A New Challenge for Blood Institutions:

DARATUMUMAB

By Meghan Delaney, DO, MPH
and Connie Westhoff, SBB, PhD

Daratumumab, an anticancer drug that was approved by the FDA last year, targets the CD38 molecule expressed on plasma cells. The drug is a monoclonal antibody used to treat patients with resistant multiple myeloma. It is also currently being tested in clinical trials to treat patients with earlier stage myeloma, B-cell leukemia and lymphoma.

While daratumumab (Darzalex) offers an important new oncologic treatment option, it creates a new challenge for blood centers. Daratumumab targets the CD38 molecule expressed on plasma cells. In addition to plasma cells, CD38 is widely expressed on tissues and red blood cells (RBCs). This creates a challenge for blood centers because it effects antibody detection and crossmatch. The presence of anti-CD38 circulating in a patient's plasma sample will bind to the antibody screening cells and to donor RBCs, resulting in a positive indirect antiglobulin antibody screen and crossmatch.

Plasma samples containing daratumumab show variability in the strength of reactivity, but are often described as 1+ to moderate reactivity with all RBCs tested, though this is often stronger in solid phase testing. The reactivity is not enhanced with enzyme treated RBCs or with other enhancement media (LISS or PEG); the reactivity remains with diluted plasma and is not removed by adsorption. Unfortunately, daratumumab will continue to interfere with testing for up to 6 months following cessation of therapy. Patients may or may not have a weakly positive direct antiglobulin test (DAT) with IgG, but an eluate is usually not reactive. Daratumumab does not interfere with ABO and RhD typing of the patient's RBCs or antigen typing for other blood group antigens in samples with a negative DAT. For the purposes of this article, the sum of these serological findings is referred to as "daratumumab interference."

Faced with the technical challenges for pre-transfusion testing, laboratorians have worked to find ways around the drug-induced agglutination reactions and to develop systems to be informed when a patient receives the drug, particularly as the drug becomes more widely used.[1,2,3]

Recognizing patients prescribed daratumumab

A critical aspect of testing samples from patients taking daratumumab is communication between the clinical team and the transfusion service laboratory indicating that a patient is going to receive—or has been treated with—the drug. Development of processes and procedures for this information to be provided in a timely fashion will ensure pre-transfusion samples are tested appropriately without excessive delays and expenditure of resources.

Many blood centers and laboratories have only recently been seeing patients who are receiving daratumumab treatment. It is important for transfusion services to be informed if patients are receiving daratumumab so that daratumumab interference can be identified early. Communication is key; it is important to develop a process to include automatic notification to the blood bank from the pharmacy or the hematology service when the drug is prescribed. This may be most efficiently done through computerized alerts or ordering systems from the electronic medical record. Updating blood bank laboratory requisitions to include a query regarding daratumumab administration may also facilitate identification of problem samples. Transfusion staff should insist on a careful drug history, especially when there is a diagnosis of multiple myeloma, or when panreactivity in the absence of a positive DAT is detected. The drug manufacturer has taken an active role in efforts to educate physicians and hospital and laboratory staff about daratumumab interference in blood bank testing, as have transfusion medicine practitioners and reference laboratory leaders.

Laboratory testing before initiating daratumumab therapy

To prepare for the possibility of future transfusion, a baseline antibody detection test should always be done to detect the presence of pre-existing RBC antibodies. In addition, consideration should be given to obtaining the patient's extended RBC antigen phenotype to be made part of their blood bank record. (This recommendation was set forth in AABB's Association Bulletin #16-02, released in January.) This will inform future antibody detection testing by revealing which antigens the patient lacks and would be at risk for sensitization. Which RBC antigens, as well as the number of RBC antigens to determine, should also be considered. At a minimum, K-typing of the patient is generally recommended if future antibody detection tests will employ DTT testing. The decision to do serologic phenotyping or performing blood group genotyping may be influenced by the availability of typing reagents or genotyping. Although most clinically important blood group antigens can be serologically determined, some clinically significant antigen do not have antisera readily available. RBC genotyping can provide the results for a majority of the antigens of interest. A policy to guide performance regarding when to perform extended RBC antigen phenotype and/or genotype is recommended. For patients with chronic transfusion needs, or those with pre-existing RBC alloantibodies, knowledge of the extended RBC antigen type is helpful and some degree of antigen-matching to prevent additional alloimmunization may be considered.

Table. Frequencies of blood group antigens destroyed on RBCs treated with DTT in the Kell, Dombrock, Lutheran and Yt systems[4]

	K	k	Kp^a	Kp^b	Js^a	Js^b	Lu^a	Lu^b	Do^a	Do^b	Yt^a	Yt^b
Whites	9%	99.8%	2%	100%	0.01%	100%	8%	99.8%	67%	82%	>99.8%	8%
Blacks	2%	100%	<0.01%	100%	20%	99%	5%	99.8%	55%	89%	>99.8%	

Antibody detection techniques for patients taking daratumumab

When there is DARA interference, the challenge is to rule out the presence of underlying alloantibodies. The positive reactivity will mask underlying antibodies and can prevent timely provision of blood for transfusion. Techniques commonly employed in immunohematology reference laboratories have now been considered by hospital transfusion service laboratories to enable testing of pre-transfusion samples from patients on daratumumab (summarized below). Challenges to implement these alternative approaches to antibody detection include implementation and validation of a new test, controls, confirmation of the test performance and training of staff. Many hospital transfusion services choose to send out samples to a reference laboratory for rule out of underlying alloantibodies.

Dithiothreitol treatment of test RBCs

Many laboratories are using 0.2M dithiothreitol (DTT), which reduces the disulfide bonds in the CD38 molecule on the RBCs. The circulating daratumumab anti-CD38 no longer binds and allows detection of underlying alloantibodies.[1] However, DTT treatment also destroys the Kell (K/k, $Js^{a/b}$, $Kp^{a/b}$), Dombrock (Do, Hy, Jo^a), Lutheran (Lu), Knops, LW, Yt, and IN blood group antigens on the RBCs. Thus, a negative test result with DTT-treated RBCs does not provide evidence that the patient's sample lacks antibodies to antigens in these blood group systems. With the exception of antibodies to K, antibodies to Dombrock antigens, Lu or Yt antigen, or to the low prevalence Js^a or Kp^a antigens, are not frequent (See Table). However, these antibody specificities should be considered in multiply transfused patients, those with other RBC alloantibodies or multiparous women. As noted previously, to avoid anti-K production, antigen-matching for the K antigen should be considered or transfusion with K– units.

The storage stability of DTT treated RBCs has not been clearly established. DTT reagent is a skin and mucus membrane irritant and must be handled with precaution. One source of commercial DTT has recently come to market.

Trypsin enzyme treatment of test RBCs

Trypsin treatment cleaves the CD38 molecule from RBCs. Trypsin-treated cells can be used to rule out antibodies to Kell antigens (K,k,$Kp^{a/b}$ and $Js^{a/b}$), Yt and LW, since these antigens are not destroyed by trypsin treatment. When used in conjunction with DTT-treated RBCs, clinically significant antibodies to all but Dombrock and Lutheran system antigens can be ruled out. Trypsin-treated RBCs are not commercially available; trypsin can be difficult to work with, and the storage stability of trypsin treated cells is limiting.

Cord Cells

Umbilical cord RBCs lack (or have very low) CD38 expression. The use of cord RBCs for antibody detection at the IAT phase has recently been described.[2] Laboratories could potentially antigen type a number of cord RBCs to use as reagent RBCs for antibody detection and identification to test against the plasma from patients treated with daratumumab.

Neutralization of anti-CD38

Use of anti-idiotype directed to anti-CD38 or recombinant CD38 have also been described to eliminate interference.[3,5] However, these are not widely available, recombinant CD38 is costly, and there are a number of variables including the blood level of the circulating drug

that would be anticipated to complicate attempts to standardize usage.

Transfusion approach for patients treated with daratumumab

It is estimated that about one-third of patients treated with daratumumab may require a transfusion.[5] For patients requiring repeat transfusions, a policy regarding the frequency of antibody screening and identification testing should be established by the medical director. For patients with no pre-existing alloantibodies, repeating testing every 3 to 7 days since the last transfusion episode would seem reasonable. These patients could be managed in the same manner as other patients presenting with warm-reactive panreactivity.

Another approach is to do extended phenotype or genotype and select antigen-matched units.[6] In the United States, this is a cost consideration to be balanced against the potential to decrease the number of repeat workups. This approach will prevent antibody production against the specific blood group antigens matched with the patient, but uncommon antibodies to other antigens will not be prevented. Prophylactic matching to prevent Rh antibodies (C,c, E, or e), in addition to K, has been implemented by some transfusion services.

Daratumumab in the "real world"

Claudia Chapuy, MD and Rick Kaufman, MD, from Brigham and Women's Hospital in Boston,

Daratumumab brings new challenges to blood centers that will likely become familiar as the use of the drug expands.

conducted a recent study of daratumumab interference with blood compatibility testing.[1] Their laboratory now performs a baseline ABO/RhD, antibody detection test and a RBC genotype for patients who are about to start receiving daratumumab. On subsequent samples with daratumumab interference, "we use a 6-cell panel of DTT-treated cells selected to rule out common alloantibodies and to also act as controls for DTT treatment, i.e. the K+ cell must type antigen-negative after treatment, and the E+ cell must remain antigen positive," they said. "If the mini E/K DTT-treated panel is negative, which is the most common case, we issue ABO/RhD and K-matched units by electronic crossmatch."

At the University of Rochester Medical Center, doctors take a different approach. "Our cord blood reagent RBCs [protocol] continues to work very well," said Amy Schmidt, MD. "To extend the use of each cord cell panel, the cells are frozen and stored in liquid nitrogen. Patients with clinically significant alloantibodies may be tested with DTT treated cells to confirm or rule-out additional specificities." Schmidt said it is essential to have a communication system. "Before a patient starts daratumumab treatment, the blood bank is notified and a type and screen is collected so that we can establish a baseline antibody screen and perform a complete RBC phenotype. If the patient will be traveling, we can provide them with a card stating that they have positive antibody screen due to anti-CD38. The patient could present the card at any other hospital they visit. After starting daratumumab, antibody screens and additional workups would only be performed when the patient is confirmed for transfusion."

All agree that knowing the patient's medical history is essential. "It pays to pay attention to the medical history and to get an accurate drug history," Kaufman said. "As soon as we see 'multiple myeloma,' we immediately become suspicious of anti-CD38 treatment. Many immunohematology reference laboratories are becoming experts in recognizing daratumumab interference, but there is no substitute for upfront communication and an accurate drug history."

Summary

Daratumumab brings new challenges to blood centers that will likely become familiar as the use of the drug expands. The drug manufacturer, Janssen Pharmaceuticals, is actively working to educate blood centers and health care providers about daratumumab interference in pre-transfusion testing. Process challenges for the hospital blood bank include development of a mechanism to identify patients scheduled to receive the drug so that at a minimum, the critical information is passed to the blood centers, and initial testing can be performed. In laboratories that will receive samples with daratumumab interference, significant vigilance must be maintained to obtain drug history. Further, the potential regulations for laboratory developed tests may make testing more complicated in the future. It is critical that we in the health care field keep open lines of communication and continue to work together to ensure safe accessible and timely procurement of blood products for the patients on daratumumab.

REFERENCES:

1. Chapuy CI, Nicholson RT, Aguad MD, Chapuy B, Laubach JP, Richardson PG, Doshi P, Kaufman RM. Resolving the daratumumab interference with blood compatibility testing. Transfusion 2015, 55:1545-54.
2. Schmidt AE, Kirkley S, Patel N, Masel D, Bowen R, Blumberg N, Refaai MA. An alternative method to dithiothreitol treatment for antibody screening in patients receiving daratumumab. Transfusion 2015, 55:2292-3.
3. Oostendorp M, Lammerts van Bueren JJ, Doshi P, et al. When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. Transfusion 2015, 55:1555-62.
4. Reid ME, Lomas-Francis C, Olsson ML. *The Blood Group Antigen Factsbook*. Third ed: Academic Press; 2012.
5. Lokhorst HM, Plesner T, Gimsing P, et al. Phase I/II dose-escalation study of daratumumab in patients with relapsed or refractory multiple myeloma. ASCO Meeting Abstr 2013;31(15_Suppl):8512.
6. Hannon JL, Clarke G. Transfusion management of patients receiving daratumumab therapy for advanced plasma cell myeloma. (Letter) Transfusion 2015, 55:2770.

Meghan Delaney, DO, MPH, is the medical director of the Immunohematology and Red Cell Genomics Reference Laboratory at Bloodworks NW in Seattle. Connie Westhoff, SBB, PhD, is the director of immunohematology and genomics at the New York Blood Center in New York City.