ORIGINAL REPORT

Use of standard laboratory methods to obviate routine dithiothreitol treatment of blood samples with daratumumab interference

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Daratumumab is an antibody currently used in the treatment of patients with refractory multiple myeloma. Blood samples from patients being treated with daratumumab may show panreactivity during pre-transfusion testing. To facilitate the provision of blood components for such patients, it is recommended that a baseline phenotype or genotype be established prior to starting treatment with daratumumab. If patient red blood cells (RBCs) require phenotyping after the start of daratumumab treatment, dithiothreitol (DTT) treatment of the patient's RBCs should be performed. The medical charts of four patients treated with daratumumab were reviewed. The individual number of doses ranged from 1 to 14; patient age ranged from 55 to 78 years; two men and two women were included in the review. Type and screen data were obtained from samples collected over 33 encounters with a range of 1 to 13 encounters per patient. All samples were tested initially by automated solid-phase testing. Any reactivity with solid phase led to tube testing with either low-ionic-strength saline, polyethylene glycol, or both. If incubation failed to eliminate the reactivity, the sample was sent to a reference laboratory for DTT treatment and phenotyping. Of the 33 samples tested, 23 (69.7%) samples had reactivity in solid-phase testing. In 8 of the 10 samples that did not react in solid-phase, testing was conducted more than four half-lives after the last dose of daratumumab. Of the 23 that had reactivity in solid-phase, 16 (69.6%) samples demonstrated loss of reactivity using common laboratory methods. For the seven patients whose sample reactivity was not initially eliminated, six were provided with phenotypically matched blood based on prior molecular testing. Only one sample was sent out for DTT treatment. These results suggest that daratumumab interference with pre-transfusion testing can be addressed using common laboratory methods. This finding could save time and money for laboratories that do not have DTT available. ***Immunohematology* 2017;33:22–26.**

Key Words: daratumumab, pre-transfusion testing, interference

Multiple myeloma is a malignancy of plasma cells that highly express CD38. There have been many improvements in patient survival recently with the advent of new treatment strategies, although many patients still die from refractory disease.[1] Targeted therapies are highly sought after to improve treatment and minimize toxicity.[2] Daratumumab is an IgG1k monoclonal antibody that binds CD38 and is currently used in the treatment of refractory multiple myeloma.[1,3] The attachment of the therapeutic antibody may induce tumor killing via antibody-mediated complement activation, antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis, or direct effects of blocking the antigen function.[4]

In brief, human CD38 is a 45-kDa single-chain transmembrane glycoprotein with a single membrane-spanning region. In adults, the CD38 protein is present on the majority of natural killer cells, T cells, B cells, monocytes, and macrophages and to some extent on platelets and red blood cells (RBCs). CD38 is diffusely expressed on hematopoietic cells early in their differentiation, and expression levels decline on many cells lines as the cells terminally differentiate. The exception to this is plasma cells, where CD38 becomes more strongly expressed. CD38 shares structural homology with adenosine diphosphate (ADP)-ribosyl cyclase. ADP-ribosyl cyclase catabolizes nicotinamide adenine dinucleotide (NAD) to cyclic ADP ribose (cADPR), a naturally occurring metabolite of oxidized NAD (NAD+) that serves as a second messenger for Ca^{2+} mobilization from intracellular sites. With this function, CD38 plays a role in protein phosphorylation, inflammation/cell growth/differentiation, and cell attachment.[5]

CD38 also has weak expression on RBCs; as a result of this expression, it has been demonstrated that this therapeutic antibody may cause agglutination during pre-transfusion testing.[2–4] This reactivity has been reported to occur in a variety of media, including low-ionic-strength saline (LISS) and polyethylene glycol (PEG), as well as various test methods (solid-phase, tube, and gel). This interfering reactivity can be found when using antihuman globulin (AHG) in pre-transfusion testing of such patients' plasma or serum (indirect antiglobulin test [IAT]; namely, antibody detection testing, antibody identification panels, and AHG crossmatches) and/or testing of the patient's RBCs (auto control and direct antiglobulin test [DAT]). It has been noted that this reactivity is generally weak (1+) but may be stronger in solid-phase testing.[3,6,7] Studies that have reported this interfering reactivity in a variety of media have not tried multiple media on a single

sample. There is also much variability within the literature as to the frequency of this interfering reactivity in the various media. Additionally, it is not known how comorbidities or other medications may influence the interference that is seen. Substances that interfere with pre-transfusion testing have the potential to cause increased patient morbidity, mortality, and costs. These substances have the potential to mask a clinically relevant antibody. In addition, attempts to remove interfering reactivity from the patient's RBCs might disturb the antigen structure to the degree that phenotyping may be indeterminate. There are also increased costs associated with resolving the panreactivity caused by this interfering substance, as well as potential delay for patients to receive the transfusion components they need.

It is recommended that a baseline phenotype or genotype of the patient's RBCs be established prior to the patient starting treatment with daratumumab so that, if needed, phenotype-matched, antigen-negative blood can be provided. However, if the patient's RBCs require phenotyping after the start of daratumumab, routine dithiothreitol (DTT) treatment of these RBCs should be performed.[3,6] This method was recently evaluated and supported in an international validation.[7] Using DTT as the principle strategy for eliminating this interfering reactivity requires additional steps. DTT is a thiol-based reagent that cleaves sulfhydryl bonds and is commonly used when testing samples with cold agglutinins to disrupt the structure of IgM so possible underlying clinically significant IgG antibodies may be found.[8] Several antigens, including CD38, contain sulfhydryl bonds that are susceptible to this reagent, which is the basis for using DTT as a treatment to eliminate this reactivity. Some notable antigens with disulfide-exposed bonds include those in the Kell, Lutheran, Landsteiner-Wiener, and YT blood group systems.[3] Serologic typing for these antigens may show false-negative results as a byproduct of using DTT treatment to eliminate CD38 reactivity.

Additionally, DTT is not routinely used in many hospital-based blood banks, necessitating send-out testing, which may be costly and time-consuming. Although aliquots of DTT are available for a relatively small acquisition cost, there are other costs to consider: additional technician time to perform DTT treatment, time required to create and implement new protocols, special disposal of reagents, and issues with reimbursement between in-house testing and send-out testing. Another factor to consider is the small number of patients receiving daratumumab at an institution. After carefully considering all of these factors, we decided that it was more cost-effective for our lab to send out samples for DTT treatment than to perform DTT treatment in-house.

At our institution, genotyping results can take up to 2 weeks to receive and cost $975 plus the additional $97/antigen/unit for antigen-negative blood. DTT treatment may take several days for a result and costs our laboratory $1450 for each treatment. Another proposed strategy is the use of antibodies with specificity against CD38[3,6] as well as using panels with cord blood cells.[9] These antibodies and panels, also, are not widely available.

Our institution is a 676-bed academic, tertiary-care hospital in the Midwest, surrounded geographically by low-population-density rural areas. We have an active solid organ and bone marrow transplant program. In a typical year, we perform approximately 26,000 type and screen tests with a staff of 18 full-time employees.

We propose an alternative initial strategy for dealing with daratumumab interference that meets our institution's needs and may be helpful for other similarly situated institutions. Both solid-phase and gel testing are known to enhance serologic reactivity. Retesting samples, that were initially reactive by solid-phase or gel testing with tube testing using various media or incubation times to identify whether the reactivity persists, is a strategy implemented to address nonspecific reactivity in many testing algorithms for various interfering substances.[10] Rather than routinely moving to DTT treatment after identifying initial panreactivity with a three-cell antibody screen (as is currently recommended), we moved to tube testing with various media or incubation times to determine if the use of DTT is routinely warranted for testing samples from patients who are receiving daratumumab.

Materials and Methods

To evaluate if standard laboratory methods were effective in eliminating the interfering reactivity caused by daratumumab, the medical charts of patients being treated with daratumumab (dose 16 mg/kg) were reviewed. The inclusion criterion for review was patients being treated with daratumumab for multiple myeloma that needed pre-transfusion testing. The review included four patients, two men and two women, ranging in age from 55 to 78 years. The individual number of doses ranged from 1 to 14. Type and screen data were collected from 33 blood samples collected from these four patients, with each patient contributing from 1 to 13 samples. All samples were initially tested by automated solid-phase testing (Galileo Echo, Immucor, Norcross, GA) using a three-cell antibody screen (Capture-R Ready Screen 3, Immucor) following the manufacturer's instructions. The agglutination strength by solid-phase testing was graded via

the analyzer's interpretation algorithm. The agglutination strength for the tube testing was graded via methods 1–9 of the AABB Technical Manual.[10] Any reactivity by solid-phase testing led to additional tube testing with either LISS, PEG, or both. With continued reactivity, tube testing with a 60-minute incubation with no enhancement was done.

For the tube testing with enhancement, two drops of patient plasma or serum and one drop of thoroughly mixed antibody screening cells were added to their respective tubes. Then, two drops of PEG or LISS were added to each tube and mixed. The tubes were incubated at 37°C ± 1°C for 10–30 minutes. If no enhancement was used, the tubes were incubated at 37°C ± 1°C for 60 minutes.

When PEG was used, the tubes were washed four times in an automated cell washer, after which two drops of AHG reagent was added. The tubes were then mixed, centrifuged, and read for agglutination. When LISS or no enhancement was used, the tubes were centrifuged and read after incubation, and washed in an automated cell washer four times, followed by the addition of two drops of AHG reagent. The tubes were mixed, centrifuged, and read again for agglutination.

When the reactivity persisted, the sample was sent out for DTT treatment and genotyping. In addition, the results were compared with those from samples tested from their last dose of daratumumab (half-life 21 days).

The DTT treatment was done using 0.2 M DTT, which was prepared by dissolving chemical (powdered) DTT in phosphate-buffered saline, pH 8.0. The DTT solution was then divided into 1-mL aliquots that were stored at –18°C.

Prior to DTT treatment, panel cells and control (K–k+) cells were washed in saline once. Four volumes of 0.2 M DTT was added to one volume of test RBCs. The tubes were then mixed well and incubated at 37°C ± 1°C for 30 minutes. After the incubations, the treated RBCs were washed in normal saline (pH 7.3) and resuspended to 3–5 percent.

Quality control of the prepared DTT reagent was performed by testing DTT-treated and -untreated (K–k+) cells with anti-k antisera to ensure that the DTT was functioning as expected. The patient's serum or plasma sample was then tested with the 0.2 M DTT-treated cells using antibody identification methods such as tube, gel, or solid-phase.

Genotyping was carried out by our local reference laboratory (HEA Bead Chip, Immucor) following the manufacturer's instructions.

Results

Of the 33 samples tested, 23 (69.7%) had reactivity in solid-phase testing (Table 1). It was noted that 8 of the 10 samples that did not react by solid phase were collected more than four half-lives after the last dose of daratumumab. Results of the remaining two samples were reported as uninterpretable; the automated solid-phase analyzer generated a report of "results failed" due to operational error.

The 23 samples with solid-phase reactivity were then tested with other methods. Sixteen (69.6%) samples demonstrated loss of panreactivity using common laboratory methods. There were seven samples in which the reactivity was not initially eliminated. For the patients associated with six of these samples, either phenotypically matched blood from prior testing was provided or the transfusion was no longer indicated. Only one sample was sent for DTT treatment.

For patient 1, elimination of the interfering reactivity was seen with the use of LISS on the first sample, and a genotype was obtained. On testing of the sixth and seventh samples, the reactivity could not be eliminated, and antigen-negative blood was provided based on the initial genotyping, eliminating the need for costly DTT treatment. For samples 8–11, reactivity was present and was eliminated using common laboratory methods. On testing of the twelfth sample, the initial solid-phase testing was negative; this sample was collected 4.3 half-lives from the patient's last dose.

For patient 2, the interfering reactivity could not be eliminated in the first sample. There was not enough sample left to send for DTT treatment, and a redraw could not be obtained. On testing of the second sample, the reactivity remained, but a transfusion was no longer clinically indicated. On testing of samples 3–7, the reactivity was eliminated with common laboratory methods. The sixth sample required DTT treatment, and a genotype was performed. On testing the ninth and tenth samples, the reactivity remained, and antigen-negative blood was provided. Because of the implementation of this study within routine clinical laboratory processes, a 60-minute incubation was not done on these two samples. The reactivity seen in samples 11–13 was eliminated with common laboratory methods.

The first sample from patient 3 showed interfering reactivity, but a transfusion was no longer clinically indicated. On testing of the second sample, the reactivity was eliminated with common laboratory methods. The remainder of the samples were negative by the initial solid-phase testing despite all of them being collected within two half-lives of the patient's last dose.

Table 1. Results of pre-transfusion testing on samples from patients undergoing daratumumab treatment

Patient	Sample number	Solid-phase*			PEG†§			LISS†§			Incubation‡§	DTT treatment	Half-lives¶
		Panel 1	Panel 2	Panel 3	Panel 1	Panel 2	Panel 3	Panel 1	Panel 2	Panel 3			
1	1	4+	4+	4+	W+	W+	W+	0	0	0	NT	No	—
	2	3+	2+	3+	0	0	0	0	0	0	NT	No	—
	3	4+	3+	3+	0	0	0	NT	NT	NT	NT	No	—
	4	3+	3+	3+	2+	2+	2+	0	0	0	NT	No	—
	5	4+	4+	3+	0	0	0	NT	NT	NT	NT	No	—
	6	3+	4+	3+	2+	2+	2+	2+	2+	2+	NT	No	—
	7	3+	4+	3+	W+	W+	W+	2+	2+	2+	NT	No	—
	8	4+	3+	3+	1+	1+	1	0	0	0	NT	No	—
	9	3+	3+	2+	0	W+	0	W+	W+	W+	All negative	No	—
	10	2+	3+	3+	NT	NT	NT	1+	2	2+	All negative	No	—
	11	1+	1+	0	NT	NT	NT	0	0	0	NT	No	3
	12	0	0	0	NT	NT	NT	NT	NT	NT	NT	No	4.3
2	1	3+	4+	3+	2+	1+	W+	2+	2+	2+	W+	No	—
	2	3+	3+	1+	1+	1+	1+	1+	1+	1+	NT	No	—
	3	3+	3+	3+	0	0	0	NT	NT	NT	NT	No	—
	4	3+	4+	3+	W+	1+	0	W+	W+	W+	All negative	No	—
	5	3+	3+	2+	2+	W+	1+	1+	W+	W+	All negative	No	—
	6	3+	2+	1+	3+	3+	3+	2+	2+	2+	All negative	Yes	—
	7	3+	2+	0	0	0	0	NT	NT	NT	NT	No	—
	8	3+	2+	0	2+	2+	2+	1+	2+	1+	All positive	No	—
	9	1+	2+	2+	W+	1+	1+	W+	W+	W+	NT	No	—
	10	3+	3+	3+	1+	W+	1+	0	1+	1+	NT	No	—
	11	3+	2+	2+	MF	2+	W+	W+	W+	W+	All negative	No	—
	12	4+	3+	3+	0	0	0	0	W+	W+	NT	No	—
	13	2+	3+	3+	0	0	0	NT	NT	NT	NT	No	—
3	1	Results failed			W+	1+	1+	0	1+	1+	W+	No	—
	2	Results failed			NT	NT	NT	0	0	0	NT	No	~1
	3	0	0	0	0	0	0	NT	NT	NT	NT	No	—
	4	0	0	0	NT	NT	NT	NT	NT	NT	NT	No	—
	5	0	0	0	NT	NT	NT	NT	NT	NT	NT	No	—
	6	0	0	0	NT	NT	NT	NT	NT	NT	NT	No	—
	7	0	0	0	NT	NT	NT	NT	NT	NT	NT	No	—
	8	0	0	0	NT	NT	NT	NT	NT	NT	NT	No	—
4	1	0	0	0	NT	NT	NT	NT	NT	NT	NT	No	8

*Initial antibody detection by solid-phase testing using reagent antibody screening cells from three cell lines. Reaction grading results were provided by the automated solid-phase analyzer's interpretation algorithm.

†Additional tube testing using PEG and LISS.

‡Additional tube testing using 60-minute incubation with no enhancement.

§Reaction grading results for tube testing were done according to Methods 1–9 in the AABB Technical Manual, 18th ed.[10]

¶Number of half-lives since last collected sample.

PEG = polyethylene glycol; LISS = low-ionic-strength saline; DTT = dithiothreitol; W+ = weak positive; NT = not tested; MF = mixed-field reactivity.

There was only one sample tested from patient 4, and it was negative on the initial solid-phase screen. Though this patient's sample did not have any reactivity by any test method, the results were included to highlight the relationship between the timing of daratumumab treatment and the presence of interfering reactivity. This sample was collected eight half-lives from the patient's last dose.

Conclusions

Here we demonstrate that using multiple standard laboratory methods may obviate the need for routine DTT treatment up to 70 percent of the time. Under the current recommendations, the majority of samples with daratumumab-related reactivity would have needed either send-out testing or in-house DTT treatment. We propose that obtaining a baseline RBC phenotype or genotype from the first patient sample combined with the use of standard laboratory methods can be a more efficient and cost-effective strategy than directly moving to DTT treatment.

Accurate pre-transfusion testing is necessary for patient safety. Being able to manage samples with daratumumab-related reactivity in an efficient and cost-effective manner that still allows for detection of clinically significant alloantibodies is important for patient care. DTT treatment, although effective, may add significant cost and increased turnaround time for patients. Although a complete cost comparison and analysis is beyond the scope of this report, one can appreciate that for a laboratory similar to ours, using DTT testing as the primary method to manage samples with daratumumab-induced reactivity could prove to be costly. Each laboratory needs to consider their workflow and patient population to determine if bringing DTT treatment in-house is cost-effective.

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