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# Red blood cell concentrates treated with the amustaline (S-303) pathogen reduction system and stored for 35 days retain post-transfusion viability: results of a two-centre study

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**Background and Objectives** Pathogen reduction technology using amustaline (S-303) was developed to reduce the risk of transfusion-transmitted infection and adverse effects of residual leucocytes. In this study, the viability of red blood cells (RBCs) prepared with a second-generation process and stored for 35 days was evaluated in two different blood centres.

**Materials and Methods** In a single-blind, randomized, controlled, two-period crossover study ($n$ = 42 healthy subjects), amustaline-treated (Test) or Control RBCs were prepared in random sequence and stored for 35 days. On day 35, an aliquot of $^{51}$Cr/$^{99m}$Tc radiolabeled RBCs was transfused. In a subgroup of 26 evaluable subjects, 24-h RBC post-transfusion recovery, mean life span, median life span ($T_{50}$) and life span area under the curve (AUC) were analysed.

**Results** The mean 24-h post-transfusion recovery of Test and Control RBCs was comparable (83·2 ± 5·2 and 84·9 ± 5·9%, respectively; $P$ = 0·06) and consistent with the US Food and Drug Administration (FDA) criteria for acceptable RBC viability. There were differences in the $T_{50}$ between Test and Control RBCs (33·5 and 39·7 days, respectively; $P$ < 0·001), however, these were within published reference ranges of 28–35 days. The AUC (per cent surviving × days) for Test and Control RBCs was similar (22·6 and 23·1 per cent surviving cells × days, respectively; $P$ > 0·05). Following infusion of Test RBCs, there were no clinically relevant abnormal laboratory values or adverse events.

**Conclusion** RBCs prepared using amustaline pathogen reduction meet the FDA criteria for post-transfusion recovery and are metabolically and physiologically appropriate for transfusion following 35 days of storage.

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**Key words:** amustaline, pathogen reduction, red blood cells, S-303, viability.

## Introduction

Despite the use of donor screening tests and donor deferral policies, the risk of transfusion-transmitted infectious diseases (TTID) persists due to undetected low titres of classical transfusion-transmitted infectious agents and emerging pathogens such as dengue, chikungunya and Zika viruses [1–4]. In addition,

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patients at risk of transfusion-associated graft-versus-host disease (TA-GVHD) may not be recognized as such, and consequently may not be given irradiated blood products [5]. The risk of TTID must also be considered from the perspective of extensive cumulative exposure that some patients may experience due to the need for chronic transfusion support [6].

An alternative means to reduce the risk of TTID and TA-GVHD is pathogen inactivation (PI) treatment of blood components targeted to nucleic acid. Currently, pathogen-reduced platelet and plasma products prepared with photochemical technology to inactivate nucleic acids are licensed for use in the United States and in many other countries [7, 8]. Pathogens and viable residual leucocytes are inactivated by inhibition of nucleic acid replication [9, 10]. However, as yet, there are no approved pathogen reduction technologies for RBC components.

Nucleic acid-targeted technology that does not require photochemical activation was developed using a small molecule (amustaline, S-303) that reacts rapidly with nucleic acid and then decomposes into unreactive by-products [11]. Published pathogen inactivation results show that amustaline treatment of RBCs effectively inactivates multiple bloodborne pathogens ($\geq 4\ \log_{10}$) and residual leucocytes [6, 12–16]. To prevent non-specific reactions of amustaline with RBC membrane proteins, glutathione (GSH) is used in conjunction with the amustaline treatment.

RBCs treated with a first-generation amustaline process (0·2 mM amustaline and 2 mM GSH) were examined in six clinical studies [17–20]. In one study which enrolled patients requiring repeated transfusion support for chronic anaemia, two patients developed treatment-emergent antibodies to amustaline-treated RBCs [19, 20]. Although these low-titre antibodies did not cause clinical haemolysis, or support *in vitro* phagocytosis of sensitized RBCs, the process was modified to reduce the reactivity of amustaline with the RBC surface by increasing the concentration of GSH from 2 to 20 mM and using a neutralized form of GSH to control pH after the addition of GSH, and by incorporating a post-treatment replacement step with a standard RBC additive solution prior to storage for 35 days [21, 22]. A prior recovery and life span study with a prototype of the current system was conducted with processing in a centralized laboratory facility that required shipment of RBC components and delayed amustaline treatment up to 5 days after collection [23]. The primary objective of the current study was to assess the *in vivo* viability of RBC components treated with the intended commercial components of the amustaline process in two regional blood banks.

## Materials and methods

### Preclinical *in vitro* studies

Prior to the conduct of the clinical study, and to demonstrate transfer of the PI process, two *in vitro* studies were conducted. The effect of the PI protocol on the biochemical properties of treated RBCs and the effect of the PI hold time and temperature on the *in vitro* quality of amustaline-treated RBCs compared to ABO-matched controls were assessed in these studies. The first study was performed at PI incubation temperature of 22·5–25°C and post-amustaline treatment hold time of 18–24 h. The second study was performed using the PI incubation temperature range of 20–25°C and upper bound of the post-amustaline treatment hold time of 23–24 h.

### Preparation of Test and Control RBCC

The RBC concentrates (RBCC) in this *in vitro* study were locally prepared by the blood centres from whole blood as described by Cancelas *et al.* [23].

Briefly, RBCC used in the preclinical *in vitro* trials and in the clinical trial were obtained from whole blood (475 ± 25 ml), drawn into a primary collection container containing citrate phosphate dextrose anticoagulant (Terumo Imuflex WB-RP blood bag system with in-line leucocyte reduction filter; Terumo Corp., Tokyo, Japan). Collected blood was leucocyte-reduced and processed by centrifugation into RBCs by removing plasma and adding AS-5 (Optisol™, Terumo Corp.) to achieve a final haematocrit ranging from 50% to 65%.

The protocol for amustaline-treated RBCs consisted of diluting AS-5 RBCs in a processing solution to reach a haematocrit of approximately 40% in a closed processing set (Cerus Corporation, Concord, CA, USA). The processing solution contained GSH sodium salt (Cerus) and amustaline dihydrochloride (Cerus) dissolved in 0·9% saline for injection. Approximately 15 ml of each was added to achieve final concentrations of 20 and 0·2 mM, respectively. Given the absence of relevant biological differences between the parameters measured (Table 1) in the preclinical studies, the clinical trial used a range of temperature and hold time that was within the ranges applied in the two preclinical trials and offered logistical advantages for implementation in blood banking, as follows: storage at 20°C to 25°C for 20–22 h. For all the trials, after the incubation hold, the RBC units were centrifuged for 6 min at 4200 g and approximately 250 ml of the supernatant removed and replaced with 90 ml of SAG-M, followed by monitored storage at 1–6°C.

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**Table 1** Preclinical studies: RBCC characteristics postcollection and after 35 days of storage

| | | Study no 1[a] ($n$ = 6 pairs) | | Study no 2[b] ($n$ = 6 pairs) | |
|---|---|---|---|---|---|
| | | Test | Control | Test | Control |
| Total haemoglobin/unit (g) | D2 | 49 ± 2 | 52 ± 2[c] | 48 ± 1 | 50 ± 2[c] |
| Haemoglobin loss due to treatment (g) | D2 | 3 ± 1 | NA | 4 ± 2 | NA |
| Extracellular protein (mg/dl) | D2 | 74 ± 11 | 775 ± 80[c] | 67 ± 10 | 1075 ± 101[c] |
| Haemolysis (%) | D2 | 0·01 ± 0·01 | 0·02 ± 0·04 | 0·0 ± 0·00 | 0·0 ± 0·00 |
| | D35 | 0·19 ± 0·06 | 0·22 ± 0·21 | 0·12 ± 0·02 | 0·13 ± 0·03 |
| pH at 37°C | D2 | 6·76 ± 0·02 | 6·93 ± 0·02 | 6·78 ± 0·01 | 6·99 ± 0·03 |
| | D35 | 6·43 ± 0·01 | 6·53 ± 0·01[d] | 6·43 ± 0·02 | 6·54 ± 0·02[d] |
| pH at 22°C | D2 | 6·99 ± 0·02 | 7·51 ± 0·03 | 7·01 ± 0·02 | 7·22 ± 0·03 |
| | D35 | 6·65 ± 0·02 | 6·76 ± 0·02 | 6·65 ± 0·02 | 6·76 ± 0·02 |
| Extracellular $K^+$ (mmol/l) | D2 | 0·61 ± 0·09 | 3·66 ± 0·41 | 0·71 ± 0·06 | 4·37 ± 0·37 |
| | D35 | 49·82 ± 4·08 | 48·46 ± 3·65 | 46·02 ± 4·12 | 47·00 ± 2·59[d] |
| Extracellular $Na^+$ (mmol/l) | D2 | 145·5 ± 0·6 | 144·9 ± 1·1 | 145·4 ± 0·7 | 144·4 ± 1·9 |
| | D35 | 99·6 ± 2·8 | 104·5 ± 2·9 | 104·8 ± 2·6 | 108·3 ± 4·7 |
| Extracellular glucose (mmol/l) | D2 | 28·0 ± 1·0 | 31·9 ± 1·8 | 28·3 ± 2·3 | 30·2 ± 1·5 |
| | D35 | 20·8 ± 1·3 | 22·5 ± 2·4[d] | 18·9 ± 1·1 | 18·3 ± 1·5 |
| Extracellular lactate (mmol/l) | D2 | 4·4 ± 0·5 | 4·7 ± 0·6 | 6·3 ± 0·4 | 4·5 ± 0·6 |
| | D35 | 19·9 ± 3·0 | 22·3 ± 3·4[d] | 20·3 ± 2·0 | 26·8 ± 2·3 |
| Total ATP (μmol/g haemoglobin) | D2 | 6·22 ± 0·65 | 4·31 ± 0·45 | 7·00 ± 1·59 | 5·11 ± 1·71 |
| | D35 | 3·57 ± 0·82 | 3·59 ± 0·57 | 4·83 ± 0·90 | 4·31 ± 0·59[d] |
| Postrejuvenation total ATP (μmol/g haemoglobin) | D35 | 7·70 ± 0·78 | 7·14 ± 0·63 | 8·60 ± 0·61 | 7·42 ± 0·36[c] |
| Postrejuvenation 2,3-DPG (μmol/g haemoglobin) | D35 | 10·25 ± 1·44 | 11·48 ± 1·27 | 11·04 ± 1·59 | 14·62 ± 1·44[c] |
| Postrejuvenation oxygen dissociation curve (p50) | D35 | 30·60 ± 2·15 | 30·86 ± 2·30[c] | 26·72 ± 0·71 | 27·50 ± 1·47[c] |
| Haematocrit (%) | D2 | 62·9 ± 2·4 | 59·3 ± 1·7 | 63·8 ± 1·0 | 59·3 ± 1·7 |
| | D35 | 66·1 ± 1·3 | 62·6 ± 1·8[d] | 65·7 ± 1·4 | 63·0 ± 1·7[d] |
| Mean corpuscular haemoglobin concentration (MCHC; g/dl) | D2 | 29·5 ± 0·7 | 30·3 ± 1·4 | 28·9 ± 0·9 | 30·0 ± 1·0 |
| | D35 | 27·3 ± 0·3 | 27·3 ± 0·5 | 28·1 ± 0·9 | 28·1 ± 0·9 |
| Mean cell volume (MCV; fl) | D2 | 97·4 ± 6·5 | 95·8 ± 6·1 | 100·4 ± 3·4 | 96·9 ± 3·9 |
| | D35 | 101·6 ± 5·2 | 102·4 ± 5·3 | 104·9 ± 4·2 | 104·7 ± 3·4 |
| RBC count ($\times 10^6$ cells/μl) | D2 | 6·43 ± 0·28 | 6·20 ± 0·25 | 6·36 ± 0·20 | 6·13 ± 0·18 |
| | D35 | 6·45 ± 0·28 | 6·12 ± 0·25 | 6·27 ± 0·28 | 6·02 ± 0·20 |

NA, not applicable.
[a]PI incubation temperature of 22·5–25°C and post-amustaline treatment hold time of 18–24 h.
[b]PI incubation temperature of 20–25°C and the post-amustaline treatment hold time of 23–24 h.
[c]*P*-values (<0·05), Student's paired *t*-test two-tailed) used for non-repeated data.
[d]*P*-values (<0·05) indicating statistically significant difference between overall means of Test and Control data across multiple days of testing (repeated-measures ANOVA).

An aliquot was removed from the RBCs immediately after collection (day 0), post-treatment, and on the last day of storage (day 35) using closed system sampling methods. These samples were assessed for the following *in vitro* parameters: $pH_{37°C}$, free haemoglobin, adenosine 5′-triphosphate (ATP), 2,3-diphosphoglycerate (2,3-DPG), 2,3-DPG postrejuvenation (Rejuvesol™; Citra Labs, Braintree, MA, USA), extracellular potassium, extracellular glucose, extracellular lactate, packed red cell volume and mean corpuscular haemoglobin concentration (MCHC) as previously described [24].

## Clinical trial

The study used a randomized, controlled, two-treatment-period design (Fig. 1). A total of 42 subjects were enrolled. All subjects were enrolled and consented using an informed consent document approved by the local Institutional Review Board, at two study sites [the Hoxworth Blood Center, University of Cincinnati, Cincinnati, OH (site 1), and BloodCenter of Wisconsin, Milwaukee, WI (site 2)]. The order in which each subject was transfused with Test and Control RBCC was randomly assigned at the time of enrolment. Each treatment period consisted

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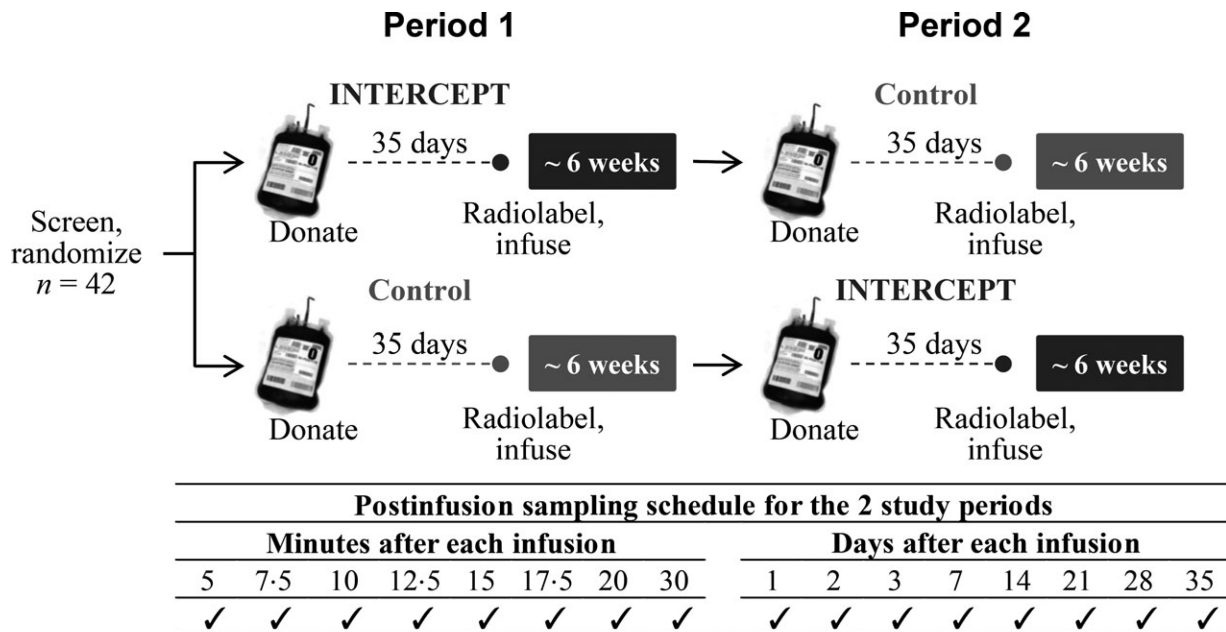


**Fig. 1** Schema of the study design. Subjects were enrolled in two treatment periods where autologous Control or Test RBCs, randomly, were radiolabeled and infused.

of autologous blood donation and preparation of RBCC according to treatment assignment. In the group of subjects evaluated for *in vivo* recovery/survival of transfused RBCC, the previous steps were followed by the infusion of $^{51}Cr/^{99m}Tc$ radiolabeled autologous RBCs and collection of blood samples for assessment of RBC recovery and life span.

The primary end-point was the 24-h post-transfusion RBC recovery. Additional end-points were as follows: mean life span, median life span ($T_{50}$) and life span $AUC_{0\text{-last}}$ (AUC up to the last measurable radioactive sample); the incidence of antibody to amustaline RBCC following transfusion of Test RBCC measured using a modified gel card IAT (indirect antiglobulin test) against amustaline RBCs; incidence of adverse events (AEs); and the *in vitro* characteristics of Test and Control RBCC after 35 days of storage.

## Radiolabeled RBC 24-h recovery and survival analyses

RBC radiolabeling, transfusion and sample collection and analysis were performed as previously described [23, 25, 26].

## Detection of antibodies to amustaline RBCs

For subjects evaluated for RBC recovery/survival, sera obtained prior to infusion on day 35 of treatment periods 1 and 2 were tested for antibodies to autologous Control and autologous Test RBCs using a commercial anti-human IgG gel card IAT modified to detect the presence of immune response to amustaline-treated RBCs (Ortho Clinical Diagnostics, Raritan, NJ). Test and Control sera, collected and frozen on days 49 and 70 of each period, were screened against three individual units of amustaline-treated and untreated allogeneic RBCs to detect specific antibodies directed against amustaline-treated RBCs. These assays were conducted at a central reference laboratory (American Red Cross Blood Services, Pomona, CA).

## Statistical methods

Sample size was determined with 80% power to detect a treatment difference of 4·4% (absolute difference) in RBC recovery and 5·2 days for median life span ($T_{50}$) at the two-sided significance level of 0·05, assuming a standard deviation (SD) of 7·5% and 8·8 days for the paired difference in RBC recovery and $T_{50}$, respectively, based on a previous crossover study of amustaline RBC recovery and life span [18].

For the *in vitro* studies, repeated-measures ANOVA (SAS 9.2, SAS Institute, Cary, NC) was used to examine the impact of storage time and treatment on various RBC test outcomes. Assays performed on Test and Control samples at the end of storage (plasma free haemoglobin, level of haemolysis, ATP, extracellular protein, postrejuvenation

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2,3-DPG and p50) were evaluated using Student's paired *t*-test (Microsoft Excel). For all statistical comparisons, a *P*-value <0·05 was considered statistically significant.

## Results

### Preclinical *in vitro* studies

Amustaline RBCC had mean haemoglobin levels of 48–49 g/unit with an average processing loss of haemoglobin of 3–4 g haemoglobin/unit (~4%) (Table 1). As expected, the prestorage exchange step reduced the extracellular protein in Test RBCC by 10- to 15-fold compared to Control RBCC. In all units, after 35 days of storage haemolysis remained below 0·3%, pH ranged between 6·4 and 6·7, and $K^+$, $Na^+$, glucose and lactate were comparable; ATP was above 3·5 μmol/g haemoglobin; and 2,3-DPG could be regenerated. No significant differences were observed between the two treatment conditions assessed.

### Clinical study

#### *Demographics and baseline subject characteristics*

A total of 42 subjects were randomized, but one withdrew before the first donation, so the safety population comprised 41 subjects, including 28 men and 13 women, ranging in age from 24 to 70 years (Fig. 2). Two subjects were withdrawn before completion, and 14 did not meet evaluable criteria for recovery/survival due to inconsistent haematocrit values that did not allow for an adequate calculation of 24-h post-transfusion recovery. For the *in vivo* efficacy study of RBC recovery/survival, a total of 26 subjects (19 men and seven women) completed both phases of the *in vivo* recovery and survival study, within the sample size required for a power ≥80%. The ABO/Rh blood groups of all subjects enrolled and those who completed the vivo studies were similar (data not shown). Direct antiglobulin tests and antibody screens were negative for all subjects enrolled.

#### *Haemoglobin content and prestorage haemolysis of Test and Control units*

A total of 40 subjects completed blood collection; one subject did not provide a full whole blood unit at the first collection and one subject was only able to donate once. The haemoglobin content and characteristics of RBCC analysed on day 35 of storage were suitable for transfusion (Table 2).

#### *24-h post-transfusion recovery*

The mean post-transfusion recoveries were 83·2% and 84·9% for Test and Control RBCs (95% CI of mean treatment difference −3·6, 0·0), respectively (Table 3). These

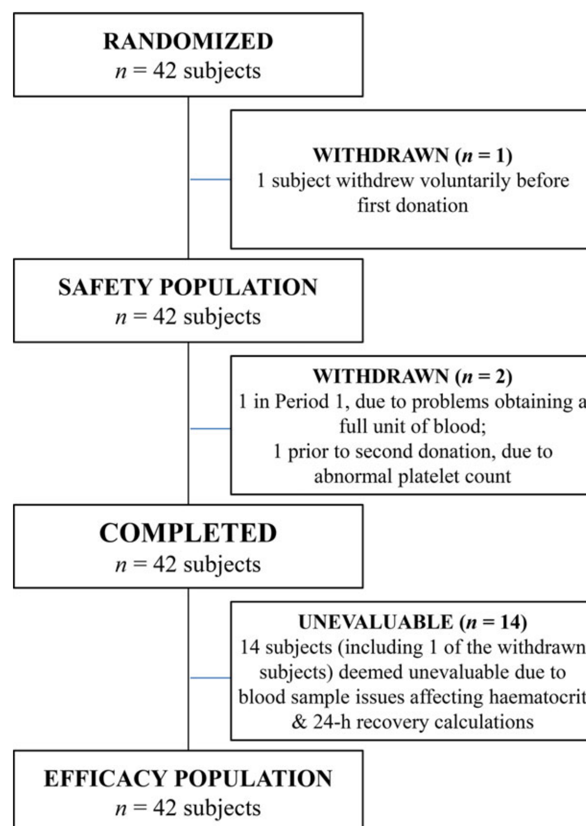


**Fig. 2** Subject disposition.

mean values were greater than the FDA guidance criteria for a minimum average recovery of 75%. The standard deviations for recovery in Test and Control were 5·2% and 5·9%, respectively, consistent with the FDA maximum criterion of 9%. There was one subject in each treatment group with recovery <75%. The lower bound of the confidence interval (CI) for the proportion of subjects with a recovery of 75% or greater exceeded the minimum requirement of 70%. The lower bounds for Test and Control were both 83%. Mean 24-h postinfusion recovery of Test and Control RBCs was not statistically significant for either the analysis performed using the dual-label method of $^{51}Cr/^{99m}Tc$ (Table 3) or single-label analysis of $^{51}Cr$ radiolabeled RBC recovery (data not shown).

#### *Red blood cell survival*

The mean (95% CI) time–course of life span proportion for Test and Control is shown in Fig. 3. The average mean life span (calculated by the weighted mean method) for Test and Control was 62·8 and 75·1 days, respectively. Although differences between Test and Control were statistically significant, mean life span of Test RBCC was within reported reference ranges indicating RBC viability [27] and within the 20% of the Control mean (Table 3).

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**Table 2** Clinical trial: characteristics of RBCC following 35 days of storage

| Characteristic | Test RBCs ($n$ = 39) Summary[a] | Control RBCs ($n$ = 40) Summary[a] | Test RBCs–Control RBCs ($n$ = 39) Summary[a] | 95% CI[b] |
|---|---|---|---|---|
| Volume (ml) | 295·9 (14·6) | 315·0 (15·9) | −18·6 (14·8) | −23·5 to −13·8 |
| Haemoglobin (g/unit) | 59·8 (4·9) | 63·0 (5·6) | −2·9 (4·3) | −4·3 to −1·5 |
| Haemoglobin concentration (g/dl) | 20·2 (0·9) | 20·0 (1·0) | 0·3 (0·6) | 0·1–0·5 |
| ATP (μmol/g) | 4·83 (0·93) | 3·89 (0·79) | 0·91 (0·61) | 0·71–1·11 |
| Haemolysis (%) | 0·22 (0·08) | 0·24 (0·14) | −0·02 (0·15) | −0·07 to 0·03 |
| Plasma free Hb (μmol/l) | 76·0 (28·2) | 85·3 (53·2) | −8·6 (55·7) | −26·7 to 9·4 |
| Haematocrit (%) | 61·3 (3·9) | 62·9 (4·9) | −1·3 (2·2) | −2·1 to −0·6 |
| MCV (fl) | 92·8 (5·1) | 96·1 (5·7) | −3·3 (2·5) | −4·1 to −2·5 |
| MCHC (g/dl) | 33·0 (1·3) | 31·8 (1·4) | 1·1 (0·7) | 0·9–1·4 |

[a]Arithmetic means and standard deviations (noted in parentheses).
[b]95% CI for the mean treatment difference (T–C) based on a paired $t$-test.

**Table 3** Clinical trial: 24-h $^{51}Cr/^{99m}Tc$ post-transfusion recovery and life span

| | Test mean (SD) ($n$ = 26) | Control mean (SD) ($n$ = 26) | Mean difference (Test–Control) | 95% CI of Mean treatment Difference (Test–Control)[a] |
|---|---|---|---|---|
| 24-h post-transfusion recovery (%) | 83·2 (5·2) | 84·9 (5·9) | −1·8 | −3·6, 0·0 |
| Life span (days) | 62·8 (10·6)[b] | 75·1 (13·7) | −12·3 | −17·4, −7·2 |
| $T_{50}$ (days) | 33·5 (7·1)[b] | 39·7 (10·2) | −6·2 | −9·7, −2·6 |
| $AUC_{0\text{-last}}$ (surviving cells × days) | 22·6 (1·9) | 23·1 (2·2) | −0·6 | −1·4, 0·3 |

Results are presented as average (SD) and 95% CI of mean difference.
[a]The 95% CIs were all constructed using the variance estimates coming from a paired $t$-test.
[b]$P < 0{\cdot}001$, Test RBCs had a ~17% lower $T_{50}$ than Control RBCs. However, the median $T_{50}$ of Test RBCs was within the reference range of 32 days to 37 days.

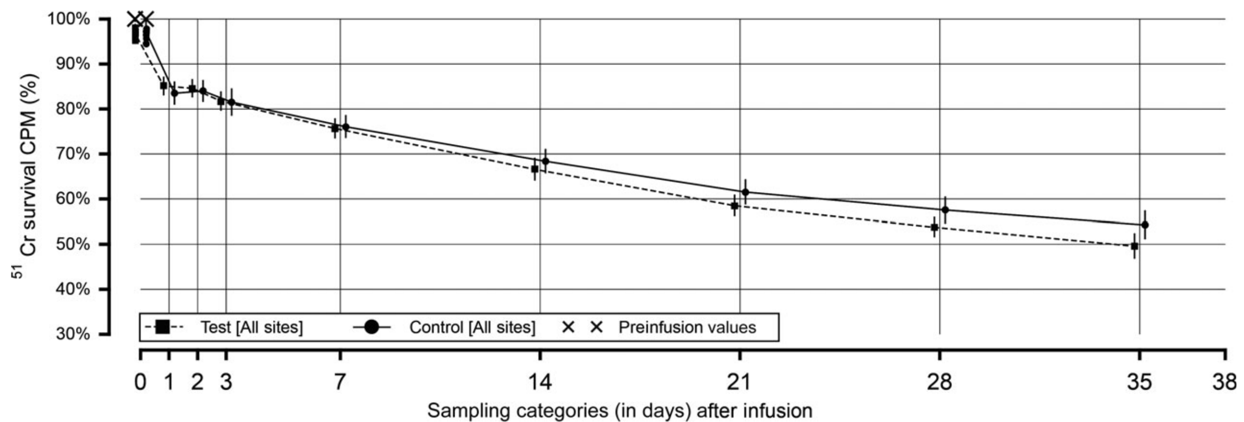


**Fig. 3** Mean time–course of life span proportion. Life span proportion was computed using the survival $^{51}$Cr CPM/g over 35 days postinfusion (day 0–35). Mean life span proportion is plotted at each sampling time-point, and the corresponding 95% CI is displayed in vertical bars.

The average $T_{50}$ (calculated by the weighted mean method) for Test and Control was 33·5 days and 39·7 days, respectively. The average $AUC_{0\text{-last}}$ for surviving cells, an integrated measure of RBC survival, was 22·6 and 23·1 (per cent surviving cells × days) for Test and Control groups, respectively, with a non-statistically significant difference of 0·6. Results were comparable between the two investigational sites (data not shown).

### Serology studies

IAT analyses for subjects' sera were negative for all subjects prior to infusion on day 35. After infusion, on days

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49 and 70 sera from all subjects were collected and were tested against a panel of three allogeneic amustaline RBCC. All IAT analyses, except for one subject, were negative; one subject had a weakly positive reaction (1+) for one of three Test RBCC, at day 49 after infusion of Control RBCs in period 2, and the period 1, day 70 serum sample after infusion of amustaline-treated RBCC was negative, suggesting that this reactivity was not likely to be specific for amustaline-treated RBCC.

### *Adverse events*

AEs collected up to 24 h following the start of the study blood donation and until 24 h post-study infusion were analysed for all 41 subjects. Overall, 15 subjects experienced an AE in period 1 or period 2; nine subjects experienced an AE during the Test period and eight subjects experienced an AE during the Control period. The most common AEs were haematomas and dizziness. None were related to the RBC autologous infusion. All AEs were mild. No serious adverse events (SAEs) occurred during this study. The overall incidence of AEs (including possibly or probably related) was not different between the Test and Control groups.

## Discussion

*In vitro* studies conducted in preparation for the recovery and life span study demonstrated that the range of incubation time and temperature for amustaline treatment of RBCC (18–24 h and 22·5–25°C, respectively) did not significantly impact the metabolic status of amustaline-treated RBCC. Additionally, the use of an overnight hold of 20 h at the highest temperature range (25°C) did not significantly impair the RBC recovery at 24 h and resulted in similar recovery and life span parameters to those observed in a previously completed trial in which whole blood was processed into RBCC at a single centre with delayed treatment (up to 5 days) and shipped to the blood centres for radiolabeling and infusion [23]. The in vitro data indicated that the amustaline treatment system provided RBCC with adequate total haemoglobin content, and these components remained physiologically intact and suitable for transfusion through 35 days of storage.

As in the previous clinical study with centrally processed RBCC and PI treated within 5 days with a prototype of this technology [23], RBCC treated with amustaline-based PI met all FDA criteria for RBC recovery after having been stored for 35 days. This was true regardless of the labelling method used to calculate red blood cell recovery (dual label [$^{51}$Cr/$^{99m}$Tc] or single label [$^{51}$Cr]), or the study site. These results indicate that RBCs treated with amustaline remained in the circulation at comparable levels to Control RBCs 24-h post-transfusion.

Mean potential life span has been considered a poor indicator of RBC life span because it does not evaluate how long a substantial population of transfused RBCs remains in circulation and instead measures the clearance of the oldest cells from the circulation. In addition, it requires extrapolation from the initial clearance curve after samples no longer contain measurable radioactivity and utilizes an empirical correction factor for $^{51}$Cr elution. Mean remaining life span has been proposed as a new parameter to quantify the duration of donor RBCs that remain in the recipient's circulation [28]. However, its use has yet to be validated in studies with radiolabeled RBCs. A more robust parameter to assess the bioavailability of radiolabeled autologous RBCs transfused is the AUC as it quantifies the extent of RBC exposure and clearance from the body during the period with measurable radioactivity [29].

All AEs were graded mild and comparable between the two arms. There were no clinically relevant abnormal laboratory values following infusion of Test RBCs. All cross-match assays of autologous amustaline RBCs were non-reactive. In the context of infusion of single small doses of amustaline-treated RBCs, there was no evidence of immune response to 35-day-stored amustaline-treated RBCs up to 70 days postinfusion. Importantly, this study had limited power to assess safety events due to the small sample size and small volume exposure to the Test RBCs.

The current study has some limitations. The dual- and single-label methods for measuring RBC survival allow radiolabeling of autologous RBCs to relatively high specific activity, permitting RBC survival to be performed with small infusions. However, spontaneous elution of label from RBCs and the potential for variability in the rate of label elution between subjects and treatments cannot be measured. In this study, label elution was corrected using the empirical methods recommended by ICSH [30–31] and relies on the assumption that the labels interact similarly with Test and Control RBCC. The standard elution correction of approximately 1% per day was applied to both Test and Control based on historical data [32]. This assumption has not been tested, and if the label were to elute faster or slower from Test than Control RBCC, all calculations of survival would be impacted. The lack of a difference in post-transfusion recovery and in the AUC indicated that small differences in label elution rates may be the most plausible explanation for the observed differences in life span with Control values being greater than the reference range and Test RBC values being within the expected reference range. Small differences in the elution rates could result in a large cumulative error over time, impacting the calculation of RBC life span.

In summary, the 24-h post-transfusion recovery of RBCs prepared using the amustaline PI process was not

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significantly different than that of Control RBCs and met the FDA guidance criteria for 24-h RBC recovery at the end of the 35-day storage period. The Test RBC survival assessed by three different parameters fell within 20% of Control RBCs, indicating bioequivalence of RBC viability.

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## Conflict of interests

The study was supported with funding from Cerus Corporation. MA Schott, A North, N Huang, N Mufti, A. Erickson, S Rico and L Corash are employees of Cerus Corporation. JA Cancelas, JL Gottschall, N Rugg and S Graminske have no conflict to declare.

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