輸血効果に有意差はみられなかった（表 II）。保存期間が 6 日および 7 日であった PC の割合には群間差はみられず、血漿 PC 群、PASIII-PC 群および PR-PASIII-PC 群に輸血された PC のそれぞれ 24%、21%および 26%に相当した。いずれの被験群においても、1-h CCI、24-h CCI とも保存期間が長くなるにつれて低下した。しかし、各保存日数における両 CCI は、PR-PASIII-PC 群が血漿 PC 群より有意に低かった（図 2A、B）。PASIII-PC 群と血漿 PC 群の 1-h CCI および 24-h CCI は、保存期間 7 日まで有意差を示さなかった。輸血 1 時間後および 24 時間後の血小板数を線形回帰分析したところ、PR は血小板数に用量非依存的な影響を示した（図 2C、D、表 III）。製剤関連および患者関連のいくつかの共変量と CCI の関連性は、群に関する補正後に分析した（表 IV）。保存期間、脾腫および発熱は、CCI 低値と有意性の高い関連性を示した一方、ステロイド前投与は 1-h CCI 高値、出血を適応とする輸血は 24-h CCI 低値と関連性を示した。

出血およびその他の臨床的合併症

初回輸血開始以降、試験期間中に認められた新規出血は患者 60 例に発生した計 67 件（CTCAE グレード 1～3）で、PR-PASIII-PC 群では発生頻度（$P = 0.034$）およびグレード（$P = 0.044$）が有意に高かった（表 V）。出血部位の分布には被験群間で差は認められなかった。出血時に抗凝固療法中であった出血例は 14 例で、その割合に群間差は認められなかった。プロトコール治療の期間中に致死性の出血性合併症は認められなかったが、PR-PASIII-PC 群の 1 例は、プロトコール治療中止後に頭蓋内出血により死亡した。血小

板投与量、保存期間およびγ線照射と、出血（全グレード）発生の間に関連性はみられなかった。赤血球輸血回数に関して群間差はみられず、平均回数は血漿 PC 群 4 ± 3 回に対し、PASIII-PC 群および PR-PASIII-PC 群はそれぞれ 5 ± 3 回および 4 ± 3 回であった。患者 25 例に発生した輸血副作用 28 件は大多数が軽度で、有意な群間差はみられなかった（表 V）。感染および SAE の発生率に群間差はみられなかった。SAE のうち PC 輸血に関連するかもしれないと判定されたのは 3 件で、発生件数は各群 1 件であった。血漿 PC 群の 1 例には重度の全身性皮膚反応が発生し、PASIII-PC 群では輸血関連急性肺障害と考えられる症例が 1 件報告された。また、PR-PASIII-PC 群の 1 例には急性声門水腫が発生したが、抗ヒスタミン薬およびステロイドによる治療が奏効した。

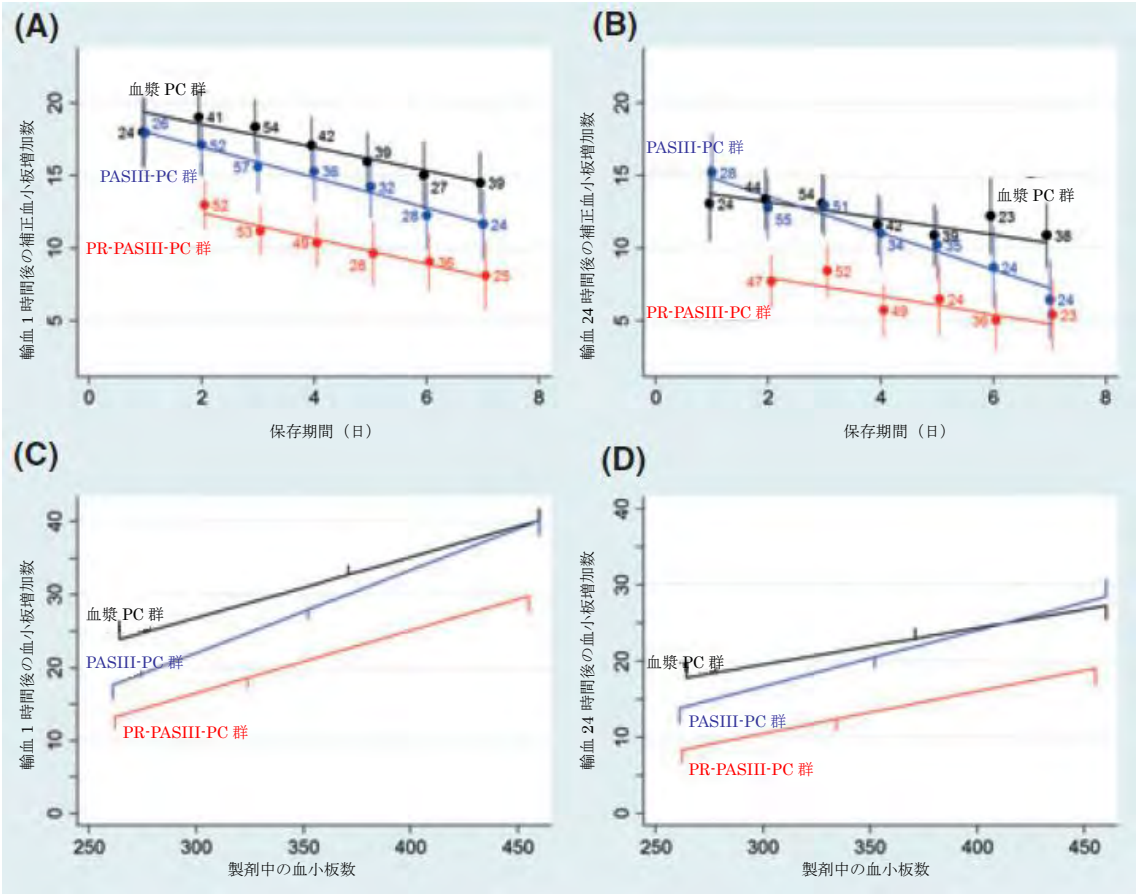

図 2.　治験実施計画書に適合する PC の輸血のみを解析対象（PP）とした線形回帰分析から得られた回帰直線。黒色、青色および赤色はそれぞれ、血漿 PC 群、PASIII-PC 群および PR-PASIII-PC 群を示す。（A、B）3 投与群における 1-h CCI および 24-h CCI と保存期間の関係。点推定値とその 95%信頼区間および輸血回数を示す。直線は、各群の CCI と保存期間が線形関係にあると仮定した回帰直線である。（C、D）体表面積 1.93 m^2、輸血前血小板

数 12 × 10^9 /L の患者に保存期間 4 日の PC を輸血した場合の、3 投与群における輸血 1 時間後および 24 時間後の血小板増加数と PC 製剤中の血小板数の線形関係。エラーバーは標準誤差を示す。

表 III. 輸血 1 時間後および 24 時間後の血小板数に関する線形回帰分析

	輸血1時間後の血小板数		輸血24時間後の血小板数	
	β*	*P*値	β	*P*値
PASIII	-2.29	0.377	1.79	0.507
PR-PASIII	-9.63	0.001	-8.95	0.003
保存期間（日）	-1.55	<0.001	-1.24	<0.001
体表面積（m^2）	-15.4	<0.001	-10.1	0.002
輸血回数（回目）	-0.38	0.047	-0.08	0.686
血小板製剤中の血小板数（×10^9）	0.09	<0.001	0.06	<0.001
輸血前血小板数（×10^9/L）	0.96	<0.001	0.96	<0.001

*β：回帰係数。患者を変量効果、輸血 1 時間後の血小板数（2 および 3 列目）および輸血 24 時間後の血小板数（4 および 5 列目）を従属変数として組み込んだ多変量線形回帰分析。同モデルに組み込んだ因子を 1 列目に示す。回帰係数の推定値をβの列に示す。回帰係数は、当該因子の単位変化あたりの影響の強さを評価するものである。例えば、保存期間が 1 日長くなるごとに、輸血 1 時間後の血小板数が平均 1.55 × 10^9/L 減少する一方、血小板製剤中の血小板数が 1 × 10^9 個増加するごとに、輸血 1 時間後の血小板数が平均 0.09 × 10^9/L 増加する。PASIII および PR-PASIII の回帰係数は、輸血後血小板数の平均値と血漿 PC 群との差を示したものである。

表 IV. 共変量と 1-h CCI および 24-h CCI の関係（群に関する補正後に分析）

	1-h CCI		24-h CCI	
	β*（SE）	*P*値	β（SE）	*P*値
保存期間（日）	-0.9 (0.1)	<0.00001	-0.9 (0.1)	<0.00001
脾腫	-5.7 (1.4)	<0.00001	-6.5 (1.5)	<0.00001
発熱	-1.7 (0.4)	<0.00001	-1.5 (0.4)	0.0003
ステロイド	2.6 (1.1)	0.02	1.0 (1.3)	0.43
出血を適応とする輸血	1.1 (1.0)	0.29	-2.5 (1.1)	0.02
介入を適応とする輸血	-0.6 (0.8)	0.39	-0.4 (0.8)	0.64
年齢（歳）	0.2 (0.3)	0.49	0.0 (0.3)	0.97
性別	1.1 (0.8)	0.17	0.3 (0.8)	0.76
血小板輸血の既往	-1.0 (0.8)	0.22	-1.0 (0.8)	0.24
赤血球濃厚液輸血の既往	-0.9 (0.8)	0.25	-0.7 (0.8)	0.42
輸血副作用の既往	-2.4 (1.5)	0.12	-0.3 (1.7)	0.84
感染	-0.5 (0.5)	0.33	-0.5 (0.5)	0.27
粘膜障害	-0.1 (0.5)	0.82	0.1 (0.5)	0.82
ABO 不適合	0.2 (0.4)	0.68	0.4 (0.4)	0.33
抗ヒスタミン薬	-1.6 (1.3)	0.21	-1.8 (1.3)	0.16
抗凝固薬	-1.3 (1.3)	0.31	-2.1 (1.4)	0.14
アセトアミノフェン	1.1 (1.3)	0.39	-1.3 (1.3)	0.31

群に関する補正後の変量効果モデルによる単変量回帰分析

保存期間および年齢を除くすべての共変量は、「無」または「有」で示される共変量である。
SE、標準誤差、β*：回帰係数

表 V. 出血、輸血副作用、感染および重篤な副作用

患者数	血漿 PC 群 99 例	PASIII-PC 群 94 例	PR-PASIII-PC 群 85 例
初回PC輸血以降の出血			
患者数（%）	19 (19)	14 (15)	27 (32)*
発生件数	19	16	32
最高グレード（%）			
グレード 1	12 (12)	10 (11)	16 (19)
グレード 2	6 (6)	4 (4)	6 (7)
グレード 3	1 (1)	–	5 (6)
輸血副作用の発生患者数、N（%）	11 (11)	8 (9)	6 (7)
輸血副作用の発生件数	13	8	7
事象の重症度			
なしまたは軽度	11	7	6
中等度	1	–	1
重度	1	1	–
感染症を合併している患者数、N（%）	40 (40)	39 (41)	42 (49)
最高グレード（%）			
グレード 1（%）	1	–	–
グレード 2（%）	3	5	6
グレード 3（%）	30	29	28
グレード 4（%）	6	4	8
グレード 5（%）	–	1	–
免疫学的不応状態、N（%）	2 (2)	–	2 (2)
SAE、N	7	3	5
PC 輸血に関連する SAE	1	1	1
死亡、N	3†	1	3

表に示す数値は、出血件数以外は患者数（%）である。出血または感染のグレード評価に関しては、出血または感染が 1 例に複数回発生した場合には、当該患者の最高グレードを使用する。
*P = 0.034（血漿 PC 群との比較）
†血漿 PC 群の 1 例は、最終回（5 回目）の輸血後、重篤な有害事象（SAE）の報告なしに死亡した。死因は基礎疾患の治療に関連すると試験中止報告書に報告され、発熱は敗血症に起因すると考えられた。

考察

我々は、血小板減少症の血液腫瘍患者を選択せずに登録した集団を対象として、PR-PASIII-PC および PASIII-PC の輸血効果を、血小板増加数、輸血無効、PC 消費量、輸血間隔、出血発生、輸血副作用に関して、血漿保存 PC と比較検討した。本試験では、PR-PASIII-PC が輸血効果関連の全エンドポイントに関して劣性を示すという結果が得られたが、この結果は SPRINT 試験と一致するも EuroSPRITE 試験とは異なるものであっ

た（van Rhenen et al, 2003; McCullough et al, 2004）。さらに、PR-PASIII-PC 群は、出血性合併症の発生例数が相対的に多かった。既報のように、両被験製剤中の血小板数は、製造工程で血小板が減少するため、対照製剤より少なかった（McCullough et al, 2004; Kerkhoffs et al, 2006; Murphy et al, 2006; Pineda et al, 2006）。CCI では血小板投与量の群間差が十分に補正されないと考えられたため、投与群、製剤中血小板数および保存期間を共変量として、輸血後血小板数の線形回帰分析を実施したところ、PR-PASIII-PC はやはり独立した影響を示した（Davis et al, 1999）。線形回帰分析を用いた推定では、PR-PASIII-PC を用いて血漿保存 PC と同等の血小板増加数を得るためには、製剤中血小板数を平均 200×10^9 個（すなわち、バフィーコート約 3 単位分）増加させる必要があると考えられた。保存期間と両 CCI の関係は保存期間の 1 日延長ごとに CCI が一定の変化を示したことから、この PR 法での処理後に生存血小板数が一定量減少を示し、その後、生き残った血小板数の正常な消失が生じることが示唆される。PASIII-PC の輸血効果は、保存期間 7 日までは血漿保存 PC と同程度の低下を示し、出血性症状にも差はみられなかった。血小板増加数が減少したという本試験の結果は、SPRINT 試験と一致するものである。EuroSPRITE 試験および大規模第 IV 相試験と異なる結果が得られた理由は、これらの他試験では、対照群の約半数に PASII 保存 PC が使用されたことにより、対照群の結果が減弱したことにあると考えられた（van Rhenen et al, 2003; Osselaer et al, 2009）。

PR-PASIII-PC 群では、他の 2 群いずれと比較しても、出血発生件数およびグレード 2 以上の出血発生件数が多かった。EuroSPRITE 試験および欧州で実施された他のより小規模なランダム化臨床試験では、出血性症状に関する被験群間差は報告されなかった（van Rhenen et al, 2003; Janetzko et al, 2005）。しかし、SPRINT 試験後の安全性報告では、グレード 2～4 の出血発生頻度が PR 群で 43%と、対照群 34%より有意に高いと判断された（$P = 0.02$）（Snyder et al, 2005）。出血性合併症に関する差は、血小板投与量が少ないために輸血後血小板数のピーク値が低くなったことだけでは説明がつかないと考えられる。PR 処理 PC では、約 1/3 の血小板が機能していなかったと推定されるが、血小板投与量は、最近発表された血小板投与量に関する試験の少量群～中等量群に匹敵するものであり、同試験の血小板投与量の少量群、中等量群、大量群間で出血性合併症に関する差はみられなかった（Slichter et al, 2010）。PR による血小板ミトコンドリア核酸の障害は、血小板の生存率のみならず、止血機能も低下させる可能性がある（Keuren et al, 2006; Apelseth et

al, 2007）。PR-PASIII-PC を用いた大規模試験では、輸血副作用に有意差を認めたが（Osselaer et al, 2008a, b）、本試験では確認されなかった。

本試験にはいくつか不十分な点がある

PR-PASIII-PC 群において治験実施計画書に適合しない輸血の回数は、本試験にとって重大な制約と考えられた。しかし、ITT、PP 両解析の結果からは、同様の結論が得られている。本試験が非盲検デザインであったことから出血の評価にバイアスが生じた可能性を完全に排除できないが、本試験の主要エンドポイント（血小板数）には影響を及ぼさなかったと考えられる。

以上のことから、PR 法には明らかな利点があり、輸血の安全性を向上させるためには PR 法の使用が望ましいという主張があるものの、本試験の結果は、同技術をルーチンな手法として導入する前に再評価が必要であることを示している。アモトサレン塩酸塩存在下での UVA 照射による PR 工程はおそらく、血小板生存率を低下させるとともに、高リスク患者における血小板輸血の主要目的である止血機能を低下させる可能性が高い。アモトサレン塩酸塩存在下での UVA 照射による血小板障害がルーチンの輸血業務においてどのように代償できるかを理解するためには、障害の種類と影響を包括的に調査する必要がある。