Received: 1 April 2022 | Revised: 15 May 2022 | Accepted: 25 May 2022

DOI: 10.1111/trf.17003

BLOOD COMPONENTS

TRANSFUSION

Evaluation of amotosalen and UVA pathogen-reduced apheresis platelets after 7-day storage

Jose A. Cancelas[1] | **Jamie R. Genthe**[2] | **Moritz Stolla**[3,4] | **Neeta Rugg**[1] | **S. Lawrence Bailey**[3] | **Shawnagay Nestheide**[1] | **Beth Shaz**[5] | **Samantha Mack**[6] | **Kadi Schroeder**[6] | **Waseem Anani**[7] | **Zbigniew M. Szczepiorkowski**[8,9] | **Larry J. Dumont**[6] | **Subramanian Yegneswaran**[2] | **Laurence Corash**[2] | **Nina Mufti**[2] | **Richard J. Benjamin**[2] | **Anna C. Erickson**[2]

[1]Hoxworth Blood Center, Cincinnati, Ohio, USA

[2]Cerus Corporation, Concord, California, USA

[3]Bloodworks Northwest, Seattle, Washington, USA

[4]Division of Hematology, Department of Medicine, University of Washington Medical Center, Seattle, Washington, USA

[5]Duke University, Durham, North Carolina, USA

[6]Vitalant Research Institute, Denver, Colorado, USA

[7]Canadian Blood Services, Ottawa, Canada

[8]Dartmouth Hitchcock Medical Center, Lebanon, New Hampshire, USA

[9]Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Correspondence
Anna C. Erickson, Cerus Corporation, 1220 Concord Ave Suite 600, Concord, CA 94520, USA.
Email: aerickson@cerus.com

Abstract

Background: Amotosalen/UVA pathogen-reduced platelet components (PRPCs) with storage up to 7 days are standard of care in France, Switzerland, and Austria. PRPCs provide effective hemostasis with reduced risk of transfusion-transmitted infections and transfusion-associated graft versus host disease, reduced wastage and improved availability compared with 5-day-stored PCs. This study evaluated the potency of 7-day PRPCs by *in vitro* characterization and *in vivo* pharmacokinetic analysis of autologous PCs.

Study Design and Methods: The *in vitro* characteristics of 7-day-stored apheresis PRPCs suspended in 100% plasma or 65% platelet additive solution (PAS-3)/35% plasma, thrombin generation, and *in vivo* radiolabeled post-transfusion recovery and survival of 7-day-stored PRPCs suspended in 100% plasma were compared with either 7-day-stored or fresh autologous conventional platelets.

Results: PRPCs after 7 days of storage maintained pH, platelet dose, *in vitro* physiologic characteristics, and thrombin generation when compared to conventional 7-day PCs. *In vivo*, the mean post-transfusion survival was 151.4 ± 20.1 h for 7-day PRPCs in 100% plasma (Test) versus 209.6 ± 13.9 h for the fresh autologous platelets (Control), (T-ΔC: 72.3 ± 8.8%: 95% confidence interval [CI]: 68.5, 76.1) and mean 24-h post-transfusion recovery 37.6 ± 8.4% for Test versus 56.8 ± 9.2% for Control (T-ΔC: 66.2 ± 11.2%; 95% CI: 61.3, 71.1).

Discussion: PRPCs collected in both 100% plasma as well as 65% PAS-3/35% plasma and stored for 7 days retained *in vitro* physiologic characteristics. PRPCs stored in 100% plasma for 7 days retained *in vivo* survival. Lower *in vivo* post-radiolabeled autologous platelet recovery is consistent with reported reduced count increments for allogenic transfusion.

KEYWORDS
amotosalen, pathogen-reduced, platelets, recovery and survival

1 | INTRODUCTION

Platelet components (PCs) treated with amotosalen and UVA-light pathogen reduction (INTERCEPT Blood System for Platelets; Cerus) were shown in multiple randomized controlled clinical trials to provide effective clinical hemostasis for patients with thrombocytopenia.[1–7] Postmarket surveillance data support efficacy in reducing the risk of bacterial sepsis,[8] preventing HIV transmission,[9] and utility in allowing continued platelet collection and distribution in the face of local viral epidemics for which no viral marker tests were available.[10,11] Preclinical and postmarket data support the product claims of reducing the risk of a broad-spectrum of pathogen transmission including cytomegalovirus, sepsis, and transfusion-associated graft versus host disease.[12–18] Amotosalen–UVA pathogen-reduced PCs (PRPCs) are now routinely transfused in >50 countries and represent all PCs currently transfused in France, Belgium, Switzerland, Iceland, and Kuwait, and the majority of PCs in Austria and other countries, establishing a new standard of care.

The FDA approved PRPCs in December 2014 for the treatment of apheresis platelets collected in 65% PAS-3/35% plasma, and in March 2016 for the treatment of apheresis platelets suspended in 100% plasma with storage for up to 5 days. INTERCEPT processing kits for treating ~725,000 PCs were sold in the United States from October 2021 to March 2022, suggesting that the majority (~58%) of US PCs are now PRPCs, assuming an annual collection of ~2.5 million whole blood or apheresis PC equivalents.[19]

In France, Austria, and Switzerland, PRPCs are routinely stored up to 7 days after collection. Postmarket studies have documented clinical efficacy with reduced wastage and increased PC availability when compared with historical 5-day-stored PCs. Furthermore, in routine practice there has been minimal change in RBC, plasma, or platelet usage attributed to the introduction of PRPCs, despite evidence of reduced platelet count increments when compared with untreated conventional platelets.[20–23]

The therapeutic efficacy of PRPCs stored for 6–7 days is supported by a Phase 3 RCT[5] and by postmarket experience.[20–25] Further studies presented in this manuscript support transfusion of PRPCs through 7 days of storage: Phase 1 studies evaluated the post-storage *in vitro* platelet properties of apheresis PRPCs suspended in 65% PAS-3/35% plasma or 100% plasma (Test) compared to untreated conventional platelets from the same donor (Control) and stored for 7 days; thrombin generation of conventional or PRPCs stored for 7 days in 100% plasma; and a Phase 2 study comparing the *in vivo* post-infusion radiolabeled recovery and survival of PRPCs suspended in 100% plasma and stored for 7 days, with fresh autologous platelets prepared from platelet-rich plasma (PRP) isolated from whole blood. This manuscript summarizes and integrates the experience with PRPC stored >5 days and was designed to evaluate the hypothesis that single-donor derived PRPCs stored for 7 days retain sufficient function for therapeutic transfusion efficacy.

2 | MATERIALS AND METHODS

2.1 | *In vitro* study design

Phase 1 *in vitro* studies were designed as prospective, randomized, open-labeled, paired, controlled, crossover studies of apheresis PCs in 65% PAS-3 (PAS-C)/35% plasma collected on the Amicus separator (Fresenius Kabi), or in 100% plasma collected on the Trima separator (Terumo BCT). The PAS-3 study was performed at Versiti Blood Center of Wisconsin, Dartmouth Hitchcock Medical Center, and New York Blood Center, and the 100% plasma study was performed at Hoxworth Blood Center, and Vitalant Research Institute. Protocols were conducted with institutional review board approval. The population consisted of healthy subjects who met the FDA, AABB, and site/institutional eligibility criteria for autologous apheresis platelet donation and the study inclusion/exclusion criteria.

Each study subject donated a single- or double- apheresis PC during each of two consecutive donation periods. Components were randomized for preparation as untreated Control PCs or Test PRPCs and processed with Small Volume (SV), Large Volume, or Dual Storage (DS) INTERCEPT Platelet Processing sets, the INT100 Illuminator, and compound adsorbing device per the manufacturer's instructions (Figure 1A). Test platelets were stored in storage containers integral to the pathogen reduction processing sets (Figure 1B). Control platelets were stored in the apheresis manufacturers' storage containers. All PCs were stored at 22 ± 2°C with agitation and evaluated for *in vitro* platelet function on Day 0/1 (Input), Day 5, and Day 7 of storage.

The primary endpoints were (i) the proportion of Test PCs with $pH_{22°C}$ ≥6.2, and (ii) platelet dose of ≥3.0×10^{11} platelets per PC. Other platelet characteristics were measured as previously described.[26–34] Baseline adjusted cell-free lactate dehydrogenase (LDH) was

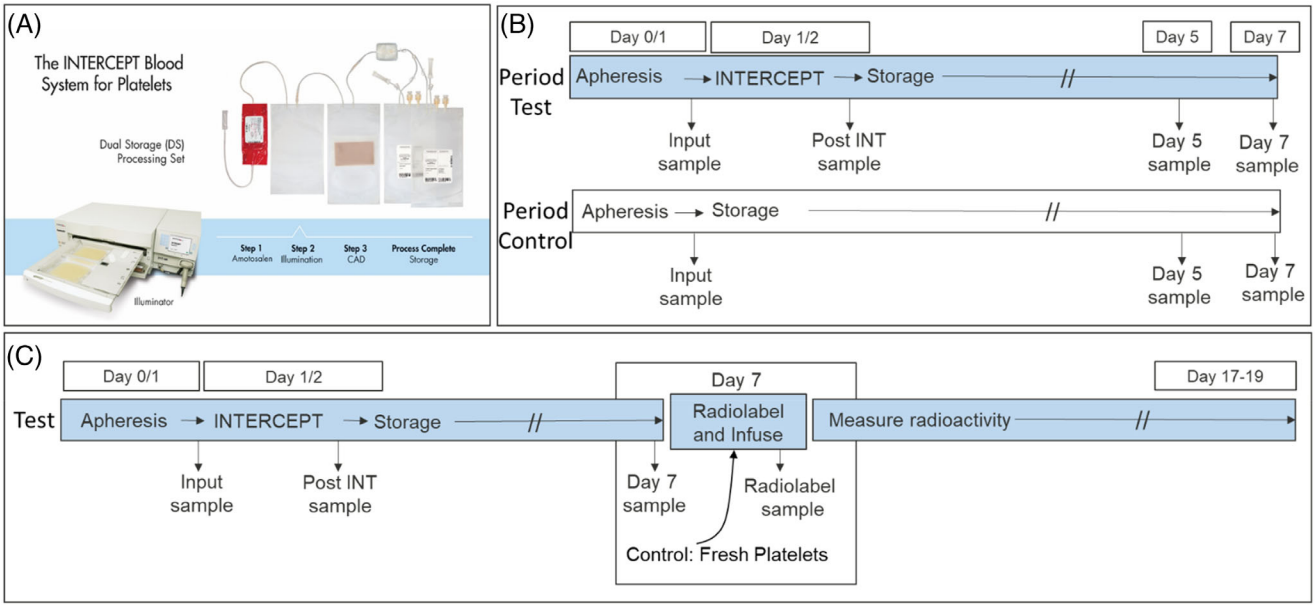

FIGURE 1 INTERCEPT Blood System for Platelets process (A) and study schemas for the *in vitro* studies (B) and the *in vivo* recovery and survival study (C). Test = INTERCEPT treated platelet components. Control = untreated platelet components [Color figure can be viewed at wileyonlinelibrary.com]

determined as the proportion (%) of total LDH calculated as follows: supernatant Day 5 or 7 LDH − baseline LDH (Day 0/1)/total LDH, where total LDH was measured after 100% induced cellular lysis by Triton-X treatment.[35]

2.2 | Thrombin generation

Thrombin generation of stored Test (INTERCEPT) and Control (untreated) PCs was measured in a separate experiment performed at Cerus Corporation. ABO matched apheresis PCs collected on the Trima apheresis separator suspended in 100% plasma were pooled (Input) and split into two components; one was untreated (Control), and the other was treated with the INTERCEPT SV platelet processing set (Test). Both Test and Control PCs had input platelet doses of 4.7 $\pm$ 0.3 $\times$ 10^{11} platelets and were stored with constant agitation for 7 days at 22 $\pm$ 2°C. Samples from six replicates (both Test and Control) were analyzed on Day 1 (Input), Day 5, and Day 7 of storage for thrombin generation capacity in a calibrated automated thrombogram assay (CAT; Stago). Thrombin levels were calibrated against an internal standard (CAT; Stago). Platelet thrombin generation was induced by the Stago PRP-Reagent which includes 5 pM of Tissue Factor and a minimal amount of phospholipid. Lag time, peak height, and endogenous thrombin potential (ETP) were assessed using platelets as the source of phospholipid.

2.3 | *In vivo* study design

The *in vivo* Phase 2 investigation was a randomized, multicenter, controlled, *in vivo* study of the recovery and survival of PRPCs suspended in 100% plasma and stored for 7 days before transfusion compared to paired donor fresh autologous platelets. The study was performed at two study sites: Bloodworks Northwest and Hoxworth Blood Center. Subjects donated a single- or double- apheresis PC, which were treated with the INTERCEPT DS set and stored for 7 days from donation (Test; Figure 1A). All females had a negative pregnancy test before any radiolabel infusion. *In vitro* platelet function was evaluated on Day 0/1 and Day 7 of storage.

Radiolabeling studies were performed using a variation on the BEST method[36–38] that excludes the RBC depletion and addition of ACD-A steps during the preparation of the apheresis Test platelets for radiolabeling, to minimize the loss of platelets. Validation studies confirmed the absence of increased RBC contamination using this preparation method. Test platelets stored for 7 days, and fresh Control platelets were radiolabeled according to randomization assignment with either ^{51}Cr (sodium radiochromate—$Na_2{}^{51}CrO_4$) or ^{111}In (indium oxine). Physical recovery of the Test PC aliquots used for radiolabeling after 7-day storage were assessed by comparing the platelet dose at input and immediately before the addition of the radiolabel to assess platelet loss during the radiolabeling process. Radiolabeled Test and Control platelets were combined, enabling simultaneous

administration into the subject through intravenous infusion (4–14 mL) into a peripheral vein.

The subjects had post-infusion blood samples (10–14 mL) collected at 1-h (±15 min), at 2-h (±15 min) post-infusion, and on Days 1, 2, 3, 5 ± 1, 7/8, and 11 ± 1 post-infusion within ±4 h of the initial infusion time (Figure 1C). Post-infusion platelet recovery (%) and survival (h) were estimated using a multiple-hit gamma-function model.[39] Post-infusion blood samples were corrected for plasma-associated radioisotope, spontaneous radiolabel elution, and RBC-associated radioisotope.[28]

The primary endpoints for the Phase 2 study, post-infusion recovery and survival of PRPCs at Day 7, were assessed using the FDA-required criteria: "fresh" autologous platelets, drawn and prepared on day of reinfusion using the BEST method of processing.[29,30]

2.4 | Statistical analysis

Data were summarized using descriptive statistics (e.g., mean, SD, median, minimum, and maximum for continuous data, frequencies, and percentages for categorical data) across all study sites and within each study site (SAS; SAS Institute). A two-sided 95% confidence interval (CI) for the mean for continuous parameters and by frequencies and percentages for categorical data variables was calculated and used to determine statistical significance between Test and Control platelets. Lactate, glucose, ATP, and supernatant LDH were normalized to the platelet content and were calculated using regression analysis. Recovery and survival were calculated from specific activities using the nonlinear multiple-hit regression model using time points greater than 20 h. Comparisons against FDA acceptance criteria were performed as previously described.[36,37] The acceptance criteria were: the lower bound of a two-sided 95% CI for the mean treatment difference in post-infusion recovery (Test 0.66 × Control) is ≥0 and in post-infusion survival (Test 0.58 × Control) is ≥0.

Data were graphed in SAS, Microsoft Excel, and/or GraphPad. Summary statistics for *in vivo* recoveries, *in vivo* survivals, and *in vitro* characteristics, were prepared in SAS. Significance threshold was *p* values of not more than 0.05.

3 | RESULTS

3.1 | *In vitro* quality of PRPCs stored for 7 days in 65% PAS-3/35% plasma

Post collection (Day 0/1), Test and Control PCs demonstrated no significant differences (Table S1). Following amotosalen–UVA treatment, the mean (±SD) platelet dose recovery was 87.3 ± 7.1% for Test components. On Day 7, Test components demonstrated no treatment differences compared to Control for pH, volume, platelet count, or platelet dose (compensated for sampling). All (72/72) of the Test units retained $pH_{22°C}$ ≥6.2; 91.7% (66/72) of Test components retained ≥3.0×10^{11} platelets. Test components exhibited lower extracellular glucose, higher lactate levels, and similar total adenosine triphosphate (ATP) compared to Controls (Figure 2E,F,H; Table S1), indicating that active metabolism was maintained up to 7 days of storage. Indices indicative of *in vivo* viability were maintained. Test components had higher morphology scores on Day 5 (280 ± 27 vs. 266 ± 29) and on Day 7 (265 ± 27 vs. 255 ± 32) than Controls ($p < 0.05$) with increase on Day 5 (174.8 ± 6.4% vs. 15.7 ± 5.9%, $p < 0.05$) or no significant changes on Day 7 of storage ($p > 0.05$) for extent of shape change (ESC) and hypotonic shock response (HSR) scores (Figure 2A–C). Platelet lysis as determined by the baseline adjusted cell-free LDH normalized level of supernatant LDH (%) was lower in the Control group on Day 5 (test: 3.2 ± 2.0%; control 2.0 ± 1.2%, $p < 0.05$) and similar between Test (4.1 ± 2.3%) and Control (4.3 ± 3.4%) PCs on Day 7, indicating the presence of a modest storage dependent platelet injury for both Test and Control (Figure 2D). On Day 7, P-selectin expression showed that Test (44.6 ± 12.4%) was higher compared to Control (37.4 ± 15.1%, $p < 0.05$) (Figure 2G).

3.2 | *In vitro* quality of PRPCs stored for 7 days in 100% plasma

Post-collection and pre-treatment (Day 0/1), the Test and Control components demonstrated no significant differences (Table S2). Following amotosalen–UVA treatment, the mean ± SD platelet dose recovery was 84.9 ± 4.4%. After 7 days of storage, 100% (68/68) of Test units had $pH_{22°C}$ ≥6.2; 92.6% (63/68) of Test components retained ≥3.0×10^{11} platelets. Test PCs demonstrated no treatment differences for pH, component volume, or dose. Platelet count was decreased ($p < 0.05$) in Test PCs (Table S2) due to processing dilution and losses. Test components contained comparable normalized extracellular glucose, lactate, and ATP compared to Controls (Figure 2E,F,H; Table S2) indicating that metabolism was maintained. Indices indicative of *in vivo* viability were maintained for morphology, while small differences were noted for ESC and HSR ($p < 0.05$) (Figure 2A–C). Test PCs had higher baseline adjusted proportional LDH on Day 5 but not on Day

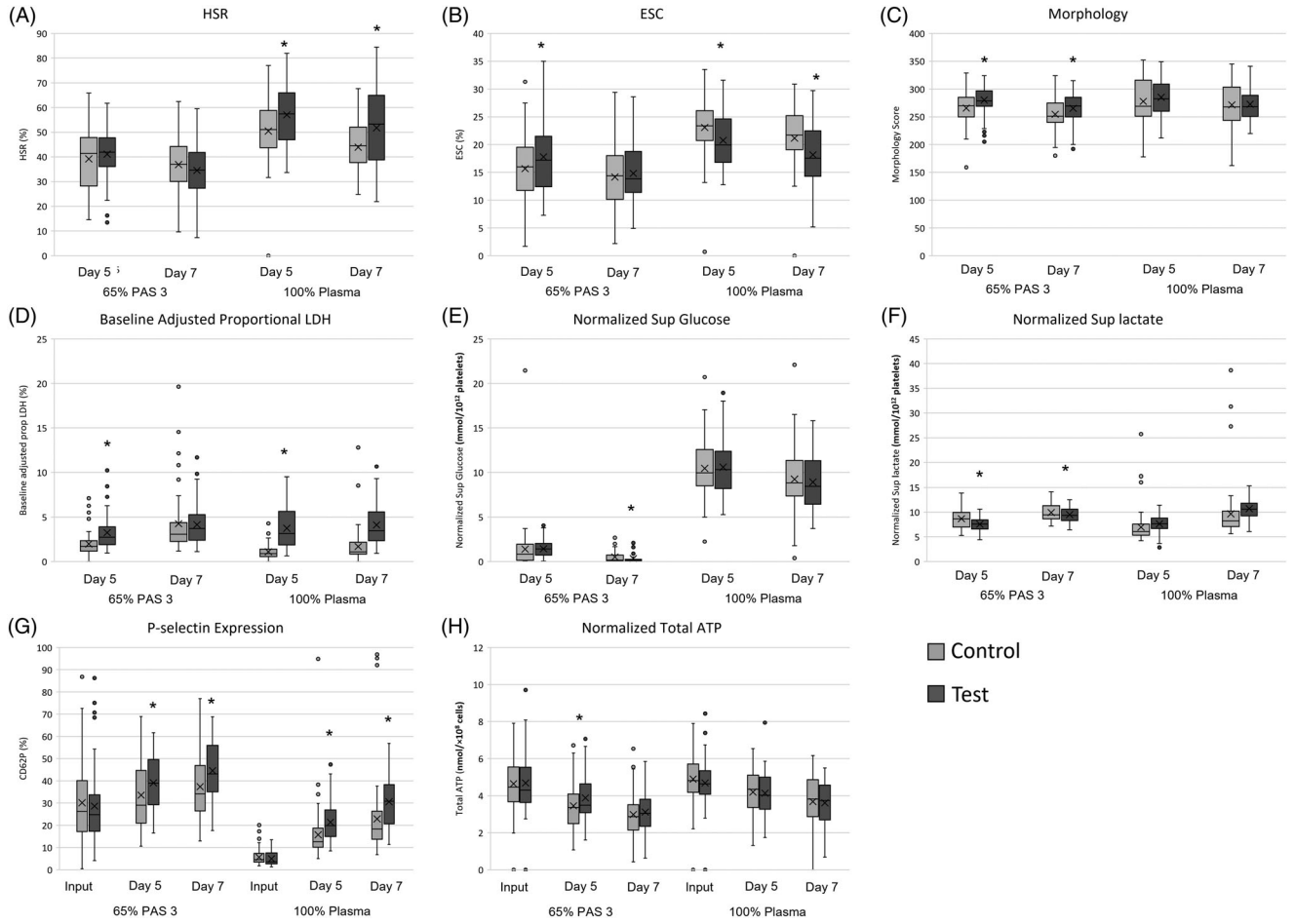

FIGURE 2 Box plots analyzing *in vitro* characteristics of Test and Control treated platelets at input (before amotosalen–UVA treatment), Day 5, or Day 7. Test = Amotosalen/UVA-treated platelet components. Controls = untreated platelet components. (A) Hypotonic shock response (HSR), (B) extent of shape change (ESC), (C) morphology, (D) baseline adjusted lactate dehydrogenase (LDH) as a % of total LDH expression, (E) normalized supernatant glucose, (F) normalized supernatant lactate, (G) P-selectin (CD62P) expression, and (H) normalized total adenine triphosphate (ATP). * signifies significantly different ($p \leq 0.05$) from paired control. Boxes represent the interval between 25 and 75 percentiles of the parameter distributions. Means are presented as transversal lines and medians as "×" ($n = 72$ for PAS-3, $n = 68$ for 100% plasma)

7 (Figure 2D). P-selectin expression increased for both Test and Control PCs between Day 5 and Day 7; although Test components showed a significant increase in on Days 5 (21.4 ± 8.8 vs. 15.8 ± 11.9%) and 7 (30.6 ± 11.7% vs. 22.9 ± 17.2%) compared to Controls (Figure 2G).

3.3 | Thrombin generation with PRPCs stored for 7 days in 100% plasma

The ETP was measured to determine the total amount of thrombin generated by a platelet sample following stimulation. Test PCs and untreated Control PCs suspended in 100% plasma had similar ETP, peak thrombin levels, and lag time at input, Day 5, and Day 7 ($p > 0.05$) (Figure 3).

3.4 | *In vivo* recovery and survival of PRPCs stored for 7 days in 100% plasma

On Day 7 of storage, all Test PCs had $pH_{22°C} \geq 6.2$, and platelet active metabolism and viability were maintained in components through 7 days of storage (Table 1). Mean *in vitro* physical recovery of the Test PC aliquots used for radiolabeling was 84%, indicating the samples used for radiolabeling were representative of the entire Test PC platelet population and platelet populations were not biased by the radiolabeling process.

In vivo platelet survival (expressed as mean lifespan) and post-transfusion recovery were assessed for Test and fresh autologous Control platelets using the ^{51}Cr and ^{111}In dual-labeling technique.[36] Post-infusion blood samples were corrected for plasma-associated radioisotope, spontaneous *in vitro* elution, and the RBC-associated

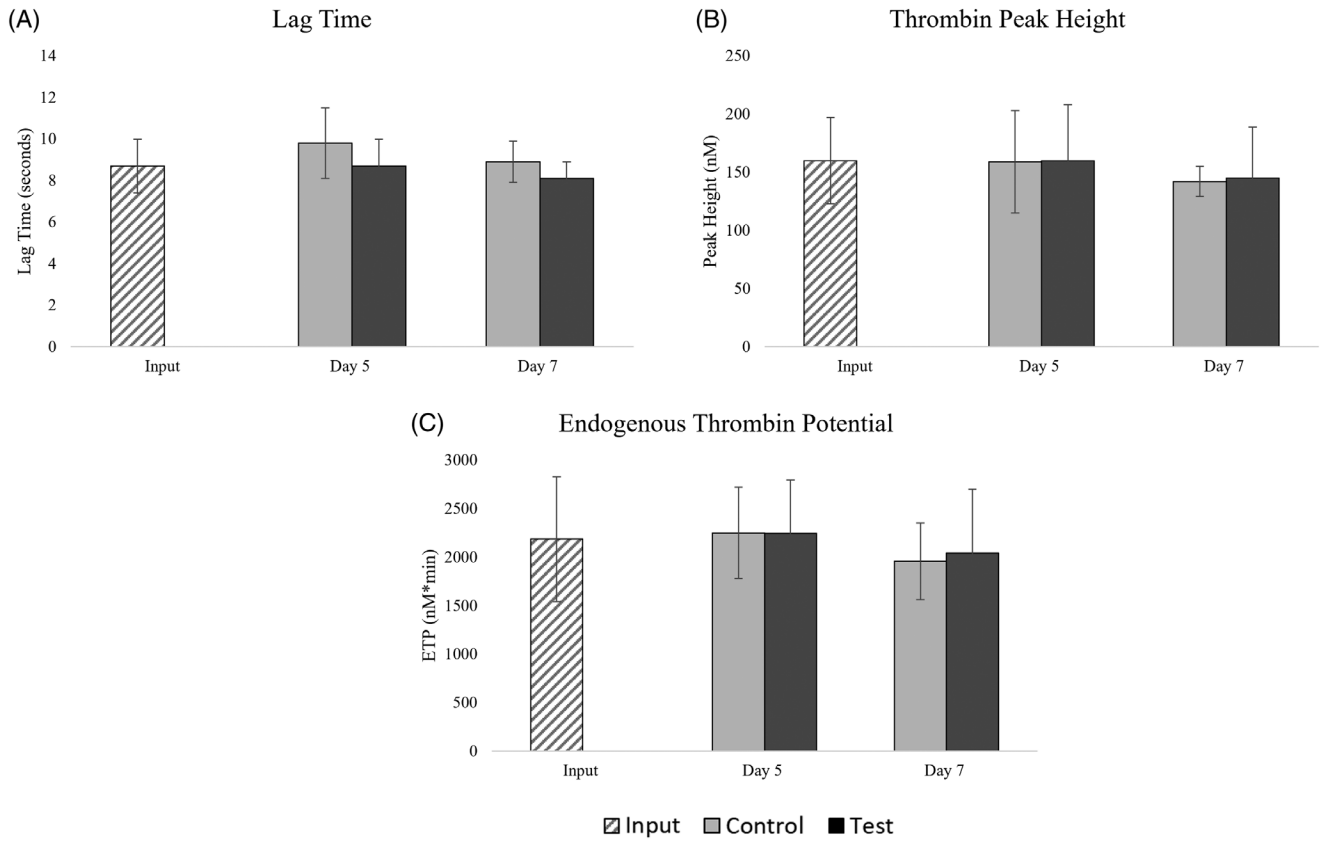

FIGURE 3 Thrombin generation in platelets stored for 7 days. Lag time (A), peak height (B), and endogenous thrombin potential (C) are plotted at input (Day 0/1), Day 5, and Day 7 post-donation. Input = platelet components prior to split into Test and Control units, treatment, and/or storage. Test = Amotosalen/UVA treated platelet components. Controls = untreated platelet components. Data are presented as mean ± one standard deviation ($n = 6$)

TABLE 1 *In vitro* analysis for PRPCs stored for 7 days in 100% plasma prior to radiolabeling

	Mean ± SD [range]	
Indices	**Input[a] (Day 0/1) ($n = 23$)**	**Day 7 ($n = 23$)**
$pH_{22°C}$	7.4 ± 0.1 [7.2–7.7]	7.1 ± 0.2 [6.8–7.3]
Platelet dose[b] ($\times 10^{11}$ platelets)	4.4 ± 0.5 [3.3–5.2]	3.7 ± 0.5 [2.1–4.6]
Component volume (mL)	336 ± 18 [302–367]	330 ± 20 [276–362]
Platelet count ($\times 10^3$ cells/μL)	1311 ± 190 [1015–1624]	1133 ± 176 [661–1401]
P-selectin (CD62P, %)	14.4 ± 9.9 [1.1–37.9]	52.2 ± 16.5 [17.6–75.4]
Total ATP (nmol/$\times 10^8$ platelets)	4.3 ± 1.3 [2.1–6.5]	3.5 ± 1.7 [0.6–7.2]
Supernatant LDH activity (U/$\times 10^{12}$ platelets)	104 ± 24 [69–162]	217 ± 90 [114–440]
Baseline adjusted LDH as a % of total LDH[c]	N/D	4.4 ± 2.9 [1.5–12.4]
Supernatant glucose (mmol/10^{12} platelets)	N/D	11.2 ± 4.4 [6.9–23.8]
Supernatant lactate (mmol/10^{12} platelets)	N/D	11.1 ± 2.8[d] [7.2–19.6]
Extent of shape change (%)	N/D	16.8 ± 5.5 [6.7–31.1]
Hypotonic shock response (%)	N/D	48.6 ± 16.9 [22.4–78.2]
Morphology score	N/D	324 ± 22 [291–370]

Abbreviation: N/D, not done.
[a]Assessed immediately before initiating amotosalen–UVA treatment.
[b]Corrected for volume loss due to sampling.
[c]Adjusted for normal plasma LDH levels measured at baseline.
[d]$N = 22$.

TABLE 2 Recovery and survival of radiolabeled amotosalen–UVA PRPCs after 7-day storage

	Test	Fresh Control	T-ΔC[a]	% Fresh Control
Recovery (%) $n = 23$				
Mean ± SD	37.6 ± 8.4	56.8 ± 9.2	0.1 ± 5.9	66.2 ± 11.2
Median	37.6	57.0	+0.0	66.1
Min to max	20.1–52.4	39.8–79.7	−8.8–18.2	45.9–101.0
95% CI for mean	[33.9, 41.2]	[52.8, 60.7]	[−2.5, 2.6]	[61.3, 71.1]
Survival (h) $n = 23$				
Mean ± SD	151.4 ± 20.1	209.6 ± 13.9	29.8 ± 18.5	72.3 ± 8.8
Median	155.9	209.3	32.8	72.7
Min to max	120.5–187.8	181.8–236.7	−6.5–63.6	55.1–88.1
95% CI for mean	[142.7, 160.0]	[203.6, 215.6]	[21.8, 37.8]	[68.5, 76.1]

[a]For recovery and survival, T-ΔC = (Test endpoint) − (Δ × Control endpoint), where Δ = 0.66 for recovery and 0.58 for survival.

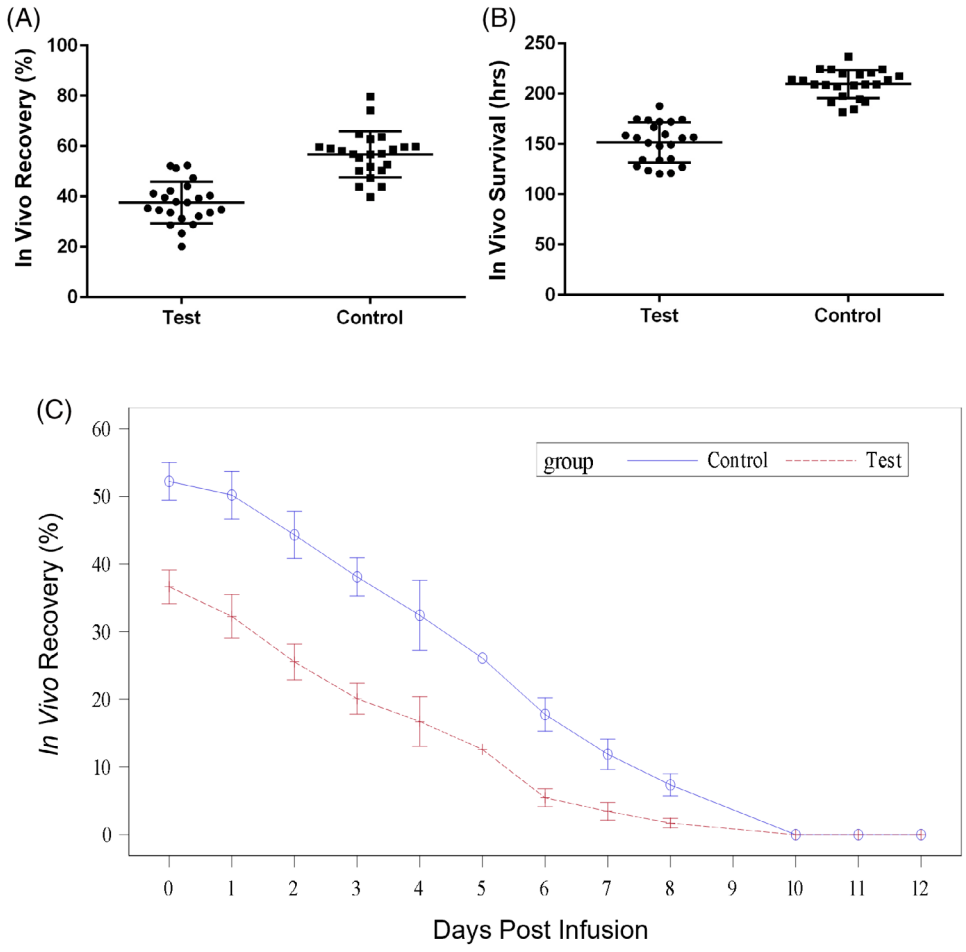

FIGURE 4 Aggregate post-infusion recovery and survival curve of Test and Control platelet components (A) platelet *in vivo* recovery at 24 h postinfusion for Test and Control. (B) *In vivo* survival of Test and Control PCs. Data are presented as individual data points and transversal lines depict average ± one standard deviation ($n = 23$). (C) Post-infusion recovery and survival of Test and Control platelet components using raw data for recovery at each day post-infusion and 95% confidence interval. The data collected on each nominal day were combined for all subjects to generate a composite curve for Test and Control where the day of infusion was Day 0. Data are presented as mean ± one standard deviation ($n = 23$). Regression analysis (Test $r^2 = .79$; Control $r^2 = .80$) shows significantly different slopes; Test *in vivo* recovery = −4.74 (days post infusion) + 36.02 and Control *in vivo* recovery = −5.82 (days postinfusion) + 54.22 (Test slope – Control slope: $p < 0.0004$). Test = Amotosalen/UVA treated platelet components. Controls = untreated platelet components [Color figure can be viewed at wileyonlinelibrary.com]

activity by subtracting the Day 11 ± 1 residual counts. Post-infusion samples greater than 20 h were used to calculate the post-infusion recovery and mean lifespan after all radioactive corrections had been made.

Test components stored for 7 days demonstrated a mean post-transfusion survival of 151.4 ± 20.1 versus 209.6 ± 13.9 h for the fresh Control (Table 2; Figure 4B). Test PRPCs stored for 7 days met the FDA criterion for the lower bound of the two-sided 95% CI of the treatment difference for 58% of the Control (21.8, 37.8) and were noninferior to the fresh Control platelets by this criterion.

Test PRPCs demonstrated a mean 24-h post-transfusion recovery of 37.6 ± 8.4% versus 56.8 ± 9.2% for the fresh Control (>66% recovery of fresh Control platelets) and did not meet the FDA criterion for the lower bound of the two-sided 95% CI of the treatment difference for 66% of the Control (−2.5, 2.6) (Table 2; Figure 4A).

The mean post-infusion recovery for the aggregated data ($n = 23$) using nominal days were plotted to examine the shape of the Test PC survival curve compared to the Controls (Figure 4C). Both survival curves are approximately linear with a terminal exponential phase, indicative of a predominantly linear senescence-driven clearance process. The Test platelet clearance is significantly less rapid ($p < 0.0004$) than the control platelet clearance rate (Table S3; Figures S1 and S2).

To evaluate the clinical impact of Test PCs that did not meet the FDA criterion for 24-h post-infusion platelet recovery, a specific analysis of the potential clinical impact of Test PCs with recovery values less than 66% of fresh Controls was performed to estimate the risk to patients of a potential inadequate CI response. The average post-infusion recovery of components with recovery <66% of the fresh Control was 33.2%, and the average difference from 66% of Control was −5%. Each of these components demonstrated *in vitro* properties (pH, ATP, morphology score, HSR, and ESC) indicative of therapeutic efficacy, indicating that low recovery did not correlate with poor *in vitro* characteristics (data not shown).

4 | DISCUSSION

We report the *in vitro* characteristics of apheresis PRPCs in 65% PAS-3/35% plasma and 100% plasma, containing the range of platelet doses collected in routine practice and stored for 7 days, demonstrated therapeutically sufficient mean platelet dose ($>3.0 \times 10^{11}$) with retention of pH and *in vitro* metabolic and functional properties consistent with post-transfusion viability. PRPC had similar thrombin generation potential as paired conventional PCs, demonstrating adequate hemostatic capacity potential; and baseline adjusted LDH as a proportion (%) of total LDH, a measure of platelet injury due to leakage or lysis, was increased in Test platelets stored for 5 days and not different ($p < 0.05$) between Test and Control platelets stored for 7 days. Absolute LDH values were similar on Day 7 of storage to those previously reported with 5-day storage of apheresis platelets in 65% PAS-3/35% plasma[30] and 7-day storage in 100% plasma[41] (both products that are approved for use in the United States) and lower than historical data for proportional LDH release in whole blood platelets stored for 48 h in plasma.[35]

The study met the primary efficacy endpoint for platelet survival *in vivo*. PRPCs retained >66% mean recovery at 24-h after infusion when compared to fresh autologous Control platelets but did not meet the FDA's criterion for lower bound of the 95% CI of the treatment difference for post-transfusion recovery (T-66%C = −2.5, 2.6). Normal platelet senescence is a linear process, in contrast to treatment-related damage, that is, random destruction, which results in nonlinear clearance.[42,43] The linear clearance of Test platelets with a significantly less steep slope ($p < 0.0004$) compared to fresh Control platelets after infusion indicates robust survival and normal senescence processes for circulating platelets consistent with minimal random destruction through the end of the Test platelet lifespan. The data suggest that 7-day-stored PRPCs that circulate for >24 h have a lifespan consistent with therapeutic efficacy and a reasonable potency for the transfusion support of thrombocytopenia.

The overall effect of reduced platelet recovery at 24-h would predict decreased post-transfusion platelet count increments in patients, as has been demonstrated with PRPCs, most recently in the study from Basel, Switzerland.[22] Clinical data from several large studies showed patients transfused at a threshold platelet count of 10×10^9 platelets/L, a 1-h post-transfusion platelet count increment of $\geq 10 \times 10^9$/L resulting in a patient platelet count of $\geq 20 \times 10^9$/L provided adequate hemostasis.[1,40] Using data from the current study, 1-h count increments were estimated using the measured radiolabeled recovery data for Test PCs with an assumed average Test PC platelet dose containing 3.0×10^{11} platelets. This analysis predicts that the estimated post-transfusion platelet count increment for a patient with a 5-L blood volume under ideal conditions would be 20×10^9/L, resulting in a post-transfusion patient platelet count of $\sim 30 \times 10^9$/L. The model estimated platelet count increments from our data are consistent with those observed in the pivotal Phase 3 trial[1] with PRPCs stored for up to 5 days, and with the PLADO study with variable dosing for 5-day-stored conventional PCs.[40,44,45] Similarly, the data from Basel, Switzerland provides real-world data confirming a mean count increment with PRPCs of $>10 \times 10^9$/L, even after 7 days of storage.[22] Taken together with the extensive postmarket experience with PRPCs worldwide and

at sites that routinely store for up to 7 days, there is no indication of increased bleeding risk to patients with PRPCs stored for 7 days.

With effective inactivation of a range of bacterial strains and demonstrated sterility at outdate,[46–48] many countries across Europe, Asia, and Latin America permit PRPC storage for up to 7 days before transfusion, facilitating improved platelet logistics, increased availability, and reduced wastage.[23,49] The efficacy of 6-7-day-stored amotosalen–UVA buffy-coat derived platelets was demonstrated in a Phase 3 RCT[5] that confirmed noninferiority of the 1-h corrected count increment (CCI) compared with conventional platelets. Post-transfusion bleeding, RBC use, and the median time to the next PC transfusion after the study PC were not different. These data were confirmed by post-market hemovigilance data from the Basel, Switzerland that compared two 5-year periods (2006–2010 vs. 2011–2016), before and after universal PRPC implementation. In that study 2809 patients received a total of 22,570 PRPCs. With 7-day storage, 16.6% of transfused PRPCs were >5 days and wastage was reduced from 8.7% to 1.5%. PRPC storage duration had no effect on the proportion of index transfusions of 6-7-day PRPCs that required the transfusion of a second PC or of RBCs on the same or the next calendar day (as an surrogate marker for hemostasis).[22] Transfusion of PRPCs >5 days compared with ≤5 days did not increase reported transfusion reactions. In a subset of patients receiving allogeneic stem cell transplants, the overall 100-day survival and treatment-related mortality were significantly improved after the implementation of PRPCs with extended storage to 7 days despite transplantation of older patients with higher EBMT risk scores.[24] Mean CCIs for PRPCs declined with increasing storage duration, although the correlation coefficient was weak ($r^2 = .005–.014$), suggesting that clinical factors were more important than PC characteristics when predicting CCIs. Mean CCIs for PRPCs stored for 5 days or less were 22.6% lower than for conventional PCs ($p < .001$). In the Swiss experience, where the minimum required platelet dose is 2.4×10^{11} platelets, the mean count increment for PRPCs was 16.5×10^9/L on Day 2 and 10.2×10^9/L on Day 7 of storage (derived from Infanti et al.[22]), indicating an acceptable therapeutic response.

Further data supporting 7-day-stored PRPCs was reported from a study at Innsbruck Hospital, Innsbruck, Austria,[20] showing no change in per patient platelet, plasma, or RBC use in hematology/oncology, cardiac surgery, pediatric, or neonatal patients in 23 months following the introduction of PRPCs stored for 7 days compared with conventional platelets in the prior 23-month period. In particular, no differences were noted in survival or time to discharge in patients undergoing massive transfusion protocols between the two time periods.[21]

Overall, although *in vitro* characteristics do not always adequately predict *in vivo* platelet recovery and survival, these studies suggest 7-day amotosalen–UVA PRPCs retained sufficient *in vitro* functional characteristics consistent with *in vivo* viability for platelets stored in both 100% plasma and 65% PAS-3/35% plasma. The survival/recovery of PRPCs in 65% PAS-3/35% plasma and stored for 7 days are currently unknown. The benefits of pathogen reduction to reduce the risk of bacterial contamination combined with the potential to reduce platelet wastage and increase availability support a favorable benefit to risk profile for 7-day storage.

ACKNOWLEDGMENTS

The authors express thanks to the subjects and research donors for agreeing to participate in these studies, the lab staff and clinical teams at the study sites for their excellent technical contributions, and Jin-Sying Lin, Ho-Lan Peng, and Stanley Bentow for statistical support. This study was funded by Cerus Corporation.

CONFLICT OF INTEREST

Jamie R. Genthe, Subramanian Yegneswaran, Nina Mufti, Laurence Corash, Richard J. Benjamin, and Anna C. Erickson are employees of Cerus Corporation. Larry J. Dumont has received research support and consulting fees from Cerus Corporation. Other authors declare no conflict of interest.

ORCID

Jose A. Cancelas https://orcid.org/0000-0002-1291-7233
Jamie R. Genthe https://orcid.org/0000-0003-4668-0606
Moritz Stolla https://orcid.org/0000-0002-2866-1674
Beth Shaz https://orcid.org/0000-0002-2270-4821
Zbigniew M. Szczepiorkowski https://orcid.org/0000-0003-2357-9564
Larry J. Dumont https://orcid.org/0000-0002-1715-0748
Laurence Corash https://orcid.org/0000-0002-8615-9869
Richard J. Benjamin https://orcid.org/0000-0001-6618-4744

REFERENCES

1. McCullough J, Vesole DH, Benjamin RJ, Slichter SJ, Pineda A, Snyder E, et al. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT trial. Blood. 2004;104:1534–41.
2. van Rhenen D, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Klüter H, et al. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood. 2003;101:2426–33.

3. Garban F, Guyard A, Labussiere H, Bulabois C-E, Marchand T, Mounier C, et al. Comparison of the hemostatic efficacy of pathogen-reduced platelets vs untreated platelets in patients with thrombocytopenia and malignant hematologic diseases: a randomized clinical trial. JAMA Oncol. 2018;4:468–75.
4. Janetzko K, Cazenave JP, Kluter H, Kientz D, Michel M, Beris P, et al. Therapeutic efficacy and safety of photochemically treated apheresis platelets processed with an optimized integrated set. Transfusion. 2005;45:1443–52.
5. Lozano M, Knutson F, Tardivel R, Cid J, Maymó RM, Löf H, et al. A multi-centre study of therapeutic efficacy and safety of platelet components treated with amotosalen and ultraviolet a pathogen inactivation stored for 6 or 7 d prior to transfusion. Br J Haematol. 2011;153:393–401.
6. Slichter SJ, Raife TJ, Davis K, Rheinschmidt M, Buchholz DH, Corash L, et al. Platelets photochemically treated with amotosalen HCl and ultraviolet a light correct prolonged bleeding times in patients with thrombocytopenia. Transfusion. 2006;46:731–40.
7. Simonsen AC, Johansson PI, Conlan MG, Jacquet M, Lin JS, Junge K, et al. Transfusion of 7-day-old amotosalen photochemically treated buffy-coat platelets to patients with thrombocytopenia: a pilot study. Transfusion. 2006;46:424–33.
8. Benjamin RJ, Braschler T, Weingand T, Corash LM. Hemovigilance monitoring of platelet septic reactions with effective bacterial protection systems. Transfusion. 2017;57:2946–57.
9. Cappy P, Barlet V, Lucas Q, Tinard X, Pillonel J, Gross S, et al. Transfusion of HIV-infected blood products despite highly sensitive nucleic acid testing. Transfusion. 2019;59:2046–53.
10. Rasongles P, Angelini-Tibert MF, Simon P, Currie C, Isola H, Kientz D, et al. Transfusion of platelet components prepared with photochemical pathogen inactivation treatment during a Chikungunya virus epidemic in Ile de La Reunion. Transfusion. 2009;49:1083–91.
11. Pierelli L, Vacca M, Zini G, Maresca M, Menichella G, Santinelli S, et al. Emergency response of four transfusion centers during the last chikungunya outbreak in Italy. Transfusion. 2018;58:3027–30.
12. Singh Y, Sawyer LS, Pinkoski LS, Dupuis KW, Hsu JC, Lin L, et al. Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function. Transfusion. 2006;46:1168–77.
13. Lin L, Hanson CV, Alter HJ, Jauvin V, Bernard KA, Murthy KK, et al. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. Transfusion. 2005;45:580–90.
14. Lin L, Dikeman R, Molini B, Lukehart SA, Lane R, Dupuis K, et al. Photochemical treatment of platelet concentrates with amotosalen and long-wavelength ultraviolet light inactivates a broad spectrum of pathogenic bacteria. Transfusion. 2004;44:1496–504.
15. Grass JA, Wafa T, Reames A, Wages D, Corash L, Ferrara JLM, et al. Prevention of transfusion-associated graft-versus-host disease by photochemical treatment. Blood. 1999;93:3140–7.
16. Grass JA, Hei DJ, Metchette K, Cimino GD, Wiesehahn GP, Corash L, et al. Inactivation of leukocytes in platelet concentrates by photochemical treatment with psoralen plus UVA. Blood. 1998;91:2180–8.
17. Lin L, Londe H, Janda JM, Hanson CV, Corash L. Photochemical inactivation of pathogenic bacteria in human platelet concentrates. Blood. 1994;83:2698–706.
18. Lin L, Londe H, Hanson CV, Wiesehahn G, Isaacs S, Cimino G, et al. Photochemical inactivation of cell-associated human immunodeficiency virus in platelet concentrates. Blood. 1993;82:292–7.
19. Mowla SJ, Sapiano MRP, Jones JM, Berger JJ, Basavaraju SV. Supplemental findings of the 2019 National Blood Collection and utilization survey. Transfusion. 2021;61(suppl 2):S11–35.
20. Amato M, Schennach H, Astl M, Chen CY, Lin JS, Benjamin RJ, et al. Impact of platelet pathogen inactivation on blood component utilization and patient safety in a large Austrian regional medical Centre. Vox Sang. 2017;112:47–55.
21. Nussbaumer W, Amato M, Schennach H, Astl M, Chen CY, Lin JS, et al. Patient outcomes and amotosalen/UVA-treated platelet utilization in massively transfused patients. Vox Sang. 2017;112:249–56. https://doi.org/10.1111/vox.12489
22. Infanti L, Holbro A, Passweg J, Bolliger D, Tsakiris DA, Merki R, et al. Clinical impact of amotosalen-UVA pathogen inactivated platelets stored for up to 7-days. Transfusion. 2019;59:3350–61.
23. Fachini RM, Fontao-Wendel R, Achkar R, Scuracchio P, Brito M, Amaral M, et al. The 4-year experience with implementation and routine use of pathogen reduction in a Brazilian hospital. Pathogens. 2021;10(11):1499.
24. Buser AS, Laura Infanti L, Holbro A, et al. Impact of amotosalen/UVA pathogen inactivated platelet components on outcomes after allogeneic Hematopoetic stem cell transplantation: a single center analysis. Blood. 2019;134:1167.
25. Sigle JP, Infanti L, Studt JD, Martinez M, Stern M, Gratwohl A, et al. Comparison of transfusion efficacy of amotosalen-based pathogen-reduced platelet components and gamma-irradiated platelet components. Transfusion. 2013;53:1788–97.
26. Holme S, Sawyer S, Heaton A, Sweeney JD. Studies on platelets exposed to or stored at temperatures below 20 degrees C or above 24 degrees C. Transfusion. 1997;37:5–11.
27. Sweeney JD, Holme S, Heaton A. Quality of platelet concentrates. Immunol Invest. 1995;24:353–70.
28. Holme S, Heaton A, Roodt J. Concurrent label method with ^{111}In and ^{51}Cr allows accurate evaluation of platelet viability of stored platelet concentrates. Br J Haematol. 1993;84:717–23.
29. Dumont LJ, Cancelas JA, Graminske S, Friedman KD, Vassallo RR, Whitley PH, et al. In vitro and in vivo quality of leukoreduced apheresis platelets stored in a new platelet additive solution. Transfusion. 2013;53:972–80.
30. Vassallo RR, Adamson JW, Gottschall JL, Snyder EL, Lee W, Houghton J, et al. In vitro and in vivo evaluation of apheresis platelets stored for 5 days in 65% platelet additive solution/35% plasma. Transfusion. 2010;50:2376–85.
31. Kunicki TJ, Tuccelli M, Becker GA, Aster RH. A study of variables affecting the quality of platelets stored at "room temperature". Transfusion. 1975;15:414–21.
32. Holme S. Storage and quality assessment of platelets. Vox Sang. 1998;74(suppl 2):207–16.
33. Holme S, Moroff G, Murphy S. A multi-laboratory evaluation of in vitro platelet assays: the tests for extent of shape change and response to hypotonic shock. Biomedical excellence for

safer transfusion working Party of the International Society of Blood Transfusion. Transfusion. 1998;38:31–40.
34. Murphy S, Rebulla P, Bertolini F, Holme S, Moroff G, Snyder E, et al. In vitro assessment of the quality of stored platelet concentrates. The BEST (Biomedical Excellence for Safer Transfusion) task force of the International Society of Blood Transfusion. Transfus Med Rev. 1994;8:29–36.
35. Snyder EL, Hezzey A, Katz AJ, Bock J. Occurrence of the release reaction during preparation and storage of platelet concentrates. Vox Sang. 1981;41:172–7.
36. BESTCollaborative. Platelet radiolabeling procedure. Transfusion. 2006;46(s3):59S–66S.
37. Yegneswaran S, Kardel S, von Goetz M, Hanson D, Benjamin RJ, Corash L, et al. Is the BEST collaborative protocol the Best for preparing stored apheresis platelets for radiolabeled recovery and survival studies? Transfusion. 2020;60: 56A–7A.
38. Dumont LJ, Cancelas JA, Dumont DF, Siegel AH, Szczepiorkowski ZM, Rugg N, et al. A randomized controlled trial evaluating recovery and survival of 6% dimethyl sulfoxide-frozen autologous platelets in healthy volunteers. Transfusion. 2013;53:128–37.
39. Lotter MG, Rabe WL, Van Zyl JM, Heyns AD, Wessels P, Kotzé HF, et al. A computer program in compiled BASIC for the IBM personal computer to calculate the mean platelet survival time with the multiple-hit and weighted mean methods. Comput Biol Med. 1988;18:305–15.
40. Slichter SJ, Kaufman RM, Assmann SF, McCullough J, Triulzi DJ, Strauss RG, et al. Dose of prophylactic platelet transfusions and prevention of hemorrhage. N Engl J Med. 2010;362:600–13.
41. Dumont LJ, Gulliksson H, van der Meer PF, Murphy S, Nixon JG, de Wildt-Eggen J, et al. Interruption of agitation of platelet concentrates: a multicenter in vitro study by the BEST collaborative on the effects of shipping platelets. Transfusion. 2007;47:1666–73.
42. Berger G, Hartwell DW, Wagner DD. P-selectin and platelet clearance. Blood. 1998;92:4446–52.
43. Quach ME, Chen W, Li R. Mechanisms of platelet clearance and translation to improve platelet storage. Blood. 2018;131: 1512–21.
44. Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, Kao KJ, et al. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood. 2005;105:4106–14.
45. Slichter SJ, Fish D, Abrams VK, Gaur L, Nelson K, Bolgiano D. Evaluation of different methods of leukoreduction of donor platelets to prevent alloimmune platelet refractoriness and induce tolerance in a canine transfusion model. Blood. 2005; 105:847–54.
46. McDonald CP, Bearne J, Aplin K, et al. Assessing the inactivation capabilities of two commercially available platelet component pathogen inactivation systems: effectiveness at end of shelf life. Vox Sang. 2021;116:416–24.
47. Schmidt M, Hourfar MK, Sireis W, Pfeiffer U, Göttig S, Kempf VAJ, et al. Evaluation of the effectiveness of a pathogen inactivation technology against clinically relevant transfusion-transmitted bacterial strains. Transfusion. 2015;55:2104–12.
48. Wagner SJ, Benjamin RJ, Hapip CA, Kaelber NS, Turgeon AM, Skripchenko A, et al. Investigation of bacterial inactivation in apheresis platelets with 24 or 30 hours between inoculation and inactivation. Vox Sang. 2016;111:226–34.
49. Infanti L, Stebler C, Job S, Ruesch M, Gratwohl A, Irsch J, et al. Pathogen-inactivation of platelet components with the INTERCEPT blood system: a cohort study. Transfus Apher Sci. 2011;45:175–81.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Cancelas JA, Genthe JR, Stolla M, Rugg N, Bailey SL, Nestheide S, et al. Evaluation of amotosalen and UVA pathogen-reduced apheresis platelets after 7-day storage. Transfusion. 2022;62(8):1619–29. https://doi.org/10.1111/trf.17003