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A multi-center study of therapeutic efficacy and safety of platelet components treated with amotosalen and UVA pathogen inactivation stored for 6 or 7 days prior to transfusion

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A multi-center study of therapeutic efficacy and safety of platelet components treated with amotosalen and UVA pathogen inactivation stored for 6 or 7 days prior to transfusion

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Supporting Information

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Manuscripts

For Peer Review

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2 **A multi-center study of therapeutic efficacy and safety of platelet components treated**
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4 **with amotosalen and UVA pathogen inactivation stored for 6 or 7 days prior to**
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6 **transfusion**
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9 Miguel Lozano¹, Folke Knutson², René Tardivel³, Joan Cid^{1,4}, Rosa Maria Maymó⁴, Helena Löf²,
10
11 Huw Roddie⁵, Jane Pelly⁶, Anthony Docherty⁶, Claire Sherman⁷, Lily Lin⁷, Meisa Propst⁷,
12
13 Laurence Corash⁷, and Chris Prowse⁶
14

15
16
17 ¹Dept. Hemotherapy Hemostasis, Hospital Clínic, Barcelona, Spain. ²Clinical Immunology and
18
19 Transfusion Medicine, University Hospital, Uppsala, Sweden. ³EFS Bretagne, Rennes, France.

20
21 ⁴Banc de Sang i Teixits, Barcelona, Spain. ⁵Western General Hospital, Edinburgh, United
22
23 Kingdom. ⁶Scottish National Blood Transfusion Service, Edinburgh, United Kingdom. ⁷Cerus
24
25 Corporation, Concord, CA, United States.
26

27
28 Running short title: 6 or 7 days old amotosalen-UVA treated platelets
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30
31 Contact author.

32
33 Dr. Miguel Lozano

34
35 Hospital Clínic Universitari

36
37 Dept. Hemotherapy and Hemostasis

38
39 Villarroel 170

40
41 08036 Barcelona

42
43 SPAIN

44
45 Tel. +34 932 275 448

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Abstract

Bacteria in platelet components (PC) may result in transfusion related sepsis (TRS). Pathogen inactivation with amotosalen of PC (A-PC) can abrogate the risk of TRS and hence facilitate storage to 7 days. A randomized, controlled, double-blinded trial to evaluate efficacy and safety of A-PC stored for 6-7 days was conducted. Patients were randomized to receive one transfusion of conventional PC (C-PC) or A-PC stored for 6-7 days. The primary endpoint was the 1-hr corrected count increment (CCI) with an acceptable inferiority of 30%. Secondary endpoints included 1 and 24-h CI, 24-hr CCI, time to next PC transfusion, RBC use, bleeding, and adverse events. 101 and 100 patients received A-PC or C-PC, respectively. The ratio of 1-h CCI (A-PC: C-PC) was 0.87 (95% CI: 0.73, 1.03) demonstrating non-inferiority ($p = 0.007$), with respective mean 1-hr CCI of 8,163 and 9,383 and; mean 1-h CI ($10^9/L$) not significantly different. Post transfusion bleeding and RBC use were not significantly different ($p = 0.44$, $p = 0.82$, respectively). Median time to the next PC transfusion after study PC was not significantly different between groups: (2.2 vs 2.3 days, $p = 0.72$). For A-PCs, storage for 6-7 days, had no impact on efficacy.

Keywords: Transfusion medicine, platelet transfusion; pathogen inactivation, amotosalen, platelet storage

Introduction

Since the inception of transfusion therapy, bacterial contamination resulting in transfusion related sepsis (TRS) has been a major cause of morbidity and mortality among transfusion recipients (Novak, 1939). With the introduction of effective platelet component (PC) transfusion therapy in the early 1960s and the discovery that the optimal conditions for PC storage for 5 or 7 days was at room temperature with agitation in improved plastic containers to retain platelet function (Murphy & Gardner, 1975), bacterial contamination of PC has persisted, and continues to limit the maximal storage time to 5 days (Esber, 1986; Murphy, 2002). Extension of PC storage > 5 days offers the potential to increase availability and reduce wastage.

In order to reduce the risk of TRS different strategies have been implemented including initial blood flow diversion, improved skin disinfection, and bacterial detection. However, despite implementing sensitive bacterial screening of PC, TRS continues to occur in the recipients of platelet transfusions (Eder *et al*, 2009; Dumont *et al*, 2010). For example, Eder *et al* reported that even with increased sample volume for bacterial culture (8 mL) with inlet-diversion at collection, there were still at least 1.2 platelet transfusion septic reactions per 100,000 transfusions (Eder *et al*, 2009). Probably the figure might be even higher because a passive report was used and it is known that, very often, septic reaction goes under recognized (Cawley *et al*, 2010). Actually, PASSPORT study found that PC collected by apheresis with negative bacterial cultures performed 24-36 hours after collection, had a positive bacterial culture if re-cultured on day 8 in 66.2 per 100,000 PC (Dumont *et al*, 2010). So as a consequence of the study USA Food and Drug Administration, requested that in spite of performing bacterial screening, 5 days is the maximum shelf life of PC due to the residual bacterial septic risk. The recent published experience of Welsh Blood Services also support the fact that initial screening of PC does not detected all the contaminated units. (Pearce *et al*, 2011).

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2 Since 1939, strategies have been proposed to decrease the bacterial burden in blood for
3 transfusion, for example addition of antibiotics such as sulfanilamide (Novak, 1939). However,
4 almost 60 years elapsed before a method for inactivating not only bacteria but also other
5 pathogens in labile cellular blood components was developed (Lin *et al*, 1997).
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9 A method for inactivating pathogens in PC utilizing amotosalen and ultraviolet A light (A-
10 UVA, Intercept™ Blood System, Cerus Corp, Amersfoort, the Netherlands) was developed to
11 inactivate a broad spectrum of viruses, bacteria, protozoa, and leukocytes in PCs and plasma
12 (Lin *et al*, 1997; Lin *et al*, 2005; Grass *et al*, 1998; Nussbaumer *et al*, 2007). PCs treated with A-
13 UVA (A-PC) have demonstrated viability in healthy volunteers (Snyder *et al*, 2004) and corrected
14 prolonged bleeding times in thrombocytopenic patients (Slichter *et al*, 2006). Three Phase III
15 trials with repeated transfusion of A-PC in thrombocytopenic patients demonstrated sufficient
16 therapeutic responses, in terms of platelet count increments (CI) (van Rhenen *et al*,
17 2003; Janetzko *et al*, 2005) and prevention of bleeding, comparable to conventional PCs (van
18 Rhenen *et al*, 2003; Janetzko *et al*, 2005; McCullough *et al*, 2004), stored up to 5 days. This
19 technology has been implemented into routine practice in European blood centers (Osselaer *et*
20 *al*, 2009; Cazenave *et al*, 2010) However, data on the therapeutic efficacy and safety of A-PC
21 stored for > 5 days prior to transfusion are limited.
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25 Under routine operating conditions only a small proportion of PC are likely to be stored >
26 5 days, thus making conduct of clinical trials to evaluate the therapeutic efficacy and safety of
27 PC stored > 5 days challenging. To mimic clinical practice hematology patients were
28 randomized to receive one transfusion of either A-PC or conventional PC (C-PC) in a series of
29 transfusions with PC stored ≤ 5 days. We designed a multicenter prospective, randomized,
30 controlled, double blinded, intent to treat study in which C-PC stored 6 or 7 days were compared
31 with A-PC stored for 6 or 7 days. The primary outcome was the 1 hour corrected count
32 increment (1-h CCI) analyzed using a non-inferiority hypothesis with a 30% margin. Secondary
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2 outcome measures included: 1 and 24 hour post transfusion platelet counts, count increments
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4 (CI), platelet transfusion interval, hemostasis, RBC use, and safety.
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Methods

Study Design and Patients

The purpose of the study was to evaluate the therapeutic efficacy and safety of PC treated with A-UVA (A-PC) and stored for 6 or 7 days in comparison to C-PC stored for 6 or 7 days. The study was designed as a prospective, randomized, double-blind parallel group non-inferiority trial enrolling hematology-oncology patients with thrombocytopenia requiring platelet transfusion support. Patients were recruited from hematology-oncology departments at the following clinical study centers: Hospital Clínic, Barcelona, Spain; Western General Hospital, Edinburgh, Scotland; Centre Hospitalier Universitaire Pontchaillou, Rennes, France and University Hospital, Uppsala, Sweden. The study protocol and consent forms were approved by the local ethics committees at each study center. Furthermore, the study was conducted in accordance with the applicable European regulations governing clinical investigations of medical devices (Council Directive 93/42/EEC, EN ISO 14155-1:2003, EN ISO 14155-2:2003) and the ICH Tripartite Guideline for Good Clinical Practice legislations (ICH-GCP). The study EudraCT number was 2005-003183-33. Additionally, the study was registered at www.clinicaltrials.gov website (ref. NCT00261924).

Patients 16 years of age and older undergoing a hematopoietic stem cell transplant or admitted for the treatment of one of the following conditions: acute or chronic leukemia, lymphoma, multiple myeloma, or myelodysplasia were eligible to participate in the study if they were thrombocytopenic or expected to develop thrombocytopenia requiring platelet transfusion within 30 days of randomization. Exclusion criteria were: prior history of clinical refractoriness to platelet transfusion; a history or diagnosis of immune-mediated thrombocytopenia, thrombotic thrombocytopenia purpura, or hemolytic uremic syndrome; treatment with IL-11 (Neumega®) or other investigational platelet growth factors; disseminated intravascular coagulation; clinically or radiologically detectable splenomegaly; and previous participation in the study.

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2 After written informed consent, eligible patients were screened and randomized in a 1:1
3 ratio (using a permuted block design), in one of two study groups, to receive a single study
4 transfusion of either A-PC or C-PC. All other platelet components given to the patient prior to
5 and after study platelet transfusion were conventional products stored for 5 days or less.
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10 11 *Platelet Products and Study Procedures*

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13 At each study center, both study A-PC and C-PC were prepared using one of the
14 following preparation modes: pooled whole blood derived buffy-coat or apheresis. All PCs were
15 prepared with leukocyte reduction according to standard operating procedures of the local blood
16 transfusion center. C-PC were suspended in platelet additive solution (PAS) with plasma
17 (nominal 65:35): T-Sol[®] (Fenwal, Mont Saint Guibert, Belgium) or SSP (MacoPharma, Mouvaux,
18 France) and A-PC were suspended in InterSol[®] (Fenwal) with plasma (nominal 65:35) and
19 treated with A-UVA according to manufacturer's instructions for use.
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26 At each center, study investigators determined whether samples of all study products (A-
27 PC and C-PC) or only C-PC underwent bacterial culture on day 3 after collection. Unless
28 specifically required by the investigator, A-PC did not require bacterial culture before release.
29 When platelet products were cultured, they were released for transfusion only if the results were
30 negative after 3 or 4 days of culture. Also at each center, the investigator determined which
31 study products (A-PC and C-PC) were gamma-irradiated, prior to release on day 6 or 7, for
32 prophylaxis against transfusion-associated graft-versus-host disease (TA-GVHD). As A-UVA
33 treatment is CE registered for leukocyte inactivation for prevention of TA-GVHD not all centers
34 used gamma-irradiation to prevent GVHD for A-PCs.
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42 The target threshold platelet content per transfusion dose was 3.0×10^{11} /unit. A sample
43 was withdrawn from all study products at day 5 of storage to determine platelet dose. For single
44 donor apheresis PC, a mean threshold dose of 3.0×10^{11} /unit after PI treatment required over-
45 collection by approximately 10% to compensate for processing losses. For whole blood buffy-
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2 coat derived platelet components, blood centers optimized platelet processing and an additional
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4 buffy-coat unit could be added to the pool prior to the PI process to compensate for processing
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6 losses. C-PC contained approximately the same platelet concentration and volume as the A-PC.

7
8 Study transfusions were ordered by primary care physicians, blinded to treatment
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10 assignment or transfusion sequence, and administered according to standard of care clinical
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12 practice for each study center. The study transfusion was postponed if the subject had a body
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14 temperature $>39^{\circ}\text{C}$ within 12 hours prior to the transfusion, a planned invasive procedure, or
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16 active hemorrhage associated with reduction in hemoglobin >1.0 g/dL or 10% within 24 hours,
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18 in the previous 12 hours prior to the study transfusion. The suggested platelet count threshold
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20 for prophylactic platelet transfusion was 10 to $20 \times 10^9/\text{L}$. After transfusion, empty PC bags were
21
22 returned to the transfusion service and keep at 4°C for at least, 24 hours in case culture was
23
24 required. A pre-transfusion platelet count was measured each day prior to transfusion. A
25
26 nominal 1-h post-transfusion count was measured between 10 minutes and 4 hours of the study
27
28 platelet transfusion. A nominal 24-h post-transfusion platelet count was measured between 16
29
30 and 24 hours after transfusion.

31 *Study Endpoints*

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33 The primary endpoint was the 1-h CCI. Secondary endpoints were 1-h post-transfusion
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35 CI, 24-h CI and CCI, 1-h and 24-h post-transfusion platelet count, the number of platelet
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37 transfusions given within 24 hours after the study transfusion, the number of RBC units
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39 transfused within 24 hours after the study transfusion, the time interval between the study
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41 transfusion and the next platelet transfusion, and clinical hemostatic assessments using the
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43 WHO Bleeding Criteria. Clinical hemostasis was assessed both pre- and post-study platelet
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45 transfusion to include the period 6 hours before and 6 hours after each study transfusion.
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47 Hemostatic assessments were performed by trained personnel blinded to treatment assignment
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49 to determine the bleeding grade on a 5-point scale of 0 (none) to 4 (maximum) for each of eight

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2 potential bleeding sites: mucocutaneous, gastrointestinal, respiratory, genitourinary, body cavity,
3 musculoskeletal, neurologic, and invasive sites (Miller *et al*, 1981; McCullough *et al*, 2004). The
4 hemostatic score was defined as the maximum score of sub-scores for the eight organ systems
5 and sites of potential bleeding.
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10 11 *Safety Assessments and the Safety Monitoring Committee*

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13 Global safety was assessed by monitoring patients for changes in health status using
14 previously described methods (McCullough *et al*, 2004; Snyder *et al*, 2005). Patients were
15 monitored for all adverse events occurring during the 4 days after each study platelet
16 transfusion. Serious adverse events (SAE) were recorded from randomization until 15 days after
17 study transfusions. An acute transfusion reaction was defined as an AE or SAE within the 24
18 hour period following a study transfusion which was attributed to the transfusion by the
19 investigator. AE occurring from 24 hours to 4 days after study transfusions also were assessed
20 for relationship to study transfusions. Patients were assessed specifically from initiation of the
21 study transfusion to 24 hours following the transfusion for events indicative of acute transfusion
22 reactions. Vital signs and transfusion related laboratory abnormalities, blood culture results and
23 platelet culture results were also recorded. The signs and symptoms of AE classified as acute
24 transfusion reactions, as well as the potential assessments to evaluate suspected transfusion
25 related sepsis, were recorded. The study investigator at each site graded and recorded the
26 overall AE and the causal relationship with the study platelet transfusion.
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38 No interim analyses were conducted in this study. A safety monitoring committee (SMC)
39 reviewed each SAE description when it occurred and performed two interim reviews of safety
40 data during the course of the study: the first after approximately 100 patients and the second
41 after 150 patients were transfused.
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2 Estimation of sample size for conduct of the study and detailed statistical methods for
3 data analysis, including the bootstrap modeling approach, are described in the Supporting
4 Information.
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9 **Results**

10 *Age and Dose of Study Products*

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12 The majority of PC (86%) were whole blood derived prepared by the buffy coat method.
13 Approximately 80% of study PC (77.1% A-PC and 78.3% C-PC) were stored for 7 days prior to
14 transfusion, the remaining products were stored for 6 days. The mean platelet content of the
15 study product at day 5 of storage was equal between groups ($4.2 \times 10^{11} \pm 0.67$, (mean \pm SD
16 $p=0.97$)). Study PC were ABO compatible with the recipient for 99.1% (A-PC) and 98.1% (C-
17 PC) of transfusions, ($p=1.0$). Study PC were gamma irradiated for 14.3% of the A-PC
18 transfusions and for 73.6% of C-PC transfusions ($p<0.0001$).
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29 *Study Populations For Analysis of Endpoints*

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31 A total of 242 patients were enrolled in four clinical sites. Most patients had hematologic
32 malignancies. A total of 211 patients received a study platelet transfusion (FAS population). Of
33 the 211 patients who received a study platelet transfusion, 199 patients were eligible for the 1-h
34 CCI per protocol analyses and 186 patients were eligible for the per protocol 24-h CCI analyses.
35 Patient demographics, alloimmunization risk factors, and primary diagnoses were balanced
36 between the treatment groups (Supporting Information: Tables 1, 2 and 3).
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42 Two control arm patients (C-PC group) with aberrantly high 1-h post-transfusion platelet
43 counts (110 and $128 \times 10^9/L$) were excluded from the per protocol group. These post-transfusion
44 platelet count increments were excluded because they were deemed implausible and, upon
45 clinical inquiry, the investigator concluded that these post-transfusion blood samples were
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2 drawn through an indwelling catheter and that the catheter's lumen had not been adequately
3 flushed following the study transfusion. As a consequence, the platelet count was falsely
4 elevated because residual platelet product was sampled from the catheter through which the
5 platelet transfusion had been administered, and the post count partially reflected the high
6 residual platelet concentration in the catheter rather than the platelet concentration in the
7 patient's peripheral blood.
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13 14 15 *Primary Endpoint*

16 The primary hypothesis of this study was whether A-PC, when stored for 6 or 7 days,
17 provided sufficient 1-h post-transfusion CCI compared to C-PC stored for 6 or 7 days. For the A-
18 PC group the mean 1- h CCI was 8,163.0 versus 9,383.1 for the C-PC group (Table 1). The
19 ratio of the means was 0.87 (95% CI: 0.732 to 1.034) Thus, the lower bound of the ratio of
20 means was greater than the pre-defined inferiority margin (0.70), and the null hypothesis of
21 inferiority of A-PC compared to C-PC was rejected (p=0.007).
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29 *Secondary endpoints*

30 *Platelet counts*

31 Similar to the primary efficacy endpoint, the 1-hr CI was not a significantly different
32 between treatment groups (p=0.268, Table 1)). Conversely, patients treated with A-PC platelets
33 had significantly lower 24-h CI (p=0.01) and 24-h CCI (p=0.003) compared to patients receiving
34 C-PC (Table 2).
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40 The covariates that were significant predictors of both 1-h and 24-h post-study
41 transfusion platelet counts were: pre-transfusion platelet count (p<0.0001), body surface area
42 <0.0001), and platelet dose (p<0.0001). An additional covariate, study center (p=0.01), was
43 determined to be a significant predictor for 1-hour post-study transfusion platelet count. The
44 effect of study center was likely a significant covariate because pre-study transfusion platelet
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1
2 counts differed significantly across the study centers. For 24-hour post-study transfusion platelet
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4 count, the number of previous transfusions ($p=0.003$) in the transfusion cycle was an additional
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6 significant covariate. These analyses indicated that study center practices and factors known to
7
8 influence response to platelet transfusion had a significant effect on the secondary outcome
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10 measures of platelet count and count increments. The effect of treatment group was not
11
12 significant when these covariates were included in the model.

13 14 15 *Additional platelet transfusions*

16
17 The number of additional platelet transfusions after the study transfusion was similar
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19 between groups. Within 24 hours after the start of the study transfusion the proportion of
20
21 patients in the FAS population who received additional platelet transfusions was 15.2% in the A-
22
23 PC group and 11.3% in the C-PC group ($p=0.398$). Being the main indication for that a low
24
25 platelet count (10 in the A-PC group; 5 in C-PC) and a bleeding event (4 in A-PC group; 6 C-
26
27 PC). Within 15 days after the study transfusion the median number of platelet transfusions
28
29 administered was 2 for both treatment groups. The median time to the next platelet transfusion
30
31 after the study transfusion was 2.2 days for the A-PC group and 2.3 days for the reference
32
33 group ($p=0.717$, log-rank test, Figure 1).

34 35 *Hemostatic scores*

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37 A majority of the patients in both treatment groups (>75%), had no bleeding observed in
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39 the 6 hours prior to study transfusion; and the distributions of pre-transfusion hemostatic scores
40
41 were comparable between the treatment groups ($p=0.801$) (Table 3). Less than 6% of the
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43 patients in both treatment groups had pre-transfusion hemostatic scores of 2 and greater. The
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45 post-transfusion hemostatic scores were similar to the pre-transfusion scores in that majority of
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47 patients did not experience bleeding in the monitored organs and sites within 6 hours after the
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1
2 study transfusion (84% A-PC, 77% C-PC, $p=0.275$). A majority of patients had no change in
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4 hemostatic scores before and after the study transfusion (86.7% A-PC, 88.7% C-PC, $p=0.244$).
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7 *RBC Transfusions*

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9 A majority of patients in both treatment groups did not require RBC transfusions within
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11 24 hours after the study transfusion (A-PC 76.2%, C-PC 78.3%) (Supporting Information, Table
12
13 4). There was no significant difference in the distribution of the number of RBC units transfused
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15 between the treatment groups ($p=0.821$).
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18 *Safety*

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20 Patients were observed for any adverse events for 24 hours after the start of the study
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22 platelet transfusion, and all events were recorded, whether or not the events were classified as
23
24 related to the study transfusion. Approximately 30% of the patients experienced at least one AE
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26 within the 24 hour after study platelet transfusion period (31.4% A-PC, 29.2% C-PC) (Table 4).
27
28 The most common AEs in any treatment group were recorded under the category of "other"
29
30 (19.0% A-PC, 23.6% C-PC, $p=0.502$) (Supporting Information, Table 5). These AE were
31
32 composed of a spectrum of signs and symptoms including, fever (14.3 % A-PC, 10.4% C-PC,
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34 $p=0.410$), chills (6.7 % A-PC, 2.8% C-PC, $p=0.214$), positive blood culture (6.7 % A-PC, 1.9%
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36 C-PC, $p=0.101$), skin rash (2.9 % A-PC, 1.9% C-PC, $p=0.683$), and nausea/vomiting (1.9 % A-
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38 PC, 2.8% C-PC, $p=1.00$) (Supporting Information, Table 5). There were no statistically
39
40 significant differences between treatment groups in the frequency of any AE deemed by the
41
42 investigator as a related to the study transfusion within the first 24 hours or during the 4 days
43
44 following study transfusion. (Table 4)

45
46 When patients experienced an elevation in temperature following the study transfusion
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48 ($>1^{\circ}$ C increased temperature with rigors or $>2^{\circ}$ C increased temperature without rigors) within
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50 24 following the study transfusion, blood and PC cultures were ordered. Positive blood cultures
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2 were recorded for 9 patients (7 [6.7%] A-PC, 2 [1.9%] C-PC). Most patients with positive blood
3
4 cultures had experienced infectious events that preceded the study transfusion. Of the 7 A-PC
5
6 patients with a blood positive culture, 5 patients had AEs indicating an infection that had started
7
8 before the study transfusion; 2 patients with a positive blood culture had no AEs coded to the
9
10 MedDRA System Organ Class Infections and Infestations(2010) before or after the study
11
12 transfusion. None of the PC cultured tested positive for bacteria.

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13 The majority of patients in each treatment group experienced at least one AE during the
14
15 4 days after the start of the study platelet transfusion (83.8% A-PC, 85.8% C-PC, p=0.705).
16
17 These AE, were coded to MedDRA and listed by preferred terms (PT), (Supporting Information,
18
19 Table 5). Two events in the Gastrointestinal System Organ Class were statistically significantly
20
21 greater in the A-PC treatment group (diarrhea, p=0.022; nausea, p = 0.024). No events were
22
23 statistically significantly greater in the C-PC treatment group. (Table 9)

24 All patients were followed from randomization until 15 days after study transfusion for
25
26 SAE. Overall, 22 patients (11.4% A-PC, 9.4% reference) experienced at least one SAE. Only
27
28 one SAE was considered possibly related to the study transfusion (a patient in the reference
29
30 group who experienced HLA alloimmunization and hemorrhagic syndrome); all other SAEs were
31
32 considered unrelated to the study transfusion.

33 Six study patients died. Five deaths occurred in the C-PC group (4.7%) and one death
34
35 occurred in the A-PC treatment group (1.0%). Three patients (all C-PC) died prior to completing
36
37 15 days of active surveillance period) and 3 patients (1 A-PC group, 2 C-PC group) died after
38
39 completing the active surveillance period. Only one death was considered at least possibly
40
41 related to a study platelet transfusion (C-PC): a female patient experienced severe HLA
42
43 alloimmunization and hemorrhagic syndrome with the outcome of death (0.9%). All other deaths
44
45 were considered related to the patient's underlying disease. (Table 8)

Discussion

Extension of PC storage beyond 5 days offers the potential to improve PC availability and reduce wastage. However, a major barrier to extension of PC storage is the risk of TRS, currently still the most common reported transfusion transmitted infection. While bacterial detection has failed to be sufficiently protective (Murphy *et al*, 2008; Eder *et al*, 2009; Dumont *et al*, 2010), robust inactivation of bacteria will facilitate storage of PC > 5 days provided that sufficient therapeutic efficacy is retained. There are data suggesting that A-PC results in acceptable in vitro platelet functional characteristics stored up to 7 days.(van Rhenen *et al*, 2000) Moreover, available paired in vitro studies performed in a perfusion model, mimicking the flow conditions found at sites of vascular damage, suggest that A-PC stored up to 7 days exhibits adhesive and aggregating capacities similar to that of C-PCs stored for the same period of time. Actually perfused surface covered by platelet was higher for A-PC compared to C-PC (27,1% vs 21,2%) although this difference did not reach statistical difference.(Lozano *et al*, 2007) Another issue to be considered when extending the storage up to 7 days of A-PC is the potential generation of inflammatory factors. Cognasse et al looked at the levels of cytokines and chemokines (CD62p, platelet-derived growth factor-AB, interleukin-8, soluble CD40 ligand, interleukin-1b and tumor necrosis factor a) during storage of paired PC, one half treated with amotosalen and the other half left as a control. The results of the study suggested that the levels of the immune modulators measured did not changed significantly between days 5 and 7 and that the observed increased during storage was very similar in A-PC compared to C-PC.(Cognasse *et al*, 2008)

Nevertheless, because there is limited clinical data on the therapeutic efficacy of A-PC stored > 5 days, we designed a study to evaluate the response to transfusion of PC prepared with extended storage. The study utilized a non-inferiority design with a transfusion protocol modeled on clinical practice where PC stored > 5 days were used to supplement PC stored for 5 days or less. We compared the response to transfusion of a single 6-7 day-old A-PC in

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2 comparison to C-PC stored for 6-7 days. Both types of products were transfused to hematology
3 patients requiring repeated platelet support within a sequence of conventional PC stored ≤ 5
4 days analogous to the anticipated intermittent use of older PC. In order to conduct this study
5 with sufficient statistical power and feasible logistics, we selected a surrogate endpoint (1-h
6 CCI), and utilized secondary supporting endpoints directly indicative for hemostasis including:
7 bleeding, time to the next platelet transfusion, and RBC use. Each of these secondary endpoints
8 is a relevant outcome measure of platelet transfusion efficacy.
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15 The inferiority margin for this trial was based on data from the prior SPRINT trial in which
16 hemostasis was evaluated in parallel with 1-h CCI responses. SPRINT was a randomized,
17 double-blinded, controlled, non-inferiority trial in which 645 patients received apheresis PC
18 treated with PI or conventional PC stored up to 5 days. The primary endpoint of the study was
19 the proportion of patients with Grade 2 bleeding (WHO) during a period of repeated platelet
20 transfusion. The SPRINT study showed that the frequency of Grade 2 bleeding was equivalent
21 between treatment groups (McCullough *et al*, 2004). The study also showed that while the mean
22 1-h CCI was 30% lower in the group receiving A-PC in comparison to C-PC, hemostasis was
23 maintained (McCullough *et al*, 2004). On the basis of this observation, we postulated that a
24 difference (A-PC versus C-PC) in mean 1-h CCI $\leq 30\%$ for 7-day old PC would be consistent
25 with maintaining hemostasis.
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35 The current study demonstrated that transfusion of PC treated with A-UVA stored for 6-7
36 days resulted in a mean 1-h CCI for the A-PC group 13% lower than the CCI of the group
37 transfused with C-PC, but was not inferior by the pre-specified margin of 30% inferiority
38 ($p=0.007$). Despite a 13% decrease in the mean 1-h CCI there was no effect on hemostatic
39 scores after transfusion, hemorrhagic AE, and the use of RBC transfusions (a surrogate marker
40 of Grade 3 bleeding) within 24 hours from the start of the study transfusion was similar between
41 groups (A-PC: 23.8% versus C-PC: 21.7 %, $p=0.821$).
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2 A statistically significant decrease of 30% was observed for the post-transfusion 24-hr
3 CCI ($p=0.003$). However this decrease did not impact the proportion of patients requiring
4 another platelet transfusion within 24 hr of the study transfusion (A-PC: 15.2%; C-PC: 11.3%,
5 $p=0.398$) and the median time to the next platelet transfusion within 15 days of the study
6 transfusion was also similar (A-PC = 2.2 days: C-PC: 2.3 days, $p=0.717$). Thus, while the 24-h
7 CCI was significantly lower for A-PC, this did not impact the need for additional transfusions or
8 the time to the next PC transfusion.

9
10 The safety profile was similar between treatment groups for the first 24 hours and for the
11 4 days following study transfusion. The majority of patients in each treatment group experienced
12 at least one AE, not unexpected considering the clinical severity of the patients enrolled in the
13 study and the high proportion (70%) undergoing stem cell transplant (> 25% allogeneic). Two
14 events were significantly more frequent in the Test group, diarrhea and nausea; but there were
15 no other significant differences within the gastrointestinal System Organ Class.

16
17 A previous pilot study with a randomized controlled crossover design, comparing A-PC
18 to C-PC, both stored for 7 days, failed to reject inferiority within the specified margin (an
19 absolute difference in 1-hr CCI of 2,100), suggesting that 7-day-old A-PC may be inferior
20 (Simonsen *et al*, 2006). In that study, the upper bound of the one-sided 95% CI for the mean
21 difference of 1-h CCI was 2,400. Paradoxically, the mean 1-h CCI was higher in the A-PC group
22 ($8,739 \pm 3,785$) than in reference group ($7,433 \pm 5,408$). However, this difference was not large
23 enough to reject inferiority. The large variance observed in the conventional PC group and the
24 small number of transfusions analyzed contributed to the failure to reject inferiority in that study.
25 The median 24 hr-CCI for Test PC (4,490) in the current study was within a range observed in
26 other studies with similar patient populations (Bishop *et al*, 1991).

27
28 A recently published report contrasts with the findings in the current study (Kerkhoffs *et*
29 *al*, 2010). Kerkhoffs *et al*, reported a multicentre, open-label, randomized, non-inferiority trial
30 using the 1-h post transfusion CCI for PC stored from 1 to 7 days in plasma (C-PC) compared

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2 with A-PC transfused to hematology patients. Secondary endpoints included bleeding and RBC
3 transfusion requirement. They found that compared to the C-PC group, patients receiving A-PC
4 had a 1-h CCI 31% lower (A-PC: 11.4 ± 5.3 versus C-PC 17.1 ± 7.3) and more frequent
5 bleeding events (grades 1-3 classified by the Common Toxicity Criteria for Adverse Events
6 (CTCAE) version 3 (A-PC: 32% versus C-PC: 19%). Most of the bleeding events were grade 1,
7 and no difference in the number of RBC units transfused was detected (4 ± 3 units in both study
8 groups).

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15 Several factors might contribute to these conflicting results. In the study by Kerkhoffs et
16 al, the mean platelet content in the A-PC (3.4 ± 0.8) was 12.8% lower than in C-PC (3.9 ± 1 ,
17 $p < 0.001$). Although CCI theoretically corrects for the platelet dose, there is evidence that
18 suggests that this correction is not complete and that lower platelet dose might yield lower CCIs
19 (Davis *et al*, 1999). Also the pre transfusion platelet count was a 11% lower in the A-PC group
20 compared to C-PC plasma (16 ± 11 , 18 ± 13 , $p = 0.04$). A remarkable difference between the
21 study reported here and that reported by Kerkhoffs et al is the fact that in our study the
22 comparison was made with PC resuspended in PAS while in the Kerkhoffs study the
23 conventional PC were suspended in 100% plasma. In 2006, Kerkhoffs et al reported the results
24 of a randomized controlled trial where PC stored in PAS II were compared with PC stored in
25 plasma (Kerkhoffs *et al*, 2006). A 19.4% lower 1-h CCI was observed in PC stored in PAS II
26 compared to PC stored in plasma (11.2 ± 6.4 PAS II, 13.9 ± 7.0 plasma, $p = 0.04$). There were no
27 significant differences between the groups regarding bleeding complications and transfusion
28 interval (Kerkhoffs *et al*, 2006). So it might be possible that the difference observed in the
29 Kerkhoffs 2010 report, may in part, be due to the fact that A-PC were re-suspended in PAS III
30 while the conventional PC was in 100% plasma. Actually there was no significant difference in
31 1-h CCI between the group receiving PC re-suspended in PAS III and that receiving A-PC
32 (Kerkhoffs *et al*, 2010).

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2 Another notable difference is the higher incidence of bleeding in the group receiving A-
3 PC compared to C-PC, observed in the Kerkhoffs' study (32% A-PC, 19% C-PC, $p=0.034$)
4 (Kerkhoffs *et al*, 2010). In the current study we have observed similar hemostatic capacity in
5
6 both treatment groups. It is pertinent to note that in the Kerkhoffs study, the evaluation of the
7
8 bleeding was performed by clinicians not blinded to the treatment assignment, and the bleeding
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10 scale was different than used in our study. Another point of note is that considering the severity
11
12 of the patients enrolled in the study (about 50% of the patients were undergoing stem cell
13
14 transplantation) grade 2 (CTCAE scale) and higher bleeding was only detected in the 7% of the
15
16 C-PC group. Several trials that have used bleeding as a primary endpoint evaluated by research
17
18 personnel blinded to treatment using daily protocol bleeding assessments reported bleeding
19
20 frequencies among the study population ranging from 57.5 % in the SPRINT trial (McCullough *et*
21
22 *al*, 2004) to 70% in PLADO (Slichter *et al*, 2010). Differences in design and execution may
23
24 account for many of the differences observed between Kerkhoffs report and ours.
25

26 In summary, our study indicates that A-PC stored for > up to 7 days provided 1-h CCI
27
28 and CI within therapeutic ranges not inferior to C-PC. A-PCs were effective by multiple clinical
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30 indices including RBC transfusions, time to next PC and hemostasis. The safety profile of A-PC
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32 did not demonstrate differences from C-PC.
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For Peer Review

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Table 1: 1-Hour Platelet Count and Count Increment – 1-Hour per protocol population

	Treatment Group		p-value
	Amotosalen-PC N = 101	Conventional-PC N = 98 ^a	
Pre-transfusion platelet count (10 ⁹ /L)			0.277 ^b
Mean (± SD)	9.8 (4.24)	9.6 (5.38)	
Median	9.0	9.0	
Min, Max	3.0, 28.0	2.0, 31.0	
1-h post-transfusion platelet count (10 ⁹ /L)			0.360 ^c
Mean (± SD)	29.2 (13.68)	31.1 (15.76)	
Median	27.0	29.0	
Min, Max	7.0, 68.0	4.0, 91.0	
1-h platelet count increment (10 ⁹ /L)			0.268 ^d
Mean (± SD)	19.4 (13.44)	21.6 (14.56)	
Median	17.0	20.0	
Min, Max	-3.0, 58.0	-3.0, 76.0	
1-h platelet corrected count increment			0.007 ^c
Mean (± SD)	8,163.0 (5,370.2)	9,383.1 (5,905.5)	
Median	7,500.0	8,859.9	
Min; Max	-1,757.3; 27,255.6	-1,350.5; 30,445.3	

a. Two patients were excluded from the 1-h per protocol population reference group due to invalid platelet counts (>100x10⁹/L)

b. Wilcoxon rank-sum test

c. Derived from bootstrap sampling distribution

d. Derived from bootstrap sampling distribution. P-value reflects rejection of inferiority hypothesis at the 0.025 significance level.

Table 2: 24-Hour Platelet Count and Count Increment – 24-Hour per protocol population

	Treatment Group		p-value
	Amotosalen-PC N = 92	Conventional-PC N = 94	
24-h post-transfusion platelet count ($10^9/L$)			0.026 ^a
Mean (\pm SD)	20.8 (9.2)	24.9 (12.48)	
Median	20.0	23.5	
Min, Max	5.0, 52.0	1.0, 54.0	
24-h platelet count increment ($10^9/L$)			0.010 ^b
Mean (\pm SD)	11.1 (8.86)	15.2 (12.19)	
Median	10.0	14.0	
Min, Max	-3.0, 36.0	-13.0, 44.0	
24-h platelet corrected count increment			0.003 ^b
Mean (\pm SD)	4,588.5 (3,522.8)	6,549.3 (5,211.1)	
Median	4,489.7	6,442.6	
Min; Max	-1,322.5; 15,053.9	-5,163.9; 24,641.8	

a. Wilcoxon rank-sum test

b. Derived from bootstrap sampling distribution

Table 3: Hemostatic Scores of all patients receiving the study transfusion (full analysis set population)

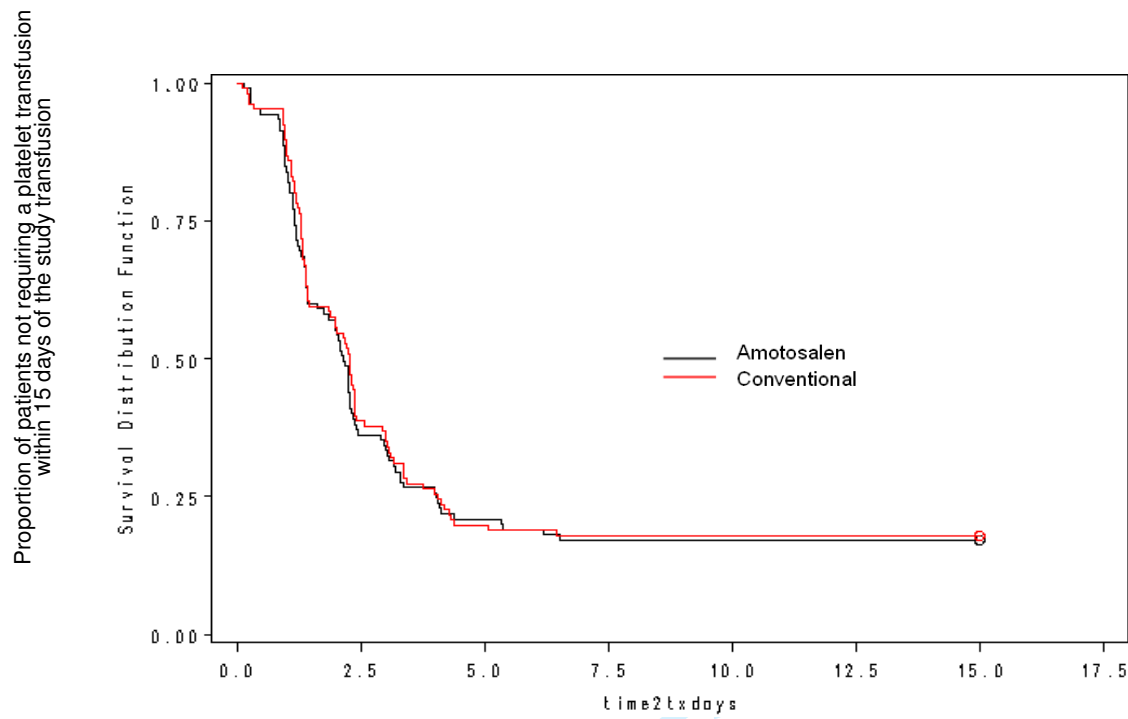
	Treatment Group		p-value
	Amotosalen-PC N = 105	Conventional-PC N = 106	
Pre-transfusion Hemostatic Score			0.801 ^a
0	81 (77.1%)	80 (75.5%)	
1	18 (17.1%)	20 (18.9%)	
2	4 (3.8%)	5 (4.7%)	
3	1 (1.0%)	1 (0.9%)	
4	1 (1.0%)	0	
Post-transfusion Hemostatic Score			
0	88 (83.8%)	82 (77.4%)	0.275 ^a
1	11 (10.5%)	18 (17.0%)	
2	4 (3.8%)	5 (4.7%)	
3	1 (1.0%)	1 (0.9%)	
4	1 (1.0%)	0	
Change in Hemostatic Score (Post-transfusion – Pre-transfusion)			0.244 ^a
-2	0	1 (0.9%)	
-1	11 (10.5%)	6 (5.7%)	
0	91 (86.7%)	94 (88.7%)	
1	2 (1.9%)	4 (3.8%)	
2	1 (1.0%)	1 (0.9%)	

a. p-values based upon Wilcoxon rank-sum test.

Table 4: Summary of Adverse Events (full analysis set population)

	Number of Patients (%)		
	Amotosalen-PC N = 105	Conventional-PC N = 106	P-value
Within 24 hours following study transfusion			
Any adverse event	33 (31.4%)	31 (29.2%)	0.766
Related adverse event	10 (9.5%)	7 (6.6%)	0.461
Within 4 days following study transfusion			
Any adverse event	88 (83.8%)	91 (85.8%)	0.705
At least one hemorrhagic adverse ever	25 (23.8%)	31 (29.2%)	0.436
Related adverse event	10 (9.5%)	7 (6.6%)	0.461
Severe adverse event	8 (7.6%)	5 (4.7%)	0.408
Within 15 days following study transfusion			
Serious adverse event	12 (11.4%)	10 (9.4%)	0.659
Death	1 (1.0%)	5 (4.7%)	0.212

Figure 1: Time to Next Platelet Transfusion within 15 days of the study transfusion (active surveillance period) – All patients receiving a study transfusion (full analysis set population)



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For Peer Review

SUPPORTING INFORMATION

Sample Size Determination and Statistical Analyses

The 1-h CCI was the primary endpoint of this clinical study. In a previous randomized, controlled study the mean 1-h CCI after transfusion of A-PC was lower compared to C-PC transfusions (McCullough *et al*, 2004). The objective of this study was to show that the observed inferiority did not exceed a clinically relevant margin. Given the known advantages of the PI treatment in reducing the risk of transfusion-transmitted infection by inactivation of viruses, bacteria, protozoa, and contaminating leucocytes, a 1-h CCI in response to A-PC not lower than 70% of the C-PC 1-h CCI (with regard to the lower bound of a 1-sided 97.5% confidence interval for the ratio of means) was considered clinically relevant because a ratio of A-PC:C-PC of 0.68 for the 1-h CCI was not associated with increased bleeding on in a prior study with sufficient power to assess bleeding in which 1-h CCI was measured in relation to the incidence of bleeding after repeated transfusions of A-PC or C-PC (McCullough *et al*, 2004).

euroSPRITE was a randomized, double blind, two-group study that used buffy-coat derived PC stored up to 5 days (van Rhenen *et al*, 2003;Cazenave *et al*, 2001). A-UVA treated PC were compared to conventional PC in 103 thrombocytopenic patients. The ratio of the A-PC mean 1-h CCI to the C-PC mean 1-h CCI was 0.88 and the coefficient of variation (CV) for the reference group was 42%. For the current study, a sample size of 94 patients per group was determined to provide 80% power to reject the null hypothesis of inferiority, i.e. $\mu_{A-PC} / \mu_{C-PC} \leq 0.70$, in favor of the alternative hypothesis $\mu_{A-PC} / \mu_{C-PC} > 0.70$. The sample size calculation assumed that the expected true ratio of the means was 0.85, the CV for the reference group was 50% and that the data would be analyzed in log-scale using a two-sample t-test at a 1-sided significance level of 0.025. A slightly higher CV as compared to the aforementioned earlier clinical studies was assumed to account for the uncertainty with respect to the influence of extended storage duration and potential differences in the study populations.

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3 The data were analyzed by intention to treat (ITT) as well as per protocol (PP). Patients
4 who received a study transfusion, had the required platelet count, and received no intervening
5 transfusions of non-study platelet components comprised the 1-h and 24-h per protocol analysis
6 set. The full analysis set (FAS) included all patients who received any PC study transfusion PC.
7
8 The statistical analysis was predicated on the sample size calculations and required use of the
9 logarithms of the CCI data to deal with skewed data distributions. However negative CCI values
10 were present in this study. Therefore the bootstrap method was used to estimate the sampling
11 distribution of μ_{A-PC} / μ_{C-PC} (Efron, 1979). With the bootstrap method, the A-PC and C-PC
12 samples are treated as the population and a Monte Carlo-style procedure is conducted on it.
13
14 The central assertion of the bootstrap method is that the relative frequency distribution of the
15 ratio of the means is an estimation of the sampling distribution of μ_{A-PC} / μ_{C-PC} . A 95%-
16 confidence interval for μ_{A-PC} / μ_{C-PC} was determined by the 2.5th and 97.5th percentiles of the
17 bootstrap sampling distribution. Thus, the aforementioned null hypothesis will be rejected if the
18 2.5th percentile of the bootstrap sampling distribution is greater than 0.70.
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33 The 24-h CCI, 1-h CI, and 24-h CI data were analyzed via bootstrap methods in a similar
34 manner as the 1-h CCI data. The bootstrap method was used to estimate the sampling
35 distribution of μ_{A-PC} / μ_{C-PC} for each endpoint. The 95%-confidence interval for μ_{A-PC} / μ_{C-PC} was
36 determined by the 2.5th and 97.5th percentiles of the bootstrap sampling distribution. To
37 determine whether there was a statistically significant difference in the means for each endpoint,
38 a 95% confidence interval was employed. If the confidence interval included the value 1.0, then
39 there was no statistically significant difference at the 0.05 level. Exact p-values were computed
40 by determining the percentile of the study sample statistic in relation to the bootstrap sampling
41 distribution under the null hypothesis of $\mu_{A-PC} / \mu_{C-PC} = 1.0$.
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53 The CCI is a ratio measure which includes the terms for platelet dose and patient size
54 (body surface area). In order to evaluate the impact of these variables, analyses of the 1-h and
55 24-h post transfusion platelet counts were conducted to examine the effects of platelet dose and
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3 patient size in addition to other variables recognized to affect platelet transfusion response. A
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5 logarithmic transformation was applied to the 1-h and 24-h platelet count data. Both 1-hour and
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7 24-hour log-transformed platelet count data were modeled via a linear ANCOVA model.
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9 Additional covariates included in the model (for adjustment) were treatment group, pre-
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11 transfusion platelet count, body surface area, the number of prior transfusions (in relation to the
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13 study transfusion) in the transfusion cycle, platelet dose, PC storage age, and study center.
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16 The number of platelet transfusions from time of randomization to study transfusion in
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18 the FAS population was compared between treatment groups using a Wilcoxon rank-sum test.
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20 Time to event (failure time data analysis) methods were used to analyze the time to the next
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22 platelet transfusion after the study platelet transfusion during both the immediate post platelet
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24 study transfusion period (24 hours of study transfusion) and the surveillance period (15 days
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26 after study transfusion) for the FAS population. The Kaplan-Meier method was used to analyze
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28 the time to next platelet transfusion after the study platelet transfusion has been administered.
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30 Failure time distributions were compared using a two-sided log rank test at the 0.05 significance
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32 level.
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35 Changes in hemostatic score were defined as the difference between post study
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37 transfusion hemostatic score and pre study transfusion hemostatic score. Changes in scores
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39 were compared between treatment groups using a Wilcoxon rank-sum test. The frequencies of
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41 clinically important bleeding between treatment groups were compared using Fisher's exact
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43 test. The number of RBC units transfused in the FAS population within 24 hours after start of
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45 study platelet transfusion was summarized and compared between treatment groups using a
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47 Wilcoxon rank-sum test.
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50 The number and proportion (%) of patients with one or more related (possibly related,
51
52 probably related, or related) and unrelated adverse events(AE) within 24 hours after study
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54 transfusion were summarized by sign/symptom and overall across all signs/symptoms. The rate
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56 of patients with one or more related adverse events was compared using a Fisher's exact test.
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Table 1: Demographics of all the patients who received a study transfusion (full analysis set population).

	Treatment Group		p-value ^a
	Amotosalen-PC N = 105	Conventional-PC N = 106	
Age (years)			0.59
Mean (± SD)	49.4 (12.57)	48.0 (13.86)	
Median	52	50	
Min, Max	19, 75	18, 72	
BSA (m ²) ^b			0.42
Mean (± SD)	1.81 (0.198)	1.83 (0.202)	
Median	1.8	1.8	
Min, Max	1.3, 2.4	1.4, 2.5	
Gender			0.21
Male	54 (51.4%)	64 (60.4%)	
Female	51 (48.6%)	42 (39.6%)	
Ethnic Origin			
Caucasian	104 (99.0%)	104 (98.1%)	1.00
Black	0	1 (0.9%)	
Asian	1 (1.0%)	1 (0.9%)	
Patient Care			0.54
ICU	0	0	
Outpatient	6 (5.7%)	4 (3.8%)	
Inpatient (non-ICU)	99 (94.3%)	102 (96.2%)	

a. p-values based upon Wilcoxon rank-sum test for quantitative data or Fisher's exact test for categorical data

b. Body Surface Area calculated by the modified DuBois and DuBois formula: $BSA = 0.007184 \times (\text{weight in kg})^{0.425} \times (\text{height in cm})^{0.725}$

Table 2: Immunohematology and Alloimmunization Risk Factors of all the patients who received the study transfusion (full analysis set population)

	Treatment Group				p-value ^a
	Amotosalen-PC N = 105		Conventional-PC N = 106		
Blood Type					0.72
A	48	(45.7%)	46	(43.4%)	
B	9	(8.6%)	9	(8.5%)	
O	41	(39.0%)	47	(44.3%)	
AB	7	(6.7%)	4	(3.8%)	
Rhesus Factor					0.54
Negative	12	(11.4%)	16	(15.1%)	
Positive	93	(88.6%)	90	(84.9%)	
Previous Transfusion					0.74 ^b
Yes	81	(77.1%)	82	(77.4%)	
No	21	(20.0%)	24	(22.6%)	
Unknown	3	(2.9%)	0		
If previous transfusion response = yes, any known adverse event?					0.50 ^b
Yes	6	(7.4%)	3	(3.7%)	
No	74	(91.4%)	77	(93.9%)	
Unknown	1	(1.2%)	2	(2.4%)	
Pregnancy History					1.00 ^b
Not applicable	54	(51.4%)	65	(61.3%)	
Yes	38	(36.2%)	28	(26.4%)	
No	13	(12.4%)	9	(8.5%)	
Unknown	0		4	(3.8%)	

a. p-values based upon Fisher's exact test

b. Categories Not applicable and Unknown are excluded from the analysis

Table 3: Primary Diagnoses and Current Therapy Contributing To Thrombocytopenia of all the patients who received the study transfusion (full analysis set population)

	Treatment Group		p-value ^a	
	Amotosalen-PC N = 105	Conventional-PC N = 106		
Diagnoses				
Hematological Malignancies	99 (94.3%)	103 (97.2%)	0.33	
Non-Malignant Hematological Disorder	4 (3.8%)	1 (0.9%)	0.21	
Solid Tumor	0	1 (0.9%)	1.00	
Other Diagnosis	2 (1.9%)	1 (0.9%)	0.62	
Therapy				
Chemotherapy without stem cell transplant	28 (26.7%)	36 (34.0%)	0.29	
Stem cell transplant with chemotherapy and/or radiotherapy	74 (70.5%)	68 (64.2%)	0.38	
Autologous	44 (41.9%)	42 (39.6%)	0.78	
Allogeneic	30 (28.6%)	26 (24.5%)	0.54	
Other Therapy	3 (2.9%)	2 (1.9%)	0.68	

a. p-values based upon Fisher's exact test.

Table 4: Number of RBC Units Transfused within 24 hours from the start of the study transfusion (full analysis set population).

	Treatment Group		p-value
	Amotosalen-UVA N = 105	Reference N = 106	
Number of RBC units transfused			0.821 ^a
0	80 (76.2%)	83 (78.3%)	
1	3 (2.9%)	1 (0.9%)	
2	22 (21.0%)	20 (18.9%)	
3	0	1 (0.9%)	
4	0	0	
5	0	0	
6	0	1 (0.9%)	

a. p-values based upon Wilcoxon rank-sum test.

Table 5: Summary of Adverse Events within 4 Days Occurring in ≥5% of the patients (full analysis set population)

Adverse Events by System Organ Class (SOC) <i>Preferred Term (PT)</i>	Number of Patients (%)		
	INTERCEPT N = 105	Reference N = 106	p -value
At least one AE^a	88 (83.8%)	91 (85.8%)	0.705
Cardiac Disorders	7 (6.7%)	11 (10.4%)	0.461
<i>Tachycardia</i>	5 (4.8%)	7 (6.6%)	0.768
Gastrointestinal Disorders	43 (41.0%)	46 (43.4%)	0.781
<i>Abdominal pain</i>	6 (5.7%)	5 (4.7%)	0.768
<i>Diarrhea</i>	22 (21.0%)	10 (9.4%)	0.022
<i>Nausea</i>	13 (12.4%)	4 (3.8%)	0.024
<i>Vomiting</i>	10 (9.5%)	13 (12.3%)	0.660
General Disorders and Administration Site Conditions	51 (48.6%)	47 (44.3%)	0.582
<i>Mucosal inflammation</i>	10 (9.5%)	12 (11.3%)	0.822
<i>Pyrexia</i>	35 (33.3%)	29 (27.4%)	0.372
Infections and Infestations	14 (13.3%)	12 (11.3%)	0.681
Injury, Poisoning and Procedural Complications	14 (13.3%)	8 (7.5%)	0.184
<i>Transfusion reaction</i>	11 (10.5%)	7 (6.6%)	0.336
Investigations	19 (18.1%)	21 (19.8%)	0.861
<i>Hemoglobin decreased</i>	17 (16.2%)	17 (16.0%)	1.000
Musculoskeletal and Connective Tissue Disorders	6 (5.7%)	6 (5.7%)	1.000
Nervous System Disorders	11 (10.5%)	13 (12.3%)	0.829
<i>Headache</i>	6 (5.7%)	11 (10.4%)	0.312
Respiratory, Thoracic and Mediastinal Disorders	15 (14.3%)	21 (19.8%)	0.361
<i>Epistaxis</i>	8 (7.6%)	15 (14.2%)	0.184
Skin and Subcutaneous Tissue Disorders	19 (18.1%)	16 (15.1%)	0.584
<i>Petechiae</i>	5 (4.8%)	6 (5.7%)	1.000
<i>Rash</i>	6 (5.7%)	2 (1.9%)	0.170
Vascular Disorders	12 (11.4%)	15 (14.2%)	0.681
<i>Hypotension</i>	7 (6.7%)	7 (6.6%)	1.000

a. All patients who experienced at least one adverse event (AE) within 4 days following the study transfusion.

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For Peer Review