Transfusion of pathogen-reduced platelet components without leukoreduction

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BACKGROUND: Leukoreduction (LR) of platelet concentrate (PC) has evolved as the standard to mitigate risks of alloimmunization, clinical refractoriness, acute transfusion reactions (ATRs), and cytomegalovirus infection, but does not prevent transfusion-associated graft-versus-host disease (TA-GVHD). Amotosalen–ultraviolet A pathogen reduction (A-PR) of PC reduces risk of transfusion-transmitted infection and TA-GVHD. In vitro data indicate that A-PR effectively inactivates WBCs and infectious pathogens.

STUDY DESIGN AND METHODS: A sequential cohort study evaluated A-PR without LR, gamma irradiation, and bacterial screening in hematopoietic stem cell transplant (HSCT) recipients. The first cohort received conventional PC (control) processed without LR, but with gamma irradiation and bacterial screening. The second cohort received A-PR PC (test) processed without: LR, bacterial screening, or gamma irradiation. The primary efficacy outcome was the 1-hour corrected count increment. The primary safety outcome was treatment-emergent ATR. Secondary outcomes included clinical refractoriness, and 100-day status for engraftment, TA-GVHD, HSCT-GVHD, infections, and mortality.

RESULTS: Mean corrected count increment ($\times 10^3$) of 33 test PC recipients was similar (18.9 ± 8.8 vs. 16.6 ± 8.4; $p = 0.296$) to that of 31 control PC recipients. Test recipients had a reduced, but nonsignificant, incidence of ATR (test = 9.1%, Control = 19.4%; $p = 0.296$). The frequencies of clinical refractoriness (0 of 33 vs. 4 of 31 patients) and refractory transfusions (6.6% vs. 19.3%) were lower in the test cohort ($p = 0.05$ and 0.02), respectively. No patient in either cohort had TA-GVHD. Day 100 engraftment, HSCT-GVHD, mortality, and infectious disease complications were similar between cohorts.

CONCLUSIONS: This study indicated that A-PR PC without LR, gamma irradiation, or bacterial screening is feasible for support of HSCT.

Hematopoietic stem cell transplant (HSCT) recipients require platelet concentrate (PC) transfusion for support of hypoplastic thrombocytopenia.[1–3] Donors are tested for a limited panel of viruses, and PCs are screened for bacterial contamination and irradiated to prevent transfusion-associated graft-versus-host disease (TA-GVHD). To mitigate the risks of acute transfusion reactions (ATRs), alloimmunization, clinical refractoriness, and cytomegalovirus (CMV) infection, PCs are commonly, but not universally, prepared with leukoreduction (LR).[4–6] These interventions require separate procedures, and while reducing the risk of transfusion adverse events (AEs), they do not provide complete protection from ATRs or CMV transfusion-transmitted infection (TTI)[7–10] or the residual risk of window period infections, untested pathogen infections, and emerging pathogen infections.[11–13]

ABBREVIATIONS: AEs = adverse events; A-PR = amotosalen–ultraviolet A pathogen reduction; ANCs = absolute neutrophil counts; ATRs = acute transfusion reactions; CCI = corrected count increment; CIs = confidence intervals; HKRCBTS = Hong Kong Red Cross Blood Transfusion Service; HSCT = hematopoietic stem cell transplant; LR = leukoreduction; PC = platelet concentrate; SAEs = serious adverse events; TA-GVHD = transfusion-associated graft-versus-host disease; TTI = transfusion-transmitted infection.

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Pathogen reduction of PC with amotosalen and UVA light (A-PR; INTERCEPT, Cerus Corporation) mitigates multiple risks of PC transfusion with a single procedure to inactivate viruses, bacteria, protozoa, and leukocytes contaminating PCs. This technology was introduced in Europe in 2003.[14,15] From inception, A-PR replaced PC bacterial culture screening, CMV serology, and gamma irradiation, but due to the established practice of leukocyte reduction, it was used with LR. Postmarketing experience provided evidence indicating A-PR efficacy for the prevention of transfusion-related sepsis and TA-GVHD.[16–18] A-PR with leukoreduced apheresis PC was licensed by the US Food and Drug Administration in 2014,[19] in 2016 A-PR replaced testing for Zika virus,[20] demonstrated efficacy to inactivate emerging pathogens,[21] and is used without bacteria screening.[22]

A-PR inactivates WBC-associated CMV[23] and inhibits WBC antigen presentation[24] WBC cytokine synthesis,[25] and T-cell proliferation.[26,27] Based on these observations and 10 years of experience showing that A-PR reduced the risk of transfusion-related sepsis and TA-GVHD, we postulated that A-PR could be used without LR. As of 2013, the Hong Kong Red Cross Blood Transfusion Service (HKRCBTS) had not implemented routine leukoreduction for conventional whole blood PCs. The HKRCBTS and Queen Mary Hospital decided to evaluate the potential to transition from the current type of PCs, platelet-rich plasma PC suspended in 100% plasma, to A-PR PC in plasma with platelet additive solution without leukoreduction of whole blood PC. We designed a prospective, sequential cohort study in HSCT recipients to characterize the efficacy and safety of A-PR PC without leukoreduction, bacterial screening, or gamma irradiation compared to conventional nonleukoreduced platelet-rich plasma PC, the current HKRCBTS standard of care, to evaluate the potential adoption of A-PR PC without LR into routine practice.

MATERIALS AND METHODS

General study design

We conducted an open-label, prospective, sequential cohort study to enroll approximately 30 HSCT recipients in each cohort. The sequential design facilitated PC inventory management and provided baseline data to characterize corrected count increment (CCI) responses with conventional PC. Cohorts were case matched for primary disease and type of HSCT at a single HSCT clinical center (Queen Mary Hospital, Hong Kong). All PCs were provided by the HKRCBTS. The same primary care physicians managed both cohorts with similar standards of care. The novel intervention was A-PR PC without LR, bacterial culture screening, or gamma irradiation compared to conventional PC without LR, but with bacterial culture screening and gamma irradiation. Both types of PC were licensed for use in Hong Kong. The active transfusion period was up to 28 days or transfusion of up to 5 PCs, with AE monitoring for 7 days after the last PC and follow-up 100 days after HSCT. Patients gave written informed consent to participate in the study, which was conducted in compliance with Good Clinical Practice according to the International Conference on Harmonization guidelines, and local ethical committee and legal requirements consistent with the Declaration of Helsinki.

Platelet components

All donors were tested for human immunodeficiency virus, hepatitis B virus, and hepatitis C virus per HKRCBTS standards. Test PCs were prepared from citrate-phosphate-dextrose solution whole blood as buffy coat platelets with platelet additive solution (SSP+, Macopharma). Five A-, B-, and O-matched buffy coat platelets suspended in nominal 35% plasma and 65% SSP+ were pooled and treated with the INTERCEPT System for platelets by the end of the first postcollection day per manufacturer's instructions to produce 3×10^{11} or more platelets per PC.[19] Test PCs were not leukoreduced, screened for bacteria, or gamma irradiated, and were stored for up to 5 days on a shaker at 22 to 24°C. Pooled test PC contained approximately 5×10^8 WBCs.[28]

Control PCs suspended in plasma, the current HKRCBTS standard of care, were prepared from citrate-phosphate-dextrose solution whole blood by the platelet-rich plasma method without leukoreduction. Five A-, B-, and O-matched concentrates in 100% plasma were pooled without LR to yield 3×10^{11} or more platelets per PC. Control PCs were screened for bacteria with the short term bacterial culture method of HKRCBTS.[29] All control PCs were gamma irradiated (2,500 cGy) and were stored up to 5 days on a shaker at 22 to 24°C. Control pooled PCs contained approximately 5×10^9 leukocytes.[28]

Study procedures

HSCT patients aged 18 years or older expected to require PC were screened for inclusion. Exclusion criteria were prior documented history of clinical refractoriness to platelet transfusion (two successive 1-hour CCI $<5.0 \times 10^3$), immune thrombocytopenia, or disorders confounding 1-hour CCI determination. For both cohorts, a transfusion threshold of 10×10^9/L was specified for clinically stable patients unless adjusted to 20×10^9/L for suspected sepsis. Patients were not screened for HLA antibody at study entry. Patients enrolled in the control cohort were not later reenrolled in the test cohort. Patients enrolled in the test cohort were supported exclusively with A-PR PC. Patients in both cohorts were monitored for AEs during each 48-hour period after PC transfusions, and AEs related to PC transfusion were classified as ATRs. After transfusion, all PC containers were sealed and returned to the HKRCBTS. In the event of a posttransfusion temperature increase of 1°C with rigors or 2°C with or without rigors, the residual PC was cultured and patient blood cultures obtained. For both cohorts, 100 to 120 days' post-HSCT clinical follow-up was performed for health status, engraftment, TA-GVHD, HSCT-GVHD, immune reconstitution, and infectious AE.

Outcome measures

The primary efficacy outcome was the mean 1-hour CCI per patient.[30] Blood samples were obtained from 10 minutes to 4 hours after transfusion to allow a window for acquisition of

platelet counts. Secondary outcomes included the proportion of patients with clinical refractoriness (defined as two successive transfusions with 1-hour CCI <5.0×10^3), and the proportion of transfusions in each cohort with 1-hour CCI less than 5.0×10^3. The primary safety outcome was the incidence of ATR during 48 hours after each study transfusion. Patients in both cohorts were monitored for all AEs in the first 48 hours after each transfusion. Vital signs were measured before and within 6 hours after each study transfusion. AEs attributed to PC were classified as ATRs as defined in prior hemovigilance studies.[17] Patients were monitored for serious adverse events (SAEs) per regulatory criteria[31] and for mortality. AEs and SAEs were classified by preferred term using the Medical Dictionary for Regulatory Activities, version 19.1.

Statistical analyses

The efficacy and safety of leukoreduced pooled buffy coat platelets prepared with A-PR were evaluated in prior randomized controlled studies of patients with neoplastic hematology disorders.[17,30,32] In view of prior clinical experience with A-PR PCs over 10 years, this study was not designed with statistical power to detect differences between cohorts but instead was designed with at least 30 patients per cohort to detect potential signals of impaired efficacy (1-hour CCI <5.0×10^3) and safety (TA-GVHD) for PC prepared without leukoreduction and gamma irradiation. The data consist of quantitative and qualitative descriptors of the population transfused, the number of PC transfusions, the mean patient 1-hour CCI, the proportion of patients with clinical refractoriness, the proportion of transfusions with 1-hour CCI less than 5.0×10^3, and quantification of the incidence and type of observed ATRs, AEs, and SAEs during the active transfusion period and 100-day follow up. All patients were analyzed on an intent-to-treat basis. Point estimates with associated standard errors and treatment differences were computed to provide qualitative comparisons. Descriptive analyses were conducted for demographic and clinical variables. Continuous variables were summarized by means and standard deviations and categorical variables by frequencies and proportions based on nonmissing data. Proportions were analyzed using Fisher's exact test. Treatment differences between test and control PC recipients with 95% confidence intervals (CIs) for the differences were calculated to provide clinical context. P values were computed for information. No adjustments were made for missing data.

In addition to the descriptive analyses, the CIs and CCI endpoints were analyzed using a repeated-measures mixed-effects model to accommodate the longitudinal results over repeated PC transfusions. For CI, the treatment cohort (test vs. control), pretransfusion platelet count, patient weight, transfused dose, and platelet age were included as fixed effects, while the individual patient was selected as the random effect. The CCI endpoint was modeled in a similar fashion with the exception that patient weight and transfused dose were removed as fixed effects since they were used in the CCI derivation. All analyses were conducted with computer software (R, version 3.2.2; and SAS, version 9.4; SAS Institute).

RESULTS

The control cohort of 31 patients received 89 PCs. The test cohort of 33 patients received 76 PCs. All 64 patients completed the active transfusion period and the 100-day follow-up. Baseline population demographics for primary disease, HSCT stem cell source, HSCT type (autologous/allogeneic), HSCT HLA match (related donor, unrelated donor, mismatch) were similar between cohorts except for the proportion of females and a longer average interval to first PC in the test cohort (Table 1). The population consisted primarily of allogeneic HSCT recipients prepared with myeloablative conditioning (control, 17 of 26 vs. test, 23 of 27; Table S1). Prior PC transfusion exposure was greater for the test cohort (Table 1). The duration of PC support, number of transfused PCs, and average time of PC storage prior to transfusion were similar between the cohorts (Table 2).

Efficacy outcomes

Mean platelet doses ($\times 10^{11}$) per transfusion were similar between cohorts (test = 3.6 $\pm$ 0.4 vs. control = 3.5 $\pm$ 0.4; Table 2). Mean pretransfusion platelet counts ($\times 10^9$/L) were low in both cohorts (Table 3; test = 15.3 $\pm$ 11.4 vs. control = 11.1 $\pm$ 6.7). The mean 1-hour CIs per patient (test = 42.5 $\pm$ 21.8 vs. control = 36.5 $\pm$ 19.2), and CCIs (test = 18.9 $\pm$ 8.8 vs. control = 16.6 $\pm$ 8.4) were not different (Table 3, and Fig. 1). Analysis by the repeated-measures mixed-effects model demonstrated similar trends (Table 3). The point estimates for the least squares mean treatment difference (test – control) for CI and CCI were 6.8 (95% CI, −3.0 to 16.7) and 2.4 (95% CI, −1.9 to 6.6), respectively (Table 3). There were no substantial differences in 1-hour CCI for patients with and without HSCT-GVHD or for autologous HSCT (Fig. 1). Four of 31 patients (12.9%) in the control cohort developed clinical refractoriness to platelet transfusions compared to 0 of 33 patients in the test cohort (p = 0.05). Among the control cohort, 19.1% of PC transfusions resulted in CCI responses less than 5.0×10^3 compared to 6.6% of test PC transfusions (p = 0.02). Patients with HSCT-GVHD may have poor responses to platelet transfusion.[33] Thus, we evaluated the 1-hour CCI in this subset of patients and observed that all test cohort patients had mean CCI responses greater than 5.0×10^3, indicative of an effective CCI,[34] while two control patients had mean CCI responses less than 5.0×10^3 (Table S2).

Safety outcomes

During the active transfusion period, ATRs were reported for 3 (9.1%) test and 6 (19.4%) control patients (p = 0.296) (Table 4). All ATRs were of low severity. For test, 2 (6.1%) ATRs consisted of urticaria and one (3.0%) with pyrexia. For control, 5 (15.6%) ATR consisted of urticaria and 1 (3.2%) of chills. In both cohorts, all ATR resolved. During the active transfusion period, no patients died, and none had TA-GVHD. AEs were reported for 4 (12.1%) test and 13 (41.9%) control patients

TABLE 1. Study population patient demographics

	Test (33)	Control (31)	Test-control difference (95% CI)	p value†
Population demographics				
Age (y)	43.3 ± 12.2	47.1 ± 11.1	−3.8 (−9.6 to 2.1)	0.201
Male (%)	18 (54.5%)	11 (35.5%)	NA	0.401
BSA (m*)	1.65 ± 0.20	1.60 ± 0.16	0.04 (−0.05 to 0.13)	0.344
ABO and Rh group and type				
A (%)	7 (21.2%)	7 (22.6%)	NA	0.941
B (%)	9 (27.3%)	10 (32.3%)	NA	
AB (%)	2 (6.1%)	2 (6.5%)	NA	
O (%)	15 (45.5%)	12 (38.7%)	NA	
Rh+ (%)	32 (97.0%)	31 (100.0%)	NA	1.00*
Primary disease				
AL (%)	21 (63.6%)	20 (64.5%)	NA†	1.000*
CL (%)	2 (6.1%)	1 (3.2%)	NA†	
Lymphoma (%)	6 (18.2%)	7 (22.6%)	NA†	
Other* (%)	4 (12.1%)	3 (9.7%)	NA†	
HSCT source				
PBSCs (%)	17 (51.5%)	16 (51.6%)	NA	1.000
Bone marrow (%)	16 (48.5%)	15 (48.4%)	NA	
Autologous (%)	6 (18.2%)	5 (16.1%)	NA	1.000
Allogeneic (%)	27 (81.8%)	26 (83.9%)	NA	
Related (%)	19 (57.6%)	17 (54.8%)	NA	
Unrelated (%)	8 (24.2%)	9 (34.6%)	NA	
HSCT HLA match status				0.491
Matched related	19 (70.4%)	17 (65.4%)		
Matched unrelated	6 (22.2%)	8 (30.8%)		
HLA mismatched (%)	2 (3.8%)	1 (3.8%)	NA	
Prior PC transfusion	28 (84.0%)	16 (53.0%)	NA	0.012
Days to first study PC	9.6 ± 3.7	7.0 ± 3.7	−2.6 (−0.8 to 4.5)	0.006

Continuous variables are summarized by the mean ± standard deviation.
AL = acute leukemia (AML and ALL); BSA = body surface area; CI = confidence interval; CL = chronic leukemia (CLL and CML); HSCT = hematopoietic stem cell transplant; NA = treatment difference not applicable; PBSCs = peripheral blood stem cells; PC = platelet concentrate.
* Other = solid tumor, multiple myeloma, and myelodysplasia.
† P values are based on a t test (with unequal variances) and Fisher's exact test for continuous and categorical variables, respectively.

TABLE 2. Patient exposure to study platelet components

	Test	Control	Difference (95% CI)	p value
Number of patients	33	31		
Number of platelet transfusions	76	89		
Duration of support (days)	8.3 ± 8.7	7.4 ± 6.3	0.9 (−2.8 to 4.7)	0.618
Median days of PC support	6.0 (1–35)	6.0 (1–23)	NA	NA
PC transfused (N)	2.3 ± 1.4	2.9 ± 1.5	−0.6 (−1.3 to 0.2)	0.129
Median (N) PC transfused	2.0 (1–5)	2.0 (1–5)*	NA	NA
Mean PC platelet dose ($\times 10^{11}$)	3.6 ± 0.4	3.5 ± 0.4	0.1 (−0.1 to 0.3)	0.232
PC storage duration (days)	3.0 ± 0.7	3.0 ± 0.2	0.0 (−0.2 to 0.3)	0.860

Data expressed as mean ± standard deviation.
NA = treatment difference not applicable; PC = platelet concentrate.
* Range (minimum and maximum).

(p = 0.021). One control patient had an SAE unrelated to PC (Table 4). The most common AEs were chills and pyrexia in seven control patients, urticaria in five control and two test patients, HSCT-GVHD in one control patient, and sepsis unrelated to PC in two test patients. There were no hemorrhagic AEs reported in either cohort.

Health status at 100-day follow-up

At 100-day follow-up, three test patients (9.1%) and two control patients (6.5%) were deceased. The causes of death for test patients were relapsed primary disease (two) and primary graft failure with sepsis (one). For test patients, death occurred on Study Days 21, 69, and 96. The causes of death for control patients were sepsis (one) and pneumonia (one). For the control patients, death occurred on Study Days 47 and 111. No deaths between the active transfusion period and the 100-day follow-up were attributed to PC exposure. Five test patients had primary disease relapse compared to two control patients.

To examine the possibility that GVHD observed at Day 100 was a delayed form of TA-GVHD, we examined individual absolute neutrophil counts (ANCs) and platelet counts at Day

TABLE 3. Mean per-patient 1-hour CI and CCI responses to study platelet transfusions

	Test (N = 33)	Control (N = 31)	Difference (95% CI)	p value
Pre platelet count ($\times 10^9$/L)	15.3 ± 11.4	11.1 ± 6.7	4.2 (−0.5 to 8.8)	0.080
1-hr platelet count ($\times 10^9$/L)	57.8 ± 24.4	47.7 ± 21.4	10.1 (−1.4 to 21.5)	0.085
1-hr CI ($\times 10^9$/L)	42.5 ± 21.8	36.5 ± 19.2	5.9 (−4.3 to 16.2)	0.254
Median, range 1-hr CI	36.7 (11.5–113.0)	36.5 (−0.2–78.0)	6.8 (−3.0 to 16.7)*	0.173*
1-hr CCI ($\times 10^3$)	18.9 ± 8.8	16.6 ± 8.4	2.3 (−2.0 to 6.5)	0.296
Median, range 1-hr CCI	19.4 (6.6–41.6)	18.4 (−0.1–34.4)	2.4 (−1.9 to 6.6)*	0.272*
1-HR CCI <5.0 ($\times 10^3$)	6.6%	19.1%		0.022†

Data expressed as mean ± standard deviation.
CCI = corrected count increment; CI = confidence interval.
* Assessed by repeated measures (test-control).
† Fisher's exact test.

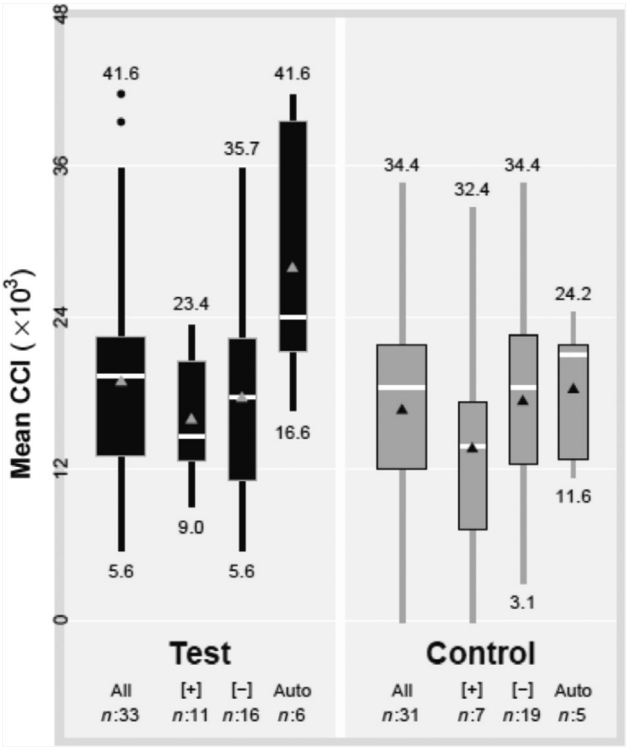

Fig. 1. Mean 1-hour CCI responses for test and control cohort patients during the active transfusion period. Data and number of patients (n) are presented for all patients, allogeneic HSCT test and control patients with GVHD(+), for allogeneic HSCT test and control patients without GVHD(−), and for autologous HSCT test and control patients. Mean values are represented by the ▲, median values are represented by the bar, and the interquartile ranges with outliers are indicated.

100 or last available follow-up (Table S2). Two test patients had ANC less than 0.5×10^9/L. Patient 121 with Grade 3 GVHD was in remission with a platelet count greater than 20×10^9/L and alive at Day 525 after HSCT. Patient 142 had Grade 4 GVHD, with relapsed acute myeloid leukemia, and died on Study Day 69. The mean ANC ($\times 10^9$/L) at Day 100 for test and control patients with GVHD (2.0 ± 1.3) and those without GVHD (2.1 ± 2.1) were similar (Fig. S1). One test patient (ID 143) with Grade 2 GVHD and a Day 100 platelet count less than 20×10^9/L had previously engrafted and was alive without relapse at Day 211. The mean platelet counts (10^9/L) at Day 100 for test and control patients with GVHD (63.8 ± 48.1), and for those without GVHD (114.7 ± 68.9) indicated reconstitution of endogenous thrombopoiesis (Table S2 and Fig. S2).

TABLE 4. Treatment-emergent AEs reported per patient during the active transfusion period*

	Test (N = 33)	Control (N = 31)	p value
All grades of AE	4 (12.1%)	12 (38.7%)	0.021
Excluded as related to PC	1	6	NA
Related to PC exposure	3	6	NA
Medication required	3	7	NA
Recovered from AE	3	12	NA
Recovered with sequelae	1	0	NA

AE = adverse event; PC = platelet concentrate.
* The relationship of AEs to PC transfusion during the 48-hour period after PC transfusion was assessed by primary care physicians. All AEs defined as possibly, probably, or certainly related were classified as related to PC exposure.

Neutrophil engraftment, ANC of 0.5×10^9/L or greater for 3 days, was attained in 97% of test and 100% of control patients. Platelet engraftment and platelet count of 20×10^9/L or greater for 3 days without PC transfusion was attained in 87.9% of test and 90.3% of control patients. The median times to neutrophil engraftment and platelet engraftment were similar in the cohorts (Table S3). The mean ANCs and mean platelet counts of test and control patients were similar at 100-day follow-up (Figs. S1 and S2). Furthermore, ANC and platelet count levels were similar between cohorts for patients with and without HSCT-GVHD (Figs. S1 and S2). Immune reconstitution at Day 100 by most proximate serum IgG and IgM levels (Table S4) for test (n = 27) and control (n = 22) patients with data were within adult reference ranges (IgG, 700–1600 mg/dL; IgM, 40–230 mg/dL).

No patients had a diagnosis of TA-GVHD at the 100-day follow-up. Eleven of 27 (40.7%) test patients had HSCT-GVHD, compared to 7 of 26 (26.9%) control patients

TABLE 5. Infectious AEs after HSCT through 100-day follow-up

Clinical status	Test (33)	Control (31)	p value
Infectious AE	22 (66.7%)	13 (41.9)	0.078 *
Serious infectious AE	9 (27.3%)	6 (20.0%)	0.564 *
Recovered from AE	27 (81.8%)	9 (77%)	
Recovered with sequelae	1 (3.0%)	1 (3.3%)	
Death from infectious AE	1 (3.0%)	3 (10.0%)	

AE = adverse event; HSCT = hematopoietic stem cell transplant.
* Fisher's exact test.

(p = 0.387). In the test cohort, maximum severity was Grade 2 for seven patients, Grade 3 for three patients, and Grade 4 for one patient. In the control cohort, the maximum severity was Grade 2 for five patients, Grade 3 for one patient, and Grade 4 for one patient. In both cohorts the most common organs involved were skin, bowel, and liver.

A substantial proportion of patients had an infectious AE following HSCT through 100-day follow-up (Table 5), and more test patients had infectious AEs reported than did control patients. The primary difference between the cohorts was due to laboratory detection of CMV antigen (5 of 33 test vs. 0 of 31 control). However, the AE of active CMV infection requiring therapy was not different between cohorts (6 of 33 test vs. 7 of 31 control). The detection of CMV antigen was most likely due to prior infection based on high prevalence of CMV in the Hong Kong population. The incidence of infectious SAE was not different between cohorts (Test, 27.3%; control, 20.0%). Most patients recovered from infectious SAEs, and only a small proportion in each cohort died as the result of an infectious AE (Table 5).

DISCUSSION

LR of PC is a common practice for HSCT patients to reduce the risk of alloimmunization, clinical refractoriness,[5,35] transfusion reactions,[36] and TTI CMV infection.[37] However, it is not completely effective in mitigating these adverse transfusion effects. In a study of LR and ultraviolet B light irradiation, lymphocytotoxic antibodies were reduced, but 17% to 18% of patients exposed to leukoreduced PC developed antibodies, and 7% to 8% of all patients in the LR cohorts developed refractoriness to PC transfusion.[5] A large retrospective study confirmed that LR decreased the incidence of alloimmunization and clinical refractoriness, but with substantial residual frequencies for both outcomes in patients with hematologic malignancy or HSCT.[35] Similarly, prestorage LR of PC has demonstrated efficacy to reduce the incidence of a wide array of ATRs, but with residual incidence of 11.4% to 13.3%.[36] Although LR results in the removal of approximately 2 log of cell-associated CMV, it is not completely effective for prevention of CMV TTI.[7,38,39] Importantly, LR alone is not sufficiently effective for prevention of TA-GVHD.[40,41]

A-PR of PC offers the potential to reduce further the risk of ATR, CMV TTI, and emerging pathogen TTI, and has been utilized in conjunction with LR for more than 10 years in Europe.[18] This technology inactivates greater than 4 log of both cell-free and cell-associated CMV with prevention of CMV TTI in sensitive murine models.[23,42] In addition, A-PR inhibits WBC cytokine synthesis and has demonstrated a reduction in ATR.[25,43] Murine donor splenocytes treated with low levels (2–20 nm) of amotosalen and ultraviolet A failed to stimulate responder T cells, did not elicit proliferative responses, and prevented TA-GVHD.[26] in vitro T-cell activation antigen expression was inhibited by amotosalen–ultraviolet A treatment.[44] In a large randomized clinical trial, recipients of A-PR PC who developed clinical refractoriness showed a reduction in lymphocytotoxic antibodies.[45] Most importantly, A-PR effectively inactivates greater than 5 log of T cells[27] and has been adopted for prevention of TA-GVHD in place of nuclear source irradiation in Europe and the United States.[18,46]

To date, all clinical trials and postmarketing studies of A-PR were conducted with LR. The current study was intended to assess the efficacy and safety of A-PR PC without LR, gamma irradiation, or bacterial screening to support routine implementation. In vitro bacterial inactivation data and extensive postmarketing experience supported the use of A-PR to replace bacterial screening in Europe and the United States.[14,15,47–49] Robust inactivation of high levels of T cells and more than 10 years of clinical experience with A-PR PC with LR in place of gamma irradiation for prevention of TA-GVHD indicated the potential for the use of A-PR without LR.

In view of extensive prior clinical experience evaluating the hemostatic efficacy and safety of A-PR PC,[18,30,32,45] we elected to evaluate A-PR PC without LR in a limited HSCT patient population at risk for TA-GVHD. The test intervention selected was the buffy coat PC method with platelet additive solution because the HKRCBTS sought to evaluate pathogen reduction in place of LR using a process well characterized in Europe, and prior experience had shown that ultraviolet B light was effective to reduce alloimmunization.[5] The observation of a trend toward reduced allergic transfusion reactions with test PC was not unexpected, with reduced exposure to plasma proteins compared to the control suspended in 100% plasma. We selected the 1-hour CCI to evaluate transfusion efficacy and ATR to evaluate safety. Both measures have been used to assess transfusion responses and safety of LR in prior studies.[5] To our knowledge, this is the first study conducted with A-PR without LR, bacterial culture screening, or gamma irradiation for transfusion of PC.[9] The test and control cohorts had similar primary disease and HSCT profiles with minimal demographic differences, and most received allogeneic grafts with myeloablative conditioning. Of interest, the proportion of patients with clinical refractoriness and the proportion of refractory transfusions were lower in test cohort patients. The mean 1-hour CI and CCI responses

were similar between cohorts and consistent with 1-hour CCI responses observed in other studies of HSCT patients transfused with LR gamma-irradiated PC.[2,5] The safety profiles of test and control PC recipients were not substantially different. The 100-day follow-up confirmed that the majority of patients in both cohorts achieved engraftment. Deaths up to Day 100 were attributed to expected causes: disease relapse, HSCT graft failure, and bacterial infections not related to PC exposure. No patients in either cohort had TA-GVHD. The 100-day ANC and platelet count data indicated that the GVHD observed in both cohorts was related to HSCT and was not clinically consistent with a diagnosis of TA-GVHD bone marrow failure.

More patients in the test cohort had HSCT-GVHD, generally of Grade 2 severity. Of interest, all of the test cohort patients with GVHD had average 1-hour CCI responses greater than 5.0×10^3 during the active transfusion period, while two of the control cohort had mean 1-hour CCI responses less than 5.0×10^3, indicative of clinical refractoriness to platelet transfusion. The incidence of clinical infectious AE through Day 100 follow-up was not substantially different between the cohorts, and the majority of patients recovered without sequelae.

The primary limitation of this study was size. However, conduct of a small study met the objectives of the primary care physicians (J.S. and A.L.) and the HKRCBTS to evaluate nonleukoreduced A-PR PC. The study was not designed to conclusively demonstrate prevention of TA-GVHD, but the patient population enrolled was at risk for TA-GVHD, and extensive prior experience with leukoreduced A-PR PC had shown efficacy for prevention of TA-GVHD in at-risk patient populations.[46] The primary care physicians concluded that 30 patients per cohort primarily treated with ablative HSCT conditioning would be sufficient to characterize clinical CCI responses, ATR incidence, and to detect TA-GVHD, if A-PR failed to be protective. Despite the limited study size, we did detect multiple 1-hour CCI responses less than 5.0×10^3 and patients with clinical refractoriness. HLA antibody status was not measured during the study, and thus we could not determine the incidence of immune-mediated refractoriness. Hence, the study size does not allow a definitive conclusion that A-PR PC without LR are superior to conventional PC without LR for either transfusion efficacy or alloimmune refractoriness. Prior large studies comparing CCI responses of A-PR PC with untreated PC have shown lower CCI responses; thus, we cannot conclusively generalize the observations from this study to indicate no difference in CCI responses with A-PR PC.[45] Nonetheless, within the scope of this study, we did not detect any differences in efficacy or unexpected safety signals in the health status of HSCT recipients supported with nonleukoreduced A-PR PC. Surveillance of a larger population supported with A-PR PC without LR during routine use will be useful to extend the safety profile of nonleukoreduced A-PR PC. Within the limitations of this study, replacement of LR, bacterial culture screening, CMV serology, and gamma irradiation with A-PR whole blood–derived PC appears feasible and offers the potential benefit to reduce the cost of PC transfusion, simplify PC production and inventory, and reduce the risk of TTI from a broad spectrum of known and emerging pathogens.

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CONFLICT OF INTEREST

JS, WCT, CKL, RL, CCKL, and AKWL have disclosed no conflicts of interest. Cerus Corporation, manufacturer of the INTERCEPT Blood Systems for Platelets, provided institutional research support for performance of the study. CK, AYL, NH, RJB, HJV, AS, and LC are employees of Cerus Corporation and beneficial owners of Cerus equities or options during the conduct of this study, analysis of the data, and preparation of the manuscript. JS, WCT, CKL, RL, CCKL, and AKWL conducted the clinical study. JS, WCT, and AKWL determined the scope and design of the study. CK and AYL monitored study data. NH designed the data collection methods and performed the biostatistics analysis. HJV transferred the A-PR technology and the production of whole blood–derived buffy coat PC to the HKRCBTS. AS cowrote the clinical protocol, monitored PC production, and coordinated interactions with the sponsor. RJB reviewed study data and edited the manuscript. LC wrote the clinical trial protocol, reviewed clinical data, and wrote the manuscript.

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SUPPORTING INFORMATION

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

Table S1. Conditioning regimen for HSCT patients in control and test cohorts.
Table S2. 1- HR CCI responses during the active transfusion period, Day 100 ANC, and Day 100 platelet counts for patients with HSCT-related GVHD.
Table S3. Time to neutrophil and platelet engraftment and 100-day ANC and platelet count.
Table S4. Serum IgG and IgM levels at 100-day follow-up.
Fig. S1. Absolute neutrophil counts for test and control cohort patients at Day 100 follow-up.
Fig. S2. Platelet counts for test and control cohort patients at Day 100 follow-up.