Amustaline-glutathione pathogen-reduced red blood cell concentrates for transfusion-dependent thalassaemia

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Summary

Transfusion-dependent thalassaemia (TDT) requires red blood cell concentrates (RBCC) to prevent complications of anaemia, but carries risk of infection. Pathogen reduction of RBCC offers potential to reduce infectious risk. We evaluated the efficacy and safety of pathogen-reduced (PR) Amustaline-Glutathione (A-GSH) RBCC for TDT. Patients were randomized to a blinded 2-period crossover treatment sequence for six transfusions over 8–10 months with Control and A-GSH-RBCC. The efficacy outcome utilized non-inferiority analysis with 90% power to detect a 15% difference in transfused haemoglobin (Hb), and the safety outcome was the incidence of antibodies to A-GSH-PR-RBCC. By intent to treat (80 patients), 12.5 ± 1.9 RBCC were transfused in each period. Storage durations of A-GSH and C-RBCC were similar (8–9 days). Mean A-GSH-RBCC transfused Hb (g/kg/day) was not inferior to Control (0.113 ± 0.04 vs. 0.111 ± 0.04, P = 0.373, paired t-test). The upper bound of the one-sided 95% confidence interval for the treatment difference from the mixed effects model was 0.005 g/kg/day, within a non-inferiority margin of 0.017 g/kg/day. A-GSH-RBCC mean pre-transfusion Hb levels declined by 6–0 g/l. No antibodies to A-GSH-RBCC were detected, and there were no differences in adverse events. A-GSH-RBCCs offer potential to reduce infectious risk in TDT with a tolerable safety profile.

Keywords: thalassaemia, transfusion, infection, RBC, iron.

Transfusion-dependent thalassaemia (TDT) requires regular transfusion to prevent complications of anaemia and excessive erythroid expansion (Cappellini & Motta, 2017). However, utilization of red blood cell concentrates (RBCC) in TDT must be managed to minimize transfusion iron (Fe) burden within the limits of chelation therapy (Cazzola et al., 1997; Cohen et al., 2008; Porter & Garbowski, 2018). Transfusion is associated with the risk of transfusion-transmitted infection (TTI) due to undetected known pathogens (Candotti et al., 2019), emerging pathogens (Stramer et al., 2013) and unrecognized bacterial contamination (Damagaard et al., 2015). Despite improved donor testing, long-term transfusion support has a substantial cumulative life-time residual risk of TTI (Kleinman & Stassinopoulos, 2015) and splenectomized TDT patients, in particular, may have increased TTI morbidity (Teawtrakul et al., 2015). Pathogen reduction of RBCC offers potential to reduce the risk of TTI.

Nucleic acid targeted pathogen reduction of RBCC using amustaline-glutathione (A-GSH) to inactivate viruses, bacteria, protozoa and leucocytes has been evaluated in healthy subjects demonstrating post-transfusion RBC recovery and life-span suitable for transfusion, and for support of acute anaemia during cardiovascular surgery (Cancelas et al., 2017; Brixner et al., 2018). A prior study with the original pathogen-reduced (PR)-RBCC process in TDT patients was halted due to the unexpected finding of antibodies to treated RBCC without evidence of clinical haemolysis (Benjamin et al., 2005; North et al., 2007; Geisen et al., 2018). A modified process was developed to reduce the risk of A-GSH RBCC immune responses. The present Phase 3 clinical trial was designed to evaluate the efficacy and safety for TDT using A-GSH RBCC (Test) prepared with this modified process. The study utilized repeated transfusion exposure to compare haemoglobin (Hb) use of A-GSH and conventional RBCC (Control), and to assess the incidence of antibodies to A-GSH RBCC within the range of RBC alloimmune responses (4–20%) reported for patients with congenital anaemia disorders (Chou et al., 2013). 

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Methods

Study design

The study (EUDRA: number 2012-002920-33) was conducted at 3 clinical centres in 2 countries (Italy and Turkey) with good clinical practice compliance in conformity with the International Conference on Harmonization (ISO 14155:2011) and the Helsinki Declaration. The research protocol was approved by the respective institutional human research review boards at each clinical site. All participants gave informed consent. A Data and Safety Monitoring Board provided oversight and the following prospectively defined stopping rules: if 3 subjects developed antibody with defined specificity to A-GSH RBCC, enrolment would be halted until the antibody haemolytic potential was defined, but subjects on study were continued as long as crossmatch-compatible RBCC were available. The study would be stopped if 2 subjects experienced a haemolytic transfusion reaction with accelerated RBC clearance and defined specificity to A-GSH RBCC.

Randomization and masking

Randomization to study treatment sequence was conducted by clinical site personnel using the electronic data capture system. Test and Control RBCC were prepared in identical containers. All clinical trial personnel were blinded to the randomized treatment sequence. Only the blood centre personnel that prepared the RBCC were not blinded: they did not have access to clinical trial data.

The trial utilized a randomized, double-blind, active-controlled 2-treatment period cross-over design to evaluate A-GSH RBCC, enrolment would be halted until the antibody haemolytic potential was defined, but subjects on study were continued as long as crossmatch-compatible RBCC were available. The study would be stopped if 2 subjects experienced a haemolytic transfusion reaction with accelerated RBC clearance and defined specificity to A-GSH RBCC.

Study treatments: preparation of RBCC

Blood centre personnel, blinded to clinical data, prepared all components. Both A-GSH RBCC (Test) and Control RBCC were prepared from CPD whole blood with leucocyte reduction and suspension in Saline-Adenine-Glucose-Mannitol (SAG-M) solution. The total haemoglobin Hb content (g) and volume (ml) were measured for each Test and Control RBCC prior to storage at 4–6°C for up to 35 days after collection. Pathogen reduction was performed in a functionally closed system of plastic containers within 24 h of collection using final concentrations of Amustaline (0.2 mmol/L) and Glutathione (20 mmol/L) as described. (Cancelas et al, 2017; Brixner et al, 2018) Process validation studies of Test RBCC at each production site were conducted prior to initiating clinical transfusions. Post-production and quality control studies were performed on a subset of Test RBCC on day 35 of storage (Erickson et al, 2018). Control RBCCs were processed in compliance with each centre’s standard operating procedures and respective national regulations.

Clinical study procedures

Patients were screened up to 30 days prior to randomization, stratified by country, and randomized to a transfusion sequence of Test-Control (T-C) or Control-Test (C-T) for six transfusion episodes in each treatment period over approximately 8–10 months with follow-up for 45 days after the last transfusion of the second period. Baseline clinical data included subject medical history, pre-transfusion haemoglobin (Hb) levels and target Hb threshold for the 6 months prior to enrolment, concomitant medications, pregnancy test for females, ABO and Rh, a blood sample reserved for RBC antigen genotype, and gel-card antibody

positive direct anti-globulin test (DAT) > 2+ with poly-specificity or indirect anti-globulin test (IAT) with pan-reactivity precluding definition of allogenic or auto antibody specificity, documented human immunodeficiency virus or hepatitis C virus infection, breast feeding, current cancer chemotherapy, other chronic medical disorder precluding study participation to completion, history of poor study compliance and participation in another study concurrently or in the preceding 28 days. Study withdrawal and stopping rules were pre-specified.

Study design. The study design for 2 wash-in transfusions (TXNS) followed by 4 efficacy evaluation transfusions (TXNS) is indicated.
screen to A-GSH treated and untreated RBC panel antigens to detect pre-existing native antibodies to A-GSH red cells (Data S1). Withdrawal was permitted for: patient request, pregnancy, medication associated with haemolysis, antibody to A-GSH RBCC preventing transfusion, antibody to A-GSH RBCC with haemolysis, antibody to RBC antigens limiting available phenotype matched RBC and grade ≥3 allergic transfusion reactions. Randomized subjects who withdrew from the study before receiving any study RBC transfusions for the first efficacy evaluation period were replaced; and subjects withdrawn in the first treatment period could be replaced.

Patients were managed with compatible RBCC with a target pre-transfusion Hb threshold of approximately 90–100 g/l, or as designated by treating physicians blinded to treatment and Hb content of RBCC. Each treatment period commenced with 2 wash-in transfusion episodes followed by 4 efficacy evaluation transfusion episodes (Fig 1). Within 72 h prior to each transfusion episode, subjects were evaluated for: serological crossmatch to the assigned RBCC, antibodies to A-GSH RBC, haematology full blood count panel, clinical chemistry panel. Direct anti-globulin tests (DAT) and indirect anti-globulin tests (IAT) were performed within 3–7 days before scheduled transfusion. Treating physicians, blinded to treatment assignment and Hb content of Test and Control RBCC, prescribed the number of RBCC required at each episode, and regulated the RBCC dose and interval between transfusion episodes to achieve physician-defined Hb levels. The transfused volume of RBCC was recorded for each transfusion episode. Patients were contacted 14 ± 2 days after each transfusion to assess adverse events (AE), serious AE (SAE) and delayed haemolytic transfusion reactions (DHTR). After completion of the last transfusion episode of the second treatment period, patients reverted to non-study conventional RBCC and were followed for two additional transfusions or at least 45 days for AE and SAE, antibodies to A-GSH and untreated RBC, and evidence of haemolysis (haematology and chemistry panels). Fe chelation therapy was maintained as prior to study entry, and could be adjusted per standard of care.

Outcome measures

The primary efficacy outcome was average Hb use expressed as Hb mass (g) transfused per kg body weight per day in transfusion episodes 3–6 after 2 wash-in transfusions for each treatment period. Average Hb use over all 6 transfusion episodes in each treatment period also was summarized. The primary safety outcome was the incidence of treatment-emergent antibodies to A-GSH RBCC during all transfusion episodes in each period and through the 45 day post-study surveillance. Secondary safety outcomes included the incidence of AE, SAE, antibodies to RBC alloantigens and acute transfusion reactions.

Statistical analyses

The primary efficacy analysis was conducted on the intent-to-treat (ITT) patients using a non-inferiority test with a pre-determined non-inferiority margin ≤15% of the Control mean value for average Hb transfused. Non-inferiority was assessed by comparing the upper bound of a one-sided 95% confidence interval (CI) for the treatment difference (Test-Control) of average Hb use of the observed Control mean. The study was designed with 90% power with 70 patients completing both treatment periods. For the primary efficacy analysis, the least squares (LS) means treatment difference (Test-Control) and a one sided 95% CI were estimated using a mixed effects model, where treatment, period, and treatment sequence were fitted as fixed effects, and patient as a random effect. A sensitivity analysis was conducted using a similar mixed effects model as specified for the primary efficacy analysis, with patient pre-transfusion Hb level included as an additional fixed effect. Continuous variables were summarized descriptively by mean, median and treatment difference (Test-Control), and compared by paired t test. For the primary safety endpoint, the 95% Clopper-Pearson CI was computed for the incidence of treatment-emergent antibodies. Proportions of patients with treatment-emergent AEs were compared between treatment periods using the Fisher’s exact test.

Results

Patient demographics

Eighty-six patients were randomized (45 to Test-Control and 41 to Control-Test, Fig 2). No screened patients were excluded due to pre-existing antibodies to A-GSH red cells. Eighty-one patients received at least one study RBCC (safety population). Eighty patients received RBCC evaluable for efficacy (transfusion episodes 3–6), and constituted the ITT population. Nine patients either: withdrew voluntarily (n = 1), received off-protocol RBCC (n = 6) or incorrectly assigned RBCC (n = 2). Thus, 71 patients received all RBCC per randomization assignment, and were summarized as per-protocol only (PPO). Baseline demographics demonstrated differences between countries in the proportional distribution of ABO groups, splenectomy status (Italy 1 of 13; Turkey 39 of 67), and prior transfusion management with phenotype matched RBCC (Table 1). Haemoglobin mutations and clinical phenotypes were consistent with TDT (Table S1). Among the total ITT population, 11-1% of patients had a history of alloantibodies to red cell antigens at entry to the study, and no patients were withdrawn due to unavailability of compatible RBCC. All patients were maintained on iron chelation therapy.

Baseline clinical characteristics

For the total ITT population, baseline clinical laboratory characteristics at entry to the first treatment period were not
different between randomization sequences (Table II). ITT patient baseline haemoglobin levels were lower in Turkey than in Italy; and lower for splenectomized patients, who were primarily enrolled in Turkey. In both countries, baseline mean corpuscular haemoglobin (MCH) values were within the normal range (27–31 pg), consistent with predominantly circulating donor RBC indicative of effective erythropoiesis suppression. Mean baseline platelet and leucocyte counts were higher in Turkey due to the inclusion of more splenectomized patients. Mean baseline LDH and total bilirubin levels were similar between regions, and between splenectomized and non-splenectomized patients (Table II).

**RBCC haemoglobin content and transfusion exposure**

During the study, 1024 Test and 1008 Control RBCC units were transfused. Post-production Test and Control RBCC demonstrated small differences in volume and mean Hb content due to expected average PR processing Hb losses of ~1 g (Table III). Total Hb content (g) of Test RBCC ranged from 39 to 73 g; and for Control RBCC from 35 to 74 g (Fig 3).

For the ITT population for all transfusion episodes, a mean of 12.5 ± 1.9 Test and Control RBCC were transfused per patient; however, the total Hb mass (g) of Test RBCC (683 ± 98) transfused was slightly less than Control RBCC (697 ± 109), but not statistically different (Table IV). The mean volume of Test RBCC transfused was statistically lower (P = 0.021). The mean storage age of RBCC was slightly shorter in Italy than Turkey, but overall similar for Test and Control RBCC (Table IV). The mean total Hb mass transfused per patient over all transfusion episodes was larger in Italy than Turkey. However, patient exposures to Test and Control RBCC components were similar within the non-splenectomized and splenectomized patient sub-groups; although splenectomized patients received less total Test Hb than non-splenectomized patients (Table IV). Only 11 (0.5%) of 2043 RBCC units transfused were off-protocol, primarily due to unavailable study RBCC (Table III).

**Efficacy outcome**

By ITT, efficacy evaluable transfusion episodes (n = 3–6) demonstrated a mean treatment difference (Test-Control) in Hb use of 0.002 g/kg/day (1.8% of Control mean), which was not significantly different (95% CI = −0.002 to 0.005, P = 0.373, paired t test) (Table V). Non-inferiority was observed with an upper bound of the 1-sided 95% CI of 0.005 g/kg/day (4.5% of Control) below the 15% inferiority margin (0.017 g/kg/day) based on the Control mean (0.111 g/kg/day). Additionally, the 2-sided 95% CI for the LS-mean treatment difference was −0.002 to 0.005 g/kg/day, within the pre-defined inferiority margin. Consistent results were observed with sensitivity analyses utilizing a paired t-test (2-sided 95% CI for the mean treatment difference = −0.002 to 0.005 g/kg/day), and a mixed effects model similar to the primary efficacy analysis with the addition of pre-transfusion Hb level included in the model (2-sided 95% CI for the mean treatment difference = −0.003 to 0.006 g/kg/day) confirming non-inferiority. From the primary mixed effects model, a significant period effect was observed (P = 0.007) where the LS mean of Hb use in Period 2 was 0.005 g/kg/day higher than Period 1, probably due to the increased Hb dose observed with the sequence of Period 1 Test to Period 2 Control RBCC (Fig 4D). When only Period 1 data were summarized, mean Hb use values were 0.115 and 0.104 g/kg/day for Test and Control RBCC, respectively, and a wider CI for the treatment difference (2-sided 95% CI = −0.004 to 0.026 g/kg/day, P = 0.155 by 2-sample t-test) was observed due to reduced statistical power. Analyses for the PPO population demonstrated a mean treatment difference (T-C = 0.003 g/kg/day, 95% CI = −0.001 to 0.006, P = 0.162 by paired t-test (Table SIII)). Additional analyses by ITT and PPO for all transfusion episodes indicated no difference in Hb use between Test and Control RBCC (Table VI and SIV).

Half of the patient population was splenectomized prior to study entry; these patients received significantly less (P = 0.019) Test RBCC Hb (673 ± 24 g) than Control RBCC (696 ± 85 g) (Table IV). Splenectomized patients were older, had higher body weight (Table I) and longer transfusion intervals, and used approximately 30% less Hb with both Test and Control RBCC (Tables V and VI). However, within the respective sub-groups of splenectomized and non-splenectomized patients, Hb use of Test RBCC and Control RBCC was not markedly different (Tables V, VI, SIV, SV).

The mean Hb dose was similar for ITT and PPO patients during Test and Control efficacy episodes 3–6 (Table V). However, during wash-in transfusions 1 and 2 (Fig 4A), the Hb dose was significantly less for Test RBCC (222 ± 37 g vs. Control 236 ± 38 g, P < 0.001). Mean pre-transfusion Hb levels with A-GSH RBCC declined over 6 transfusion episodes during Test periods by 6.0 g/l (Fig 4B), although average mean pre-transfusion Hb levels (g/l) for Test (92 ± 7.0) and Control (94 ± 7.0) remained within the targeted range (90–100 g/l), but were statistically different (P = 0.002, paired t-test). Despite changes in pre-transfusion Hb levels, patient average MCH values (Fig 4C) remained within the normal range (27–31 pg). The impact of transfusion sequence on pre-transfusion Hb levels was evaluated (Fig 4D, E). Mean Hb doses in Period 2 Control increased following Period 1 Test in response to consistently lower Test RBCC Hb doses and declining pre-transfusion Hb levels in Period 1 Test (Fig 4D, E). With larger Control Hb doses in Period 1 followed by adequate Test RBCC Hb doses in Period 2 (Fig 4D), pre-transfusion Hb levels in Period 2 with Test RBCC declined less (Fig 4E). Across both periods with Test and Control RBCC, regardless of treatment sequence, patient MCH remained consistently >27 pg (Fig 4F).
Fig 2. Disposition of patients. The disposition of patients from eligibility screening (n = 86), to randomization (n = 86) and exclusion (n = 5) is indicated. The randomization sequence assigned (Test to Control or Control to Test) is indicated with the first period assigned. The safety population (n = 81), the intent to treat (ITT) population (n = 80), and the per-protocol only (PPO) population (n = 71) are shown. One patient withdrew after a single study transfusion and was only assessed for safety. In the ITT population, 6 patients received off-protocol red blood cell concentrates (RBCC), 2 patients received incorrectly assigned study RBCC for a single transfusion episode, and 1 patient received all of the assigned transfusions in the Control period and only 3 of 6 transfusions in the Test period, thus was included in the ITT analysis but not the PPO analysis.
Safety outcomes

No patients developed antibodies specific to A-GSH RBCC. No patients developed emergent antibodies against RBC antigens during either the treatment period or post-transfusion surveillance, and no patients had clinical or laboratory evidence of immune haemolysis. Total bilirubin values were slightly elevated above the reference range at all times, but not different between Test and Control; and serum LDH levels remained within normal reference ranges. The incidence of all grades of treatment-emergent AE was comparable between treatment periods (Table SV). The majority of AE were of Grade 1 and 2 severity, and there was no difference in the distribution of clinical severity between Test and Control RBCC (P = 0.857). Within the System Organ Class of Metabolism Disorders there was an increase in the AE of Diabetes Mellitus attributable to an increased incidence of higher fasting blood glucose levels during Control periods.

The AE of “symptomatic anaemia”, defined by a pre-transfusion Hb level substantially below 90–100 g/l requiring an unscheduled transfusion, was reported for 6 Test period and 3 Control period patients, and one patient had “symptomatic anaemia” reported in both the Test and Control periods. The pre-transfusion Hb levels for these patients ranged from 60 to 78 g/l (Table SVI; Figure S1). Each of these patients had received RBCC doses below the study average Hb dose for Test and Control RBCC over successive transfusion episodes; and 2 patients had concurrent inflammatory AEs.
Table III. Post-production characteristics of clinical inventory test and control RBCC.*

| Parameter                        | Test (n = 1024) | Control (n = 1008) | Difference (CI)† | P‡ |
|----------------------------------|-----------------|-------------------|------------------|----|
| RBCC volume (ml)                 | 271.4 (19.0)    | 278.9 (22.2)      | −7.5 (−9.3, 5.7) | <0.001 |
| RBCC Hct (%)                     | 60.5 (2.4)      | 59.0 (2.8)        | 1.5 (1.7, 1.7)   | <0.001 |
| RBCC Hb content (g)              | 54.6 (5.9)      | 55.6 (5.9)        | −1.0 (−1.5, −0.5) | <0.001 |

Hb, haemoglobin; Hct, haematocrit.
*The mean (standard deviation) for the characteristics of Test (T) and Control (C) red blood cell concentrate (RBCC) transfused.
†The mean treatment differences (T-C) and the two-sided 95% confidence interval (CI) for the observed treatment differences.
‡P values based on ANOVA controlling for treatment and site.

Discussion

Pathogen reduction of platelet and plasma components has been introduced into routine practice in a number of countries as a prospective measure to reduce the risk of TTI. (U.S. Department of Health and Human Services Food and Drug Administration 2017) The current study is part of the clinical development programme for PR A-GSH RBCC to complete licensure of pathogen reduction for all blood components. This study was designed to assess the impact of A-GSH RBCC on Hb utilization, a direct measure of efficacy in TDT which requires long-term RBC support. By ITT and PPO analyses, haemoglobin use of A-GSH RBCC was not inferior to conventional RBCC. The calculated daily transfusion Fe burden associated with Test RBCC for all patients was 0.38 mg/kg/day, in agreement with the range of 0.33–0.43 mg/kg/day reported for conventional RBCC and, as previously described, we confirmed a lower transfusion Fe
### Table V. Haemoglobin use (g/kg/day) for the ITT populations in the efficacy evaluation period (transfusions 3, 4, 5 and 6)∗

|                  | Test Control Test | Control Test Control Test Control Test Control | Test Control Test Control Test Control Test Control |
|------------------|-------------------|-----------------------------------------------|-----------------------------------------------|
| **Interval**     | P                 | P                                             | P                                             |
| **ITT (n)**      | 13                | 40                                            | 40                                            | 40                                            |
| **Dose**         | 159 (133)         | 451 (47)                                      | 461 (59)                                      | 461 (59)                                      |
| **Interval**     | 0.09 (0.04)       | 1.10 (1.06)                                   | 0.09 (0.04)                                   | 0.09 (0.04)                                   |
| **Hb use†**      | 0.010 (0.03)      | 0.010 (0.03)                                  | 0.010 (0.03)                                  | 0.010 (0.03)                                  |
| **P-values**     | 0.001 (0.01)      | 0.001 (0.01)                                  | 0.001 (0.01)                                  | 0.001 (0.01)                                  |

Mean (SD) are presented. Data are presented by country and by spleen status for informational purposes. C, Control; Hb, haemoglobin; ITT, intent to treat; SD, standard deviation.

∗Data are presented for the ITT population (n = number of patients) by country, by spleen status and for the total ITT population.

†P-values for the treatment difference (T-C) for ITT population are derived from the paired t-test.
effect of A-GSH PR on Test RBC viability may not be excluded definitively. The duration of transfusion support may have impacted our ability to detect immune responses to A-GSH RBCC. Multiple studies have indicated that the number of RBCC transfused is an important determinant of alloimmunization; and that splenectomized patients have an increased rate of alloimmunization (Thompson et al, 2011; Vichinsky et al, 2014; Franchini et al, 2019). Al-Riyami et al (2018) estimated that the rate of alloimmunization per 100 units transfused was 0.56. Thus, within the limited duration of our study, we may have expected to observe approximately 5 new alloantibodies and, despite the inclusion of a substantial number of higher risk splenectomized patients and a transfusion policy of ABO-D matched RBCC in a majority of patients, no treatment emergent alloantibodies were observed. Studies of longer duration with A-GSH RBCC will be required to confirm our observations.

Fig 4. Transfused haemoglobin dose, pre-transfusion haemoglobin and patient mean corpuscular haemoglobin. (A–C) Test and Control Transfusion Episodes by Type of RBCC. (A) Mean haemoglobin (Hb) dose (g) transfused per episode; (B) Mean pre-transfusion patient Hb (g/l) per episode; (C) Pre-transfusion patient mean corpuscular haemoglobin (MCH, pg) are indicated for all Test (▲) and Control (□) red blood cell concentrate (RBCC) transfusion episodes. (D–F) Test and Control Transfusion Episodes by Sequence and Type of RBCC: For 41 patients, Test were transfused in Period 1 (▲) for episodes 1–6 followed by Control RBCC in Period 2 (■) for episodes 7–12. For 39 patients, Control RBCC (□) were transfused in Period 1 for episodes 1–6 followed by Test RBCC (▲) in Period 2 for episodes 7–12. (D) Mean Hb dose (g) transfused per episode; (E) Mean pre-transfusion patient Hb (g/l) per episode; (F) Pre-transfusion patient MCH (pg) are indicated for Test and Control RBCC by randomized treatment sequence for all transfusion episodes.
Table VI. Haemoglobin use (g/kg/day) for the ITT population for all RBCC transfusions in each treatment period (transfusions 1–6).*

| Country             | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control |
|---------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Italy               | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control |
| Turkey              | Non-splenectomized | 76 (200) | 13 | 66 (65) | 743 (230) | 17.4 (4.0) | 0.139 (0.03) | 0.030 (0.00) | 0.255 (0.01) | 0.410 (0.02) |
|                     | Splenectomized | 76 (200) | 67 | 68 (65) | 697 (131) | 18.3 (3.1) | 0.107 (0.03) | 0.110 (0.00) | 0.112 (0.03) | 0.255 (0.01) |
| Total population    | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control |
|                    | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control | Test Control |

*Data are presented for the ITT and PPO populations (n = number of patients) by country, by spleen status, and for the total ITT population.

Within the scope of this study, the experience with A-GSH RBCC may be generalizable to other types of chronic anaemia to reduce TTI risk without impacting Hb use and Fe transfusion burden, but further studies are required to support this opinion. This study showed that there is large variability (35–75 g) in the Hb content of donor RBCC. This is likely to impact both Fe loading rates and secondary Fe distribution (Porter & Garbowski, 2018). Active management of Hb dose over extended periods of transfusion support should be carefully evaluated further in routine use to determine if selection of RBCC with knowledge of Hb content can result in more consistent pre-transfusion Hb levels and further reduce transfusion Fe burden. In conclusion, this study showed that A-GSH pathogen reduction of RBCC did not significantly increase RBCC utilization in TDT patients, and appeared to be well-tolerated and logistically feasible for chronic transfusion therapy within each country’s economic resources.

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Authorship Contributions

Yesim Aydinok, Antonio Piga and Raffaella Origa conducted the clinical trial at the respective clinical trial sites. Nina Mufti, Anna Erickson, Anne North and Katie Waldhaus conducted the development, validation and transfer of the pathogen reduction process to the respective clinical blood transfusion services. Christine Ernst served as the sponsor medical monitor and coordinated study activities with the contract clinical research organization. Jin-Sying Lin and
Norman Huang wrote the statistical analysis plan and analyzed the data. Richard J Benjamin supervised clinical conduct of the study, and wrote amended versions of the protocol. Laurence Corash designed the study protocol, wrote the initial study protocol and oversaw clinical conduct of the study. Richard Benjamin and Laurence Corash wrote the manuscript. The data and the manuscript were reviewed and edited by Yesim Aydinok, Antonio Piga, Raffaella Origa, Nina Mufti, Christine Ernst, Jin-Sying Lin, Nortman Huang, Richard F. Benjamin and Laurence Corash. Anna Eriksson, Nina Mufti and Anne North reviewed the RBCC production and RBCC quality control data.

**Disclosure of Conflicts of Interest**

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