

## ORIGINAL REPORT

# Blood component administration to multiple myeloma patients treated with daratumumab: suggesting a novel approach with use of 0.1 M dithiothreitol

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Storage of dithiothreitol (DTT)-treated red blood cells (RBCs) leads to hemolysis. The aim of this study was to compare 0.1 M DTT with 0.2 M DTT treatment of RBCs and to share our experience of providing components to seven patients on daratumumab (DARA). This prospective, observational study included patients who required RBC transfusion within 6 months of DARA administration. All patients underwent a baseline serologic evaluation followed by a repeat evaluation after DARA administration. In addition, use of 0.1 M DTT was compared with 0.2 M DTT in terms of concordance of results, hemolysis with storage of treated RBCs, and ease of use. A total of 22 RBC requisitions were received for seven patients. Antibody screen was positive for one patient (anti-C) at baseline; it was panreactive for all patients after DARA. Concordance of results between the two concentrations was 98.5 percent. Laboratory personnel found results obtained with use of 0.1 M DTT-treated RBCs easy to interpret. Supernatant hemoglobin was found to be significantly greater for 0.2 M DTT-treated RBCs at the sixth day of storage. In conclusion, component administration to patients on DARA can be done without delay if adequate policies and procedures are in place. Use of 0.1 M DTT-pretreated RBCs can be used to avoid delay in transfusion and reduce the burden on the laboratory of weekly preparation of 0.2 M DTT-treated RBCs. *Immunohematology* 2020;36:157–165.

**Key Words:** DARA, daratumumab, CD38, DTT, multiple myeloma, hemolysis

Multiple myeloma (MM) is a neoplastic disorder of the bone marrow involving the proliferation of plasma cells. High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is the mainstay of treatment for newly diagnosed, transplant-eligible patients with MM. However, for patients who are not eligible for ASCT or those who do not opt for ASCT, novel agents in the treatment of MM are being developed. Among these drugs, daratumumab (DARA), an IgG1k human monoclonal antibody that specifically targets human CD38 antigens, was approved by the U.S. Food and Drug Administration in November 2016 for cases of relapsed and refractory MM.<sup>1</sup> CD38 is an integral trans-membrane

glycoprotein that is overexpressed in myeloma cells, and the expression is shared by other lineages including red blood cells (RBCs).<sup>2</sup> Anti-CD38 mediates via a variety of immune mechanisms that are responsible for its anti-myeloma activity like complement-dependent cellular cytotoxicity, antibody-dependent cellular cytotoxicity, immunoregulatory depletion of immune-suppressive regulatory T-cells, and antibody-dependent cellular phagocytosis.<sup>3</sup>

Transfusion medicine experts have discussed DARA because of its interference in serologic testing. The mechanism for DARA's interference in pre-transfusion testing was at first unclear; it was discovered that DARA directly binds to CD38 on RBCs causing panreactivity in serologic testing.<sup>4</sup> As a result, strategies to overcome this serologic quandary were described by many centers, and now guidelines have been laid down by AABB and the British Society for Haematology (BSH) for handling pre-transfusion testing on samples from patients on DARA.<sup>4</sup> Treating RBCs with dithiothreitol (DTT) eliminates the DARA interference. DTT denatures the cell surface CD38 by disrupting the disulphide bonds, thus preventing interaction of DARA at the RBC surface and allowing for safe transfusion of patients on DARA.<sup>5,6</sup> DTT treatment of RBCs also denatures some of the RBC antigens, such as MER2 and Ge3, and those in the Kell, Cartwright, Indian, JMH, Scianna, LW, Lutheran, Dombrock, Diego, and Cromer blood group systems—of which the most important is K.<sup>7</sup> A common practice in these patients, therefore, is to transfuse K- RBC units unless the patients have been typed as K+.

DTT treatment of RBCs has the potential to cause hemolysis at certain concentrations which can interfere with interpretation of results.<sup>8–12</sup> Standard DTT treatment of RBCs is performed with 0.2 M DTT, although at this concentration hemolysis was observed and has been documented. A lower concentration (i.e., 0.1 M DTT) can be used for the same purpose. In the present study, we share a single center's

experience of pre-transfusion testing in seven patients who were candidates for DARA treatment. The aim of this study was to compare 0.1 M DTT treatment of RBCs with 0.2 M DTT treatment and to share our experience of providing components to seven patients receiving DARA.

## Materials and Methods

### Setting and Design

This prospective, observational study was conducted in the Department of Transfusion Medicine at a tertiary level health care facility from February 2017 to June 2019. In accordance with suggestions described in the literature, a protocol was formulated for the institution that required all patients suffering from relapsed and refractory MM who were prospective candidates for DARA therapy to be sent to the Department of Transfusion Medicine by the Hemato-Oncology and Bone Marrow Transplant team. Approval for this study was obtained from the institutional review board and institutional ethics committee.

### Study Population

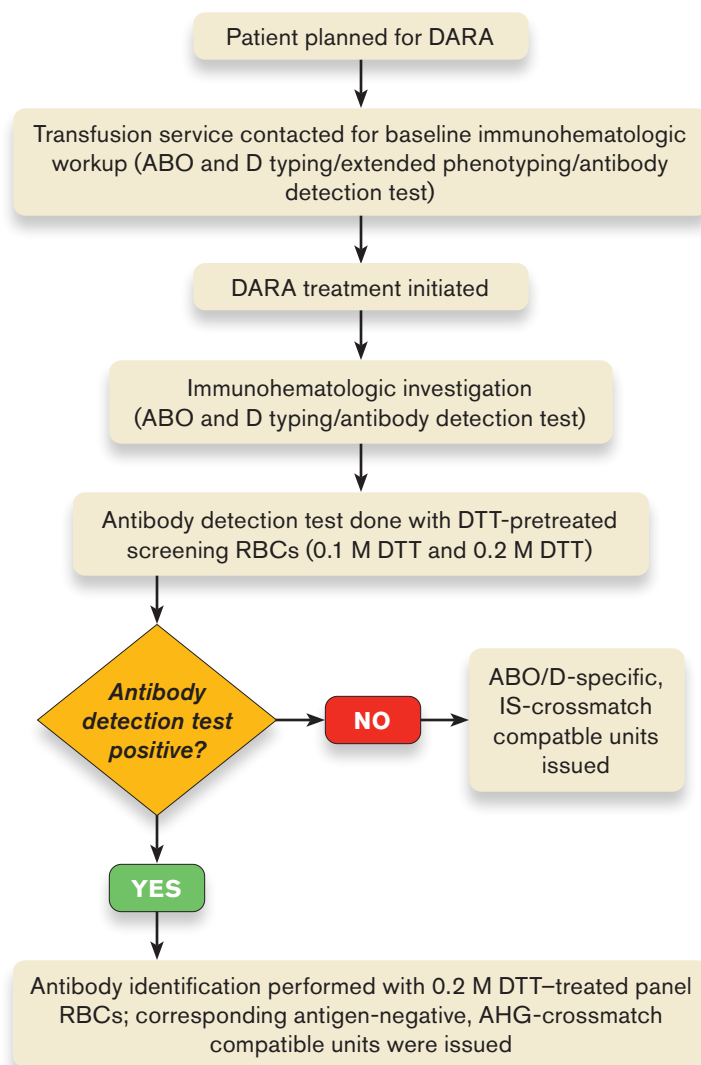
Participants were selected based on inclusion and exclusion criteria given here. The algorithm of the study is illustrated in Figure 1.

#### INCLUSION CRITERIA

- Consecutive patients with MM on DARA therapy who gave informed consent for participation.
- Patients who were being treated at the present institute only.
- Patients who required RBC transfusion within 6 months of DARA administration and whose request for RBC transfusion was received in the RBC serology laboratory of the transfusion service.

#### EXCLUSION CRITERIA

- Patients who had received RBC transfusion within 3 months at the time of baseline immunohematologic investigation.
- Patients who did not give consent for participation.
- Patients who required emergency/life-saving RBC transfusion.
- Patients for whom a requisition form for RBC transfusion was not received in the transfusion service.
- Requisitions received for patients who were admitted at other institutions.



**Fig. 1** Algorithm for the study. DARA = daratumumab; RBCs = red blood cells; DTT = dithiothreitol; IS = immediate spin; AHG = antihuman globulin.

### Serologic Investigation

All serum samples were stored at  $-80^{\circ}\text{C}$  for a maximum of 6 months for the purpose of testing at a future date in case a discrepancy was detected.

#### BASILINE INVESTIGATION

- ABO and D typing: Automated ABO and D typing were performed by solid-phase red cell adherence on an automated system (Galileo Neo, Immucor, Norcross, GA) using commercial ABO/D reagents (Anti-A, Anti-B, Anti-D; Immucor), and reagent RBCs (Referencells; Immucor).

- Antibody detection and identification: Antibody detection and identification were performed using column agglutination technology (CAT). Commercial cells for screening, (Surgiscreen; Ortho Clinical Diagnostics, Raritan, NJ), and for identification, (Panel A and Panel B; Ortho Clinical Diagnostics), were used. Antihuman globulin (AHG) polyspecific cassettes (Ortho BioVue cassettes, Ortho Clinical Diagnostics) were used as per the manufacturer's instructions on a semi-automated platform (Ortho BioVue, Ortho Clinical Diagnostics).
- Extended phenotyping (C, c, E, e, K, k, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>, M, N, S, s, Le<sup>a</sup>, Le<sup>b</sup>, P1): Extended phenotyping was performed by conventional tube test (CTT) using corresponding antisera (Anti-C, Anti-c, Anti-E, Anti-e, Anti-K, Anti-Fy<sup>a</sup>, Anti-Fy<sup>b</sup>, Anti-Jk<sup>a</sup>, Anti-Jk<sup>b</sup>, Anti-M, Anti-N, Anti-S; Immucor and Anti-k, Anti-P1, Anti-s, Anti-Le<sup>a</sup>, Anti-Le<sup>b</sup>; Ortho Clinical Diagnostics).
- Direct antiglobulin test (DAT): DAT was performed by CAT using AHG polyspecific cassettes (Ortho BioVue cassettes, Ortho Clinical Diagnostics).

#### AT TIME OF RBC REQUIREMENT

A blood sample for compatibility testing was sent with the request form as part of this center's routine type and screen policy:

- ABO and D typing
- Antibody detection and identification
- DAT
- Crossmatch: For all samples, an AHG crossmatch would be performed by CAT using AHG polyspecific cassettes (Ortho BioVue cassettes, Ortho Clinical Diagnostics) as per manufacturer's instructions.

#### DTT TREATMENT OF ANTIBODY SCREENING RBCs

For patients on DARA, antibody screening RBCs pretreated with DTT were always available.

- DTT preparation: 0.1 M DTT was prepared by dissolving 0.5 g DTT (DL-DTT, Himedia, Mumbai, India) in 32 mL phosphate-buffered saline (PBS) with pH adjusted to 8.0. Similarly, 0.2 M DTT was prepared by dissolving 1 g DTT in 32 mL PBS with pH 8.0. Both concentrations of DTT were prepared simultaneously whenever baseline immunohematologic investigations were needed for a patient on DARA therapy.
- DTT treatment of RBCs: Ten test tubes were labeled and divided into two sets of five test tubes each. One set was used for treatment with 0.1 M DTT and the other set

was used for treatment with 0.2 M DTT. For each set, screening cells 1, 2, and 3 were added to three tubes, respectively. Positive and negative controls, prepared from K<sup>+</sup> and E<sup>+</sup> reagent RBCs, respectively, were added to the fourth and fifth tubes. The controls ensured the validity of effective DTT-treatment. DTT-treated RBCs were stored at 4°C in PBS and were freshly prepared at weekly intervals. However, for the purpose of this study, the DTT-treated RBCs could be used up to 14 days from preparation.

#### CROSSMATCH REQUESTS RECEIVED, RBC UNITS ISSUED, AND TURNAROUND TIME

Every time a request form was received for these patients, an antibody detection test was performed using untreated and both 0.1 M and 0.2 M DTT-treated screening RBCs simultaneously. A positive antibody detection test warranted testing with 0.1 M and 0.2 M DTT-treated panel RBCs followed by an AHG crossmatch using 0.2 M DTT-treated donor RBCs. However, if the antibody detection test was negative with the DTT-treated RBCs, an immediate spin crossmatch was performed to identify compatible units. The turnaround time (TAT) from the time a request form was received to the time a donor unit was identified for transfusion was calculated for both 0.1 M and 0.2 M DTT-treated RBCs. Table 1 provides details of the TAT for all RBC requisitions received during the study. All transfusions were actively followed for transfusion-related adverse events.

#### Comparison of 0.1 M and 0.2 M DTT-Treated RBCs

The RBCs treated with 0.2 M DTT show hemolysis with storage, which can interfere with interpretation of results. This finding compels the laboratory to prepare 0.2 M DTT-treated RBCs frequently, which requires reagents and human resources. As an alternative to this, a lower concentration than what has been conventionally documented (i.e., 0.1 M DTT) was also tested.

#### DETECTION OF HEMOLYSIS

To measure the amount of hemolysis, hemoglobin (Hb) was measured in the supernatant of reagent screening RBCs treated with 0.1 M DTT and 0.2 M DTT using the HemoCue Plasma/Low Hb System (HemoCue India, New Delhi, India). The day of preparation of DTT-treated RBCs was considered day 0. Hemolysis was measured on the day on which a requisition form was received for RBC transfusion to a patient on DARA.

**Table 1.** Turnaround time for all RBC requisitions received during the study

Patient	Number of RBC requests received	Number of RBC units requested	Number of RBC units issued and transfused	Turnaround time (minutes)
1	1	2	2	170
2*	3	7	6	180 172 163
3	2	5	5	168 179
4*	5	12	11	173 190 184 181 195
5	2	4	4	171 160
6*	5	13	12	175 157 164 150 186
7	4	10	10	169 158 173 149
<b>Total</b>	<b>22</b>	<b>53</b>	<b>50</b>	
<b>Mean</b>	<b>3.1 ± 1.6</b>	<b>7.6 ± 4.2</b>	<b>7.1 ± 3.9</b>	<b>171.2 ± 12.2</b>

\*Patients with difference in number of RBC units requested and number of RBC units transfused. This result was due to lesser number of units being required to reach desired hematocrit than expected.

RBC = red blood cell.

#### CONCORDANCE OF ANTIBODY SCREEN RESULTS USING 0.1 M AND 0.2 M DTT-TREATED RBCs

Concordance between the results obtained with use of 0.1 M and 0.2 M DTT-treated antibody screening RBCs was recorded. All results were noted, and discrepant results were observed microscopically to confirm true agglutination.

#### SURVEY FOR EASE OF USE

Patient samples were preserved and later used for a survey. A total of 10 laboratory personnel with a minimum work experience of 2 years were given two patient samples each. They were asked to perform an antibody detection test on patient samples using two different concentrations of

DTT-treated RBCs: xM and yM. None of the participants were aware that xM RBCs were 0.2 M DTT treated and yM RBCs were 0.1 M DTT treated. Participants were asked to complete a questionnaire based on this exercise. The questionnaire had four questions with a 4-point Likert scale for each, where 1 meant strongly disagree and 4 meant strongly agree. The purpose of this questionnaire was to access whether different concentrations had any impact on the ease of use by the laboratory personnel. Table 2 shows survey results.

**Table 2.** Ease of use survey results from 10 participating staff members

Question number	Question	Total score*	Mean score
1	Visible hemolysis with use of xM DTT-treated RBCs is greater	33	3.3
2	Visible hemolysis with use of yM DTT-treated RBCs is greater	10	1.0
3	Interpretation of antibody detection test results is easier with use of xM DTT-treated RBCs	28	2.8
4	Interpretation of antibody screening results is easier with use of yM DTT-treated RBCs	37	3.7

\*Grading scores ranged from 1 (strongly disagree) to 4 (strongly agree).

DTT = dithiothreitol; xM = 0.2 M; yM = 0.1 M; RBCs = red blood cells.

#### Statistical Analysis

Mean, standard deviation, and range of the supernatant-free Hb values for both concentrations of DTT were calculated separately. Because results of two independent study sample groups were compared and the data were normally distributed, an independent Student *t* test was applied. The *p* value was calculated to determine whether the difference was statistically significant. A *p* value of < 0.05 was considered statistically significant.

#### Results

A total of seven patients were included in the study (five men, two women). Mean age of the patients was  $54.2 \pm 7.6$  years. Results of the immunohematologic investigations are provided in Table 3. A total of 22 RBC requisitions were received in the study duration for all seven patients, mean number of requested RBC units was  $7.6 \pm 4.2$ . The mean number of RBC units transfused was  $7.1 \pm 3.9$ . Details of these requisitions are provided in Table 1. Each transfusion was actively monitored by Transfusion Medicine residents. All transfusions were uneventful.

**Table 3.** Serologic workup details of patients with MM before and after DARA administration

Patient number	Before DARA administration				Days from first dose of DARA	After DARA administration				
	ABO/D*	Antibody detection test	DAT	Requisition number		ABO/D*	Antibody detection test (untreated RBCs)	Antibody detection test (0.1 M DTT-treated RBCs)	Antibody detection test (0.2 M DTT-treated RBCs)	DAT
1	A, D+	Negative	Negative	1	20	A, D+	Panreactivity	Negative	Negative	Negative
2	O, D+	Negative	Negative	1	15	O, D+	Panreactivity	Negative	Negative	Negative
				2	28	O, D+	Panreactivity	Negative	Negative	Negative
				3	45	O, D+	Panreactivity	Negative	Negative	Negative
3	AB, D+	Negative	Negative	1	21	AB, D+	Panreactivity	Negative	Negative	Negative
				2	40	AB, D+	Panreactivity	Negative	Negative	Negative
4	B, D–	Positive (Anti-C)	Negative	1	14	B, D–	Panreactivity	Positive (Anti-C)	Positive (Anti-C)	Negative
				2	30	B, D–	Panreactivity	Positive (Anti-C)	Positive (Anti-C)	Negative
				3	42	B, D–	Panreactivity	Positive (Anti-C)	Positive (Anti-C)	Negative
				4	63	B, D–	Panreactivity	Positive (Anti-C)	Positive (Anti-C)	Negative
				5	97	B, D–	Panreactivity	Positive (Anti-C)	Positive (Anti-C)	Negative
5	B, D+	Negative	Negative	1	10	B, D+	Panreactivity	Negative	Negative	Negative
				2	29	B, D+	Panreactivity	Negative	Negative	Negative
6	B, D+	Negative	Negative	1	28	B, D+	Panreactivity	Negative	Negative	Negative
				2	46	B, D+	Panreactivity	Negative	Negative	Negative
				3	71	B, D+	Panreactivity	Negative	Negative	Negative
				4	108	B, D+	Panreactivity	Negative	Negative	Negative
				5	149	B, D+	Panreactivity	Negative	Negative	Negative
7	O, D+	Negative	Negative	1	18	O, D+	Panreactivity	Negative	Negative	Negative
				2	29	O, D+	Panreactivity	Negative	Negative	Negative
				3	52	O, D+	Panreactivity	Negative	Negative	Negative
				4	84	O, D+	Panreactivity	Negative	Negative	Negative

\*No ABO discrepancy detected.

MM = multiple myeloma; DARA = daratumumab; DAT = direct antiglobulin test; RBCs = red blood cells.

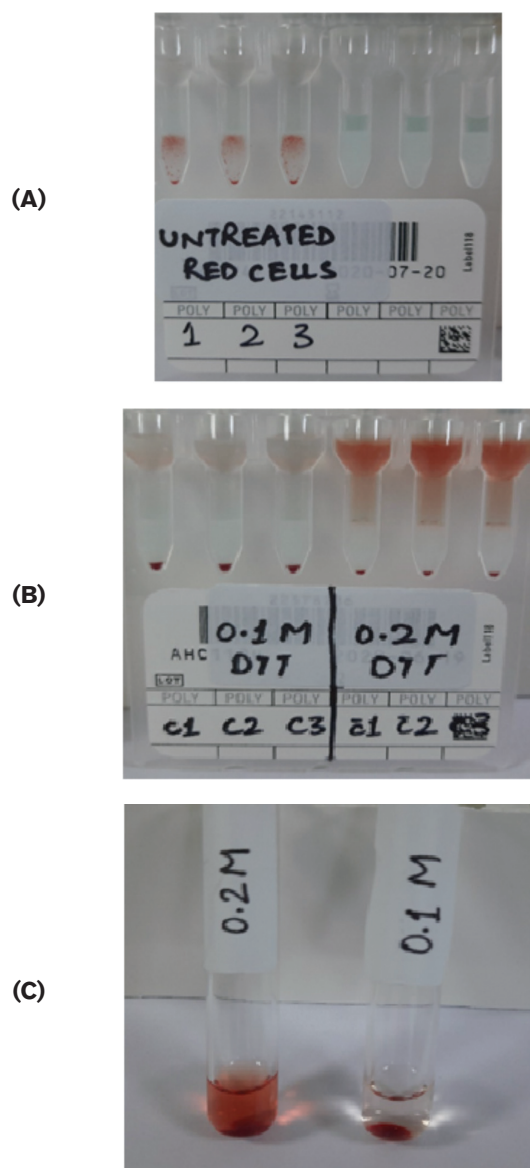
## Serologic Investigation

No ABO or D discrepancy was observed for any of the study patients, before or after DARA therapy. At baseline, the antibody detection test was negative for six patients and positive (anti-C) for one patient. After DARA administration, the antibody detection test performed with untreated screening RBCs was positive for all seven patients at the time of receipt of each of the 22 requisitions. With DTT-pretreated screening RBCs, the antibody detection test for six patients was negative. The patient with anti-C in his plasma continued to give a positive antibody detection test with DTT-treated screening RBCs, and the antibody was identified as anti-C. Antibody detection test results with untreated RBCs and with 0.1 M and 0.2 M DTT-treated RBCs are illustrated in Figure 2A and 2B.

## Turnaround Time

Because DTT-pretreated screening RBCs were always available in the RBC serology laboratory and six patients had a negative antibody screen, these six patients could receive transfusions without any delay. Patient 4 (who had anti-C) required a little more time for confirming the antibody specificity. The transfusion service routinely performs Rh and K phenotyping of all donor units; thus antigen-negative units could be identified immediately without causing a significant increase in TAT. The mean TAT for all requisitions received was  $171.2 \pm 12.2$  minutes. The details of turnaround time are provided in Table 1.





**Fig. 2** (A) Antibody detection testing showing panreactivity with untreated screening RBCs. (B) Antibody detection testing showing negative results with 0.1 M and 0.2 M DTT-treated screening RBCs using the same patient sample. (C) Visible hemolysis in supernatant of 0.2 M DTT-treated RBCs at seventh day of storage. RBCs = red blood cells; DTT = dithiothreitol.

### Comparison of 0.1 M and 0.2 M DTT

#### SUPERNATANT-FREE HEMOGLOBIN MEASUREMENT

Details of Hb estimation are given in Table 4. A statistically significant difference ( $p < 0.05$ ) was observed between the mean supernatant Hb observed in both groups. Visible hemolysis in the supernatant on the last day of storage of DTT-treated cells is illustrated in Figure 2C.

### CONCORDANCE OF ANTIBODY SCREEN RESULTS OBTAINED WITH USE OF 0.1 M AND 0.2 M DTT-TREATED RBCs

A concordance of 98.5 percent was found between the results obtained by screening RBCs treated with 0.1 M and those obtained by screening cells treated with 0.2 M DTT. There was only one RBC where a discrepancy was observed; the 0.2 M DTT-treated RBC gave a positive result whereas the 0.1 M DTT-treated RBC gave a negative result. No microscopic agglutination was observed.

### SURVEY FOR EASE OF USE

Ten laboratory personnel were included in this exercise. Based on the responses obtained from the questionnaire, it was evident that visible hemolysis was seen more with 0.2 M DTT, and the interpretation of antibody detection test results was easier with 0.1 M DTT treatment (Table 2).

### Discussion

DARA has been known to cause panreactivity with RBCs during pre-transfusion testing, and this panreactivity can last for several months. RBC treatment with the redox agent DTT has been commonly used to denature antigens in certain blood group systems, in particular K.<sup>5,13</sup> Treating RBCs with DTT destroys CD38 and hence removes the interference of DARA from pre-transfusion testing.<sup>3,4</sup>

Chapuy et al.<sup>3</sup> described the method for resolving DARA interference with blood compatibility testing. The authors found that DARA causes panreactivity *in vitro* by binding to CD38 on reagent RBCs and that treating reagent RBCs with DTT is a robust method to negate the DARA interference. The authors also found that ABO and D typing were unaffected. Similarly, Setia et al.<sup>14</sup> reported a case with panreactivity on antibody screening and identification panels that was resolved with use of 0.2 M DTT. This finding was similar to the present study, where no grouping discrepancy was observed, and panreactivity was observed with untreated screening RBCs. Oostendorp et al.<sup>15</sup> noted that DARA treatment of patients with MM resulted in false-positive indirect antiglobulin tests for 11 patients for 2–6 months after infusion. They performed DAT on samples from all 11 patients and found that DAT was negative in all of them after DARA administration. Chapuy et al.,<sup>3</sup> however, reported that the majority of patients had a positive DAT with IgG specificity. In the present study, DAT was negative for all seven patients. Extended phenotyping should be available before DARA is started. This testing is done to aid in antibody identification should the patient's

**Table 4.** Hemoglobin estimation

Patient number	Request number	Days from preparation of DTT-treated RBCs	Free plasma hemoglobin					
			0.1 M DTT-treated screening RBCs*			0.2 M DTT-treated screening RBCs*		
			Cell 1	Cell 2	Cell 3	Cell 1	Cell 2	Cell 3
1	1	2	0.0	0.0	0.0	0.0	0.0	0.0
2	1	4	0.0	0.0	0.0	0.0	0.05	0.03
	2	6	0.0	0.01	0.0	0.05	0.05	0.05
	3	9	0.0	0.01	0.01	0.06	0.05	0.05
3	1	3	0.0	0.0	0.0	0.02	0.04	0.0
	2	7	0.01	0.01	0.0	0.06	0.06	0.07
4	1	4	0.0	0.0	0.0	0.04	0.04	0.05
	2	5	0.01	0.0	0.01	0.05	0.06	0.05
	3	8	0.0	0.01	0.01	0.06	0.06	0.06
	4	9	0.01	0.01	0.02	0.06	0.06	0.06
	5	12	0.01	0.01	0.02	0.07	0.06	0.08
5	1	6	0.01	0.0	0.0	0.06	0.04	0.06
	2	8	0.01	0.01	0.01	0.08	0.07	0.08
6	1	2	0.0	0.0	0.0	0.01	0.0	0.0
	2	3	0.0	0.0	0.0	0.01	0.01	0.02
	3	6	0.0	0.0	0.01	0.05	0.05	0.05
	4	7	0.01	0.01	0.01	0.06	0.06	0.05
	5	8	0.01	0.02	0.01	0.07	0.06	0.05
7	1	5	0.0	0.01	0.0	0.05	0.05	0.05
	2	7	0.01	0.01	0.01	0.06	0.05	0.06
	3	8	0.01	0.01	0.02	0.06	0.08	0.06
	4	11	0.02	0.02	0.02	0.06	0.08	0.07
Mean ± SD	3.1 ± 1.6	6.4 ± 2.7	0.007 ± 0.007			0.05 ± 0.02		
p value					<0.05			

Colored cells highlight the instances when DTT-treated RBCs were used beyond 7 days of storage.

\*Antibody screening reagent RBCs (Surgiscreen; Ortho Clinical Diagnostics, Raritan, NJ). DTT = dithiothreitol; RBCs = red blood cells.

antibody detection test become positive after DARA administration and also to transfuse phenotypically matched units to avoid alloimmunization.<sup>16–18</sup> At our center, extended phenotyping was performed on samples from all patients, and Rh and K phenotype-matched units were issued, if possible. In the landmark SIRIUS trial conducted by Chari et al.,<sup>17</sup> 47 patients were included; none of whom had any transfusion reaction. In the present study, RBC units were transfused under direct supervision of a medical officer and Transfusion Medicine resident to look for any transfusion reactions. None of the patients experienced any transfusion-related adverse events.

The concentration of 0.2 M DTT is known to cause hemolysis of RBCs with storage.<sup>8–12</sup> The present center has

experienced the same. Treating screening RBCs every time a requisition is received delays transfusion. In an attempt to avoid this delay, DTT-treated screening RBCs were stored. With storage, however, hemolysis was observed; therefore these RBCs were replaced every week, which consumed material and manpower. In resource-constrained settings such as ours, resources have to be used judiciously. In an attempt to resolve this issue, the laboratory tried treating RBCs with a lower concentration of DTT. To validate the use of 0.1 M DTT instead of 0.2 M DTT, antibody screening was simultaneously performed using RBCs treated with both concentrations. The amount of hemolysis was also measured at both concentrations. We found that use of 0.1 M DTT provides the same results as 0.2 M DTT (concordance was 98.5%);

hemolysis was significant in the 0.2 M DTT-treated RBCs at 6 days after preparation, and the laboratory personnel found 0.1 M DTT results easier to interpret than results obtained using 0.2 M DTT because of the presence of hemolysis in the latter. Hemolysis in 0.2 M DTT-treated RBCs was observed from 4 to 28 days of preparation in various studies.<sup>8–12</sup> Use of stabilizing agents like Alsever's solution and PBS at pH 7.3 have also been tried to preserve DTT-treated RBCs.<sup>8–11</sup> In the present study, the DTT-treated RBCs were suspended in PBS. There was significant hemolysis with 0.2 M DTT-treated RBCs.

Strengths of the present study include easy component administration without any delay to patients on DARA because of the following reasons:

- Good communication between the Hemato-Oncology and Transfusion Medicine departments. Baseline immunohematologic investigations were available for all patients before DARA administration. As has been highlighted by Tiwari et al.<sup>19</sup> in their commentary about approaches to finding a compatible RBC unit for patients on DARA, communication between the treating physician and Transfusion Medicine physician is important to ensure timely patient care.
- DTT-treated antibody screening RBCs were always available. This availability led to timely results of antibody detection testing.
- The present center performs routine Rh and Kell phenotyping of all donor units, which is mentioned on the ISBT 128 label. Because most antibodies in multiply transfused patients belong to these two groups, provision of components to our alloimmunized patient was also done without delay.

Limitations of the study include exclusion of urgent or emergency requests. Also, none of the patients were found to be alloimmunized to antigens other than those belonging to Rh and Kell blood group systems. Therefore, delay in TAT for component provision to such patients could not be determined. Lastly, technical staff could discuss the results of their tests and answers to the questionnaire among themselves.

Strengths of this study include quantitative measurement of hemolysis, assessment for ease of use of both concentrations by the laboratory personnel themselves, timely administration of all components due to existence of good interdepartmental communication, and well-versed policies and procedures in place. Identification of a method for resolution of such discrepancies without observing significant hemolysis within 7 days of the preparation of 0.1 M DTT-treated RBCs was the

highlight of this study. Nevertheless, further studies need to be conducted to determine the minimum, maximum, and average duration at which 0.1 M DTT-treated RBCs show significant hemolysis.

## Conclusion

Component administration to patients on DARA can be done without delays if adequate policies and procedures are in place, there is good interdepartmental communication between Hemato-Oncology and Transfusion Medicine departments, and all laboratory personnel are well versed with use of DTT-treated RBCs. With the present study, we recommend use of 0.1 M DTT-pretreated RBCs to avoid delay in transfusion and reduce the burden on the laboratory of weekly preparation of 0.2 M DTT-treated RBCs.

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