New Methods and Approaches

Resolving the daratumumab interference with blood compatibility testing

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BACKGROUND: Daratumumab (DARA), a promising novel therapy for multiple myeloma, is an IgG1κ monoclonal antibody that recognizes CD38 on myeloma cells. During routine compatibility testing, we observed that the plasma of five of five DARA-treated patients demonstrated a positive antibody screen and panreactivity on red blood cell (RBC) panel testing. We hypothesized that the observed panreactivity reflected DARA binding to CD38 on reagent RBCs, and we investigated methods to prevent this binding.

STUDY DESIGN AND METHODS: DARA binding to CD38+ or CD38− HL60 cells was assessed by flow cytometry. To remove cell surface CD38, cells were incubated with dithiothreitol (DTT) or trypsin. Soluble CD38 or anti-DARA was used to neutralize DARA in solution. Routine blood bank serologic methods were used to test samples from DARA-treated patients and normal plasma samples spiked with DARA and/or alloantibodies.

RESULTS: Normal plasma samples spiked with DARA (0.1-10 μg/mL) and incubated with reagent RBCs recapitulated the interference observed with samples from DARA-treated patients. Flow cytometry experiments confirmed DARA binding to CD38+ HL60 cells, but not to CD38− controls. DTT treatment of CD38+ HL60 cells reduced DARA binding by 92% by denaturing cell surface CD38. Treating DARA-containing plasma with soluble CD38 or anti-DARA idiotype also inhibited DARA binding.

CONCLUSION: DARA causes panreactivity in vitro by binding to CD38 on reagent RBCs. Treating reagent RBCs with DTT is a robust method to negate the DARA interference, enabling the safe provision of blood to DARA-treated patients. Because DTT denatures Kell antigens, K− units are provided to these patients.

Daratumumab (DARA) is a promising novel therapy for multiple myeloma (MM). DARA is an IgG1κ human monoclonal antibody (MoAb) that specifically targets human CD38, which is highly expressed on myeloma cells. In preclinical studies, DARA was highly cytotoxic to tumor cells via multiple mechanisms, including complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and apoptosis.1 In the first-in-human Phase I and II clinical trial, DARA showed significant anti-MM activity as monotherapy in heavily treated patients with relapsed or refractory disease.2,3 Phase III trials of DARA are currently getting under way in the United States and across several countries internationally.

ABBREVIATIONS: DARA = daratumumab; GFP = green fluorescent protein; MM = multiple myeloma.

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On routine screening in the blood bank, we observed that five of five patients who had received DARA in a Phase I and II clinical trial had positive antibody screens. The plasma of these patients was panreactive in routine serologic tests, preventing the blood bank from providing cross-match-compatible red blood cell (RBC) units. Adsorptions using ZZAP-treated or untreated RBCs failed to remove the interference.

The expression of CD38 on human RBCs has been demonstrated previously. For example, Albeniz and coworkers performed Western blot analyses of CD38 on human RBCs of cancer patients and healthy controls. While they found increased CD38 expression on RBC membranes of cancer patients, a signal confirming weak expression on normal RBCs was also detected. We hypothesized that the panreactivity observed in the blood bank was caused by direct binding of DARA to endogenous CD38 on reagent RBCs. Previous investigators reported that CD38 is sensitive to denaturation by the reducing agent dithiothreitol (DTT) and that enzymatic digestion with trypsin can cleave CD38 from the cell surface. We explored methods to negate the DARA interference by removing RBC surface CD38 or by neutralizing DARA in solution.

MATERIALS AND METHODS

Patient samples

The Brigham and Women’s Hospital blood bank received whole blood (EDTA) samples collected as part of routine clinical care from adult patients with refractory MM receiving DARA. All of these patients were enrolled in a Phase I and II clinical trial conducted at Dana-Farber Cancer Institute (Protocol NCT00574288, Dana-Farber Cancer Institute Protocol 10-429). Baseline (DARA-free) samples were tested in all subjects (n = 11). Subsequently, DARA-containing samples were tested in the subset of enrolled subjects who required blood transfusion (n = 5). The samples were tested in the blood bank using routine serologic methods, including solid-phase (TANGO optimo automated blood bank system, Bio-Rad, Hercules, CA) and tube testing (polylevynylene glycol [PEG], low-ionic-strength saline [LISS], or no enhancement). Serologic tests performed on patient samples were as follows: ABO/Rh type, antibody screen, RBC panel, direct antiglobulin test (DAT), and antihuman globulin (AHG) cross-match. All DATs were done with monospecific testing for IgG and C3. Eluates were prepared from patient samples with a positive DAT using acid elution. Patient samples that were panreactive in routine tests were further tested using DTT-treated reagent RBCs (below). Institutional review board approval was obtained to study methods to prevent DARA binding on these patient samples.

Serologic testing of DARA-spiked plasma samples or DARA-treated patient samples

Normal (DARA-free) plasma samples with or without RBC alloantibodies were spiked with increasing concentrations of DARA (0.1-10 μg/mL; provided by Janssen R&D, Spring House, PA). These samples were analyzed using routine blood bank serologic testing with solid phase (TANGO optimo, Bio-Rad) and tube testing using PEG, LISS, or no enhancement. Agglutination was graded per routine (0 [no agglutination], M+ [macroscopically positive (weak)], 1+, 2+, 3+, 4+). Microscopic examinations were performed only on DATs. For all serologic studies, 3% to 5% cell suspensions of reagent RBCs (Bio-Rad, Biotestcell 1, Ref. 816014100, Batch ID 959) in phosphate-buffered saline (PBS), pH 7.3, were used. RBC panels used for antibody identification were from different manufacturers: Bio-Rad, Immucor (Norcross, GA), Ortho Clinical Diagnostics (Raritan, NJ), and Medion Diagnostics (Miami, FL). Cells were chosen depending on their specific antigen expression profile. Panels included five to 12 cell lines. RBC alloantibody identification required obtaining a positive agglutination reaction on three cells with the antigen present (rule-in) and a negative agglutination reaction on three cells with the antigen absent (rule-out). To demonstrate removal of CD38 from reagent RBCs, RBCs were left untreated, treated with 0.2 mol/L DTT (Sigma, St Louis, MO; detailed protocol below), or treated with 1% trypsin (incubated at 37°C for 30 min) and tested against DARA-spiked plasma samples or plasma from DARA-treated patients. For neutralization studies, DARA-spiked plasma samples were incubated at room temperature for 15 minutes with recombinant human soluble CD38 (R&D Systems [Minneapolis, MN], Cat. No. 2404-AC; final concentration, 0.05-5 μg/mL) or mouse anti-DARA idiotype (Janssen; final concentration, 5 μg/mL; reported by Ostendorp et al., submitted for publication). A mouse anti-human antibody (mouse IgG1κ, MOPC-21, Sigma-Aldrich, St Louis, MO) was used as an isotype control for the anti-idiotype. Eluates were prepared from DARA-treated RBCs using acid elution.

DTT treatment of reagent RBCs

A detailed method for DTT treatment of RBCs is described in the AABB Technical Manual. Briefly, 0.2 mol/L DTT was prepared by diluting 1 g of DTT in 32 mL of PBS, pH 8.0. K+, E+ control RBCs were used to verify that DTT treatment had denatured the K antigen while preserving the E antigen. Reagent and control RBCs (100 μL of a 3%-5% suspension) were washed four times with PBS, pH 7.3, before adding 400 μL of 0.2 mol/L DTT to each tube. The RBCs were incubated at 37°C for 30 minutes with periodic mixing by inversion (three to four times during incubation). The RBCs were washed four times with PBS, pH 7.3, and used for subsequent testing.
Cell culture

Human HL60 cells were propagated in RPMI supplemented with 10 mmol/L HEPES buffer, 2 mmol/L L-glutamine, 50 U/mL penicillin, 50 U/mL streptomycin, and 10% heat-inactivated fetal bovine serum (FBS; all from Life Technologies, Grand Island, NY).

Generation of CD38+ HL60 cells and CD38−, green fluorescent protein–positive control HL60 cells

HL60 cells were transduced with human CD38 or green fluorescent protein (GFP), the latter serving as a negative control for DARA binding. The cDNA of human CD38 was obtained in pDONR221 (HsCD00045212) from the DF/HCC DNA Resource Core (http://plasmid.med.harvard.edu/PLASMID/Home.jsp). Sanger sequencing was used to confirm that the plasmid contained the full-length open reading frame of human CD38. The human CD38 sequence was cloned into pMSCV-puro using a standard Gateway LR reaction according to the manufacturer’s directions (Life Technologies). Generation of the control vector pMSCV-puro-GFP, packaging of retroviral particles, and infection were performed as previously described.12 Two days after transduction, HL60 cells were selected for...
72 hours with 1 μg/mL puromycin (Sigma-Aldrich). Transduction efficiency of HL60-GFP and HL60-CD38 cells was assessed by flow cytometry (Fig. 1).

Detection of CD38 on RBCs and on transduced HL60 cells by flow cytometry
Reagent RBCs (Ortho Clinical Diagnostics) were incubated for 30 minutes with anti-CD235A-phycoerythrin (PE; anti-GlyA, eBioscience [San Diego, CA], 12-9987-82, 1:1000) and either a monoclonal mouse anti-CD38-fluorescein isothiocyanate (FITC; BD Biosciences [Sparks, MD], Clone HIT2) or a monoclonal mouse anti-human FITC-labeled IgG1 isotype control (BD, Clone 15H6). Enforced expression of human CD38 on HL60 cells after transduction was detected using a monoclonal mouse anti-CD38 directly conjugated to allophycocyanin (BD, 560980). This was done as follows: 5 × 10⁵ cells were washed twice with PBS, resuspended in 10 μL of antibody, and incubated for 30 minutes at room temperature in the dark. Per sample, 20,000 events were recorded. All flow cytometry data were acquired on a cell analyzer (BD LSR Fortessa, BD Biosciences). Data analysis and graphics generation were performed with computer software (FlowJo, V10.0.6 for MacOS, Tree Star, Ashland, OR).

Detection of DARA binding to transduced HL60 cells by flow cytometry
For DARA-binding studies, a mixture of HL60-CD38 and HL60-GFP cells (2.5 × 10⁵ each) was added to the same tube and incubated with increasing concentrations of DARA (0.1-1 μg/mL) for 1 hour at room temperature. An IgG isotype antibody (SouthernBiotech [Birmingham, AL]; human IgG1 kappa-UNLB, Cat. No. 0151K-01) in equal concentrations to DARA was used as a negative control. Detection of DARA or control antibody binding was assayed using a PE-labeled goat anti-human IgG (SouthernBiotech, Cat. No. 2040-09, final concentration, 0.1 μg/10⁶ cells) incubated with HL60-CD38 and HL60-GFP cells for 30 minutes at room temperature in a final volume of 100 μL. Separate gating on HL60-CD38 and HL60-GFP cells was performed to assess specific DARA binding to each cell population.

Negation of DARA binding to transduced HL60 cells
To remove cell surface CD38, mixtures of HL60-CD38 and HL60-GFP cells (2.5 × 10⁵ each) were incubated for 30 minutes at 37°C with DTT (0.1-10 mmol/L) or trypsin (1%-2%) before incubation with DARA. The DTT concentration was optimized for HL60 cells. Cell death was observed when HL60 cells were treated with the standard DTT concentration used to treat RBCs (0.2 mol/L). After DTT incubation, cells were washed twice with PBS; after trypsin incubation cells were washed once with growth medium to inactivate trypsin and then twice with PBS. The subsequent incubation with DARA and detection of DARA with PE-labeled goat anti-human IgG followed the same steps as described. For neutralization studies, DARA-containing plasma (final concentration, 0.5 μg/mL) was incubated for 15 minutes with either anti-DARA idio-type (final concentration, 5 μg/ml) or a control mouse anti-human antibody (mouse IgG1, MO-P-21, Sigma-Aldrich) at room temperature. Likewise, increasing concentrations (0.05-5 μg/mL) of recombinant soluble human CD38 (R&D Systems) or identical concentrations of bovine serum albumin (BSA, Sigma-Aldrich) were incubated with DARA before mixing with HL60 cells for 15 minutes at room temperature.

Statistical analysis
Comparison of groups was performed with a two-sided unpaired t-test using computer software (GraphPad Prism, Version 6.0c for Mac, GraphPad Software, La Jolla, CA; www.graphpad.com).

RESULTS

DARA causes plasma panreactivity in vitro
On routine screening in the blood bank, five of five patients with MM receiving DARA were observed to have a positive antibody screen and panreactive plasma in RBC
Fig. 2. Evaluation of methods to prevent DARA binding to CD38 on transduced HL60 cells. (A) DARA (0.5 µg/mL) binds selectively to HL60-CD38 cells (black) and not control HL60-GFP cells (gray). Treating the cells with increasing concentrations of DTT (0-10 µg/mL) resulted in a dose-dependent reduction in DARA binding. (B) Quantification of reduced DARA binding after pretreating HL60-CD38 cells with increasing concentrations of DTT or (C) trypsin compared to isotype control binding (gray). (D, E) Incubation of DARA-spiked plasma (0.5 µg/mL) with soluble CD38 (D) or anti-DARA idiotype (E) caused reduced DARA binding to HL60-CD38 cells, while negative controls using BSA (D) or a mouse anti-human IgG (mAb control; E) showed no effect on DARA binding. (F) Quantitative assessment of DARA adsorption by HL60 CD38+ cells. DARA-spiked plasma (0.5 µg/mL) was incubated with HL60 CD38+ cells or HL60 CD38- cells. After incubation, the plasma supernatants were assayed for residual DARA using flow cytometry. Significant adsorption of DARA was detected after incubation with the higher dose (5 x 10^6 cells), but not the lower dose (1 x 10^5 cells) of CD38+ adsorbing cells. All data show a representative example of at least three independent experiments. Error bars indicate SD.
panel testing (Table 1). All samples were initially screened using a solid-phase method (TANGO optimo); confirmatory testing was performed by tube testing with PEG enhancement. Patients requiring transfusion on multiple occasions had several longitudinal samples sent to the blood bank. In total, the blood bank observed the pan-reactivity in 18 of 18 samples from these patients. The majority of the patients (3/5) had a positive DAT (IgG only) and positive autocontrol. None of the five DARA-treated patients showed signs of hemolysis. Typically,
reaction strengths of the DARA-treated patient samples were graded as weakly positive (M\(^+\) [macroscopically positive]) to 1+ (1\(^-\)) in both solid-phase and tube. When heterologous adsorption studies were performed, the panreactivity persisted after three passes using untreated RBCs. ABO/Rh typing of patient RBCs was unaffected.

To verify that DARA was causing the observed agglutination reactions, we first confirmed by flow cytometry that CD38 is weakly expressed on human RBCs, as reported previously\(^4\)-\(^7\) (Fig. 1A). Next, we spiked normal plasma samples with increasing concentrations of DARA (0.1-10 \(\mu\)g/mL). When these samples were incubated with reagent RBCs, panreactivity was observed at antihuman globulin phase using no enhancement, PEG, and LISS, at all concentrations tested. Reaction strengths were graded as M\(^+\) (0.1-0.5 \(\mu\)g/mL) or 1\(^+\) (1.0-10 \(\mu\)g/mL). Six cycles of heterologous adsorptions\(^10\) using untreated RBCs failed to eliminate the interference. DATs performed on reagent RBCs incubated with DARA-spiked plasma were positive (IgG only) at all concentrations tested (0.1-10 \(\mu\)g/mL), with strengths varying between microscopically positive for concentrations of 0.1-0.25 \(\mu\)g/mL and 1+ for all higher concentrations.

**Negating the DARA interference in an HL60 cell model system**

To study methods of eliminating the DARA interference in the blood bank, we established a model system in HL60 cells (Figs. 1B-1D). The use of transfected HL60 cells to study CD38 function was reported previously.\(^8\) Here, HL60 cells were stably transduced with either CD38 (Fig. 1B) or GFP, which served as a CD38– control (Fig. 1C). Flow cytometry confirmed specific, dose-dependent binding of DARA in spiked plasma to CD38\(^+\) HL60 cells but not to CD38– controls (Figs. 1E and 1F).

Using this system, we evaluated methods to remove CD38 antigen from the cell surface. Incubating CD38\(^+\) HL60 cells with 10 mmol/L DTT reduced DARA binding by 92% (p < 0.001, Figs. 2A and 2B). Treating CD38\(^+\) HL60 cells with 2% trypsin reduced DARA binding by 40% (p < 0.001, Fig. 2C).

In addition to CD38 antigen removal, we attempted to inhibit DARA binding to CD38\(^+\) HL60 cells by neutralizing DARA in plasma. Soluble CD38 added to DARA-spiked plasma reduced DARA binding to CD38\(^+\) HL60 cells in a dose-dependent manner (Fig. 2D). Similarly, addition of a neutralizing mouse anti-DARA idiootype antibody decreased DARA binding by 95%. A nonspecific control antibody had no effect on DARA binding (Fig. 2E).

We also investigated using CD38\(^+\) HL60 cells as DARA-adsorbing cells (Fig. 2F). DARA-spiked plasma was incubated with either HL60 CD38\(^+\) cells or HL60 CD38– control cells. The adsorbed plasma was then assayed for the presence of residual DARA by flow cytometry. While plasma adsorbed with 1 \(\times\) 10\(^6\) HL60 CD38\(^+\) cells contained high levels of residual DARA, plasma adsorbed with 5 \(\times\) 10\(^6\) HL60 CD38\(^+\) cells contained significantly reduced levels of residual DARA (p < 0.001). Adsorbing plasma with HL60 CD38– control cells did not significantly reduce the level of DARA detected.

**DARA is present in eluates of untreated, but not DTT-treated, RBCs**

Untreated or DTT-treated RBCs were incubated with DARA and then washed (Fig. 3A, Steps 1 and 2). Eluates were then generated from untreated or DTT-treated RBCs and used for flow studies on HL60 CD38\(^+\) cells and CD38– control cells (Fig. 3A, Steps 3-5). Eluates prepared from the untreated RBCs contained specific IgG binding to CD38\(^+\) HL60 cells, while eluates prepared from DTT-treated RBCs contained no detectable IgG binding to CD38\(^+\) HL60 cells (Figs. 3A and 3B). These eluates were further serologically tested against a minipanel of five RBC lines. No reactivity was observed in the eluate of DTT-treated RBCs, but the eluate of untreated RBCs was

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**TABLE 2. Representative serology results: identification of anti-E in the presence of DARA**

| Screening cell | Plasma | Alloantibody | Antibody screen result | Panel cells | Panel result |
|---------------|--------|--------------|------------------------|-------------|-------------|
| Cell 1        | No DARA| –            | 0                      | Untreated   | No reactivity|
| Cell 2        | No DARA| Anti-E       | 1+                     | Untreated   | Anti-E      |
| Cell 1 + DTT  | No DARA| –            | 1+                     | DTT-treated | No reactivity|
| Cell 2 + DTT  | + DARA | –            | –                      | DTT-treated | Anti-E      |

* Screening Cell 1 – phenotype R\(_1\)R\(_1\), DCe; Screening Cell 2 – phenotype R\(_2\)R\(_2\), DcE.
panreactive. These results are consistent with DTT denaturing RBC CD38 epitopes, preventing DARA binding.

An RBC eluate was prepared from a DARA-treated patient sample (Patient 4, Table 1). The eluate was panreactive on RBC panels. Flow cytometry confirmed that the eluate contained binding activity to CD38^+ HL60 cells but not to CD38^− controls (Fig. 3C). Binding to CD38^+ HL60 cells was specifically inhibited by the addition of anti-DARA idiotype (Figs. 3C and 3D), confirming the presence of DARA in the patient sample.

Negating the DARA interference with blood bank tests

We performed a series of experiments on DARA-spiked plasma samples (1.0 μg/mL) using routine blood bank serologic assays. Treating reagent RBCs with DTT or trypsin eliminated the panreactivity with these samples. This allowed identification of underlying clinically significant alloantibodies (anti-E, anti-Fya,a, anti-JKa,o or anti-s) in the presence of DARA. Representative blood bank serology results for identifying anti-E are shown in Table 2. Similar results were obtained by neutralizing DARA-spiked samples with either anti-DARA idiotype or soluble hCD38 (data not shown).

Adding anti-DARA idiotype to the plasma of DARA-treated patients specifically eliminated positive antibody screen reactions (Table 3), confirming that the positive antibody screens seen in the clinical samples were directly caused by DARA. Finally, using DTT-treated reagent RBCs, the DARA interference was completely eliminated from the plasma of all five DARA-treated patients and all 18 patient samples, allowing the safe release of blood products for these patients.

| Plasma sample                | DARA dose | Days from last DARA infusion | Initial antibody screen result (Cells 1 and 2) | Neutralization | Antibody screen result (Cells 1 and 2) after neutralization |
|------------------------------|-----------|------------------------------|-----------------------------------------------|---------------|----------------------------------------------------------|
| Normal plasma spiked with DARA | 1 μg/mL   | NA                           | Positive                                      | Anti-DARA idiotype (10 μg/mL) | Negative                                                |
| DARA-treated Patient 3       | 8 mg/kg/week | 7                       | Positive                                      | Anti-DARA idiotype (100 μg/mL) | Negative                                                |
| DARA-treated Patient 5       | 16 mg/kg/week | 0                        | Positive                                      | Anti-DARA idiotype (100 μg/mL) | Negative                                                |

**DISCUSSION**

The use of anti-CD38 is a promising treatment for patients with MM.2,3 A problem with DARA is that it interferes with blood compatibility testing, complicating the safe release of blood products. Here, we show that direct binding of DARA to endogenous CD38 on RBCs causes the panreactivity observed in antibody screens and other pretransfusion tests. Several lines of evidence support this conclusion. First, a fluorescently labeled anti-CD38 was shown by flow cytometry to bind directly to reagent RBCs. Second, normal plasma samples spiked with DARA and incubated with reagent RBCs recapitulated the interference observed in the blood bank with samples from DARA-treated patients. Third, an eluate prepared from the RBCs of a DARA-treated patient bound only to CD38^+ cells and not to CD38^− control cells. Fourth, an eluate prepared from untreated RBCs that had been incubated with DARA likewise bound only to CD38^+ cells and not to CD38^− control cells (i.e., contained recovered DARA). Finally, when a specific anti-DARA neutralizing antibody was added to DARA-treated patient samples or DARA-spiked samples, RBC agglutination reactions were prevented. Surface expression of RBC CD38 appears to be relatively low, potentially explaining the weak agglutination reactions seen in vitro. Despite binding to RBCs, DARA did not cause significant hemolysis in the five treated patients.

Multiple rounds of adsorption with untreated or ZZAP-treated RBCs failed to remove the panreactivity from the plasma of DARA-treated patients. ZZAP contains DTT, so ZZAP-treated RBCs are predicted to have denatured CD38 surface antigen that would fail to bind DARA. In contrast, we speculate that adsorptions using untreated RBCs failed to remove the panreactivity from DARA-treated patient samples due to low expression of intact CD38 antigen on the adsorbing RBCs. The low expression of CD38 on RBCs is reflected in the flow cytometry results shown in Fig. 1A. Experiments using transduced CD38^+ HL60 cells as adsorbing cells (Fig. 2F) demonstrated detectable removal of DARA from spiked plasma only when a high number of adsorbing cells was used. RBCs appear to express considerably less surface CD38 than the transduced CD38^+ HL60 cells; thus we would expect RBCs to function relatively poorly as DARA-adsorbing cells.

We evaluated a number of potential methods to negate the DARA interference in the blood bank. The extracellular domain of human CD38 contains six disulfide bonds that are critical to the protein structure.13 Previous
investigators reported that the enzymatic activity of CD38 was highly sensitive to reducing agents such as DTT and 2-mercaptoethanol. Berthelier and colleagues reported that treating CD38+ HL60 cells with DTT decreased the binding of specific MoAbs to CD38 by denaturing the protein. Additionally, trypsin was reported to cleave the ectodomain of CD38 from the cell membrane. These findings provided the basis for our hypothesis that DTT or trypsin could prevent DARA binding by disrupting the extracellular domain of CD38 on RBCs. Using the HL60 model system, we confirmed that DTT is highly effective in denaturing CD38 and preventing DARA binding. CD38 binding activity was completely absent from an eluate prepared from DTT-treated RBCs incubated with DARA. We further showed using samples from DARA-treated patients that DTT pretreatment of RBCs eliminated the DARA interference with blood bank tests. Trypsin pretreatment of HL60 cells and of RBCs was also successful in reducing DARA binding, but was less efficient than DTT treatment.

Another approach to prevent DARA binding was neutralization of free DARA in plasma by adding soluble CD38 or an anti-DARA idiotype. Both methods were highly effective in preventing DARA binding, and DARA neutralization in solution is simpler to perform than DTT treatment of RBCs. Disadvantages of these neutralization methods, however, are higher costs and a lack of widespread availability of the reagents. Large quantities of soluble CD38 would be needed to treat clinical samples from DARA-treated patients. In contrast, DTT is very inexpensive and is already used by blood banks.

A potential drawback of DTT treatment is the disruption of a limited number of blood group antigens (Table 4). The sensitivity of virtually all clinically significant RBC antigens to DTT or trypsin has previously been defined. In routine clinical practice, anti-K is the only commonly encountered, clinically significant antibody to a DTT-sensitive RBC antigen. In practice, this issue is readily addressed by providing K– units to DARA-treated patients. More than 90% of all donated RBC units are K–. It is possible that using the DTT method to evaluate a DARA-treated patient could result in a potentially significant RBC alloantibody (e.g., anti-k, anti-Yt) being missed, but this would be a very rare event. Here, we have shown that the DTT method allows for the detection of alloantibodies in the presence of DARA from those blood group systems—aside from Kell—that account for the majority of clinically significant hemolytic reactions: Rh, Duffy, Kidd, and MNS.

In addition to DARA, many antibody-based cancer therapies are in various stages of development that might similarly interfere with routine blood bank tests. In the future, the DTT-based method described here may be useful to eliminate novel in vitro interferences in the blood bank, in those cases where the involved RBC antigen contains extracellular disulfide bonds.

In conclusion, we showed that DARA potently interferes with routine blood bank serologic tests by directly binding to CD38 on RBCs. DTT treatment of reagent RBCs is a robust method to negate the DARA interference, allowing the safe provision of RBC units to DARA-treated patients. Before patients are started on DARA, RBC antigen phenotyping or genotyping is recommended.

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CONFLICT OF INTEREST

PD is a full-time employee of Janssen. All other authors have disclosed no conflicts of interest.

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