Current Practice of Mitigating Monoclonal Anti-CD38 Interference in Pretransfusion Compatibility Testing

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ABBREVIATIONS: DARA - daratumumab, DTT - dithiothreitol, IAT - indirect antiglobulin test, IFE - immunofixation electrophoresis, IRL - immunohematology reference laboratory, IS - immediate spin, LIS - laboratory information system, mAb - monoclonal antibody, MM - multiple myeloma, NK - natural killer, QC - quality control, RBC - red blood cell, SPEP - serum protein electrophoresis.

Clin Lab Sci 2018;31(2):48–51

INTRODUCTION

Anti-CD38 is a high-titer human IgG1 monoclonal antibody (mAb) that binds with high affinity and specificity to CD38, a 46-kDa type II transmembrane glycoprotein expressed on lymphoid, myeloid, and nonhematopoietic tissues.[1-3] The malignant cells in multiple myeloma (MM) often express high levels of CD38.[4] Anti-CD38 mAb therapy has been shown to induce malignant cell death through antibody-dependent phagocytosis, complement-dependent cytotoxicity, and natural killer (NK) cell–mediated antibody-dependent cellular cytotoxicity.[5] Daratumumab (DARA) (Darzalex, Janssen Pharmaceuticals) is the first anti-CD38 mAb approved by the Food and Drug Administration for the treatment of MM in patients who have received at least three prior lines of therapy, including a proteasome inhibitor and an immunomodulatory agent, or in patients who are double refractory to these agents.[5-7]

MM is a plasma cell malignancy characterized by the overproduction of mAbs, most commonly IgG or IgA. In serum protein electrophoresis (SPEP), the mAb is typically detected as M protein, a light chain derivative. M protein appears as a thin, dark band that produces a sharp peak in densitometric analysis. The high concentration of M protein may result in hyperviscosity of the blood and impaired kidney function. Uncontrolled proliferation of the malignant plasma cells frequently manifests as plasmacytomas in the bone marrow. The plasmacytomas secrete macrophage-activating cytokines that induce osteolytic activity resulting in bone lesions, hypercalcemia, and neuropathy. Lumbar pain is experienced as the first symptom by a substantial percentage of MM patients. As the disease progresses, patients experience transfusion-dependent anemia. Despite recent advances in treatment, MM remains incurable.

A majority of patients receiving DARA therapy experience a significant reduction in M protein values,[6] and DARA monotherapy has been shown to induce progression-free survival for a median of 6 months.[6] However, monotherapy is less effective than combination therapy regimens.[6] Resistance to DARA has been reported in a few patients with MM cell lines that underexpress CD38 or express complement-inhibiting proteins.[7]

DARA induces the ability of FcR-bearing macrophages to eliminate MM cells through phagocytosis.[5] Rapid phagocytosis has been demonstrated in vivo through cultured MM xenografts and in ex vivo MM cells from patient samples.[5] A threshold amount of CD38 expression is necessary for DARA to induce phagocytosis, which may explain the drug's relative selectivity for MM cells over other CD38-expressing cells.[5] MM patients harbor an elevated count of macrophages within the bone marrow, which may explain the efficacy of DARA in reducing bone lesions associated with MM.[5]

DARA also eliminates MM cells through the classical complement cascade, antibody-dependent cytotoxicity, and direct cellular apoptosis.[5,6] The Fc fragment of DARA recruits C1q and triggers the formation of the membrane attack complex. FcR-bearing effector cells, such as NK cells, have a high affinity for the Fc fragment of DARA. The FcR-mediated crosslinking of multiple DARA antibodies has been demonstrated to induce apoptosis in vitro.[6]

The presence of DARA in a patient's specimen can interfere with a number of laboratory tests, including SPEP, immunofixation electrophoresis (IFE), and the indirect antiglobulin test (IAT).[6] In SPEP and IFE, the mAb comigrates with the M protein, which can negatively affect accurate quantification of the M protein.[8] Because red blood cells (RBCs) express low levels of CD38 molecules, DARA induces panreactivity with all cells tested with the IAT. MM patients receiving DARA therapy often require blood transfusions to treat the symptoms of anemia secondary to their disease progression. The detection of clinically significant alloantibodies is necessary to provide safe and efficacious RBC transfusions. The presence of DARA in the patient's specimen will induce positive reactions with

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antibody-screening cells, panel cells, and donor cells crossmatched through the antihuman globulin phase.[6] However, in most cases the direct antiglobulin test is nonreactive. ABO, Rh(D) testing, and immediate spin (IS) crossmatches are not affected by DARA.[6]

Dithiothreitol (DTT) treatment of RBCs prior to testing has been shown to remove the interference of DARA in the IAT by denaturing the extracellular domain of CD38 from the RBCs used in testing.[9,10] However, several important blood group antigens are also sensitive to DTT treatment, most notably the Kell system antigens. Anti-K (K1) antibody is a relatively common clinically significant alloantibody that can be detected in the sera of individuals who have received previous blood transfusions or who have been pregnant. Therefore, the denaturation of Kell system antigens with DTT would impair the ability to detect these alloantibodies if present in the specimen. Several other blood group system antigens are sensitive to DTT, including Indian, John Milton Hagen, Yt^a, Lutheran, MER2 (previously known as Raph), Knops, Scianna, Dombrock, Cromer, Landsteiner-Weiner, and some Diego system antigens.[11] Although antibodies directed against these blood group antigens are rare, the risk of an incompatible transfusion may be mitigated by a complete genotyping for RBC antigens.

Other methods can be employed to reduce or remove the interference of DARA, but these methods vary in sensitivity and cost. Pretreatment of RBCs with trypsin cleaves CD38 from the cell membrane and does not affect Kell system antigens, allowing for the detection of any antibody specificities directed against antigens in the Kell blood group system. However, trypsin treatment of RBCs has been shown to be much less effective than DTT treatment in the removal of CD38.[10]

Soluble CD38 can be added to the patient's serum or plasma specimen to neutralize the drug. The soluble CD38 will not interfere with the detection of alloantibodies against various blood group antigens. However, soluble CD38 is considerably more expensive in comparison to the DTT or trypsin methods.

Neutralizing mAbs directed toward the binding regions of DARA are also available, but because these neutralizing antibodies are idiotype-specific, they will only be effective at neutralizing DARA. A number of different monoclonal anti-CD38 drugs are currently undergoing clinical trials and may eventually be used in therapy, which may preclude the use of DARA-specific antibodies as a long-term strategy in managing the interference caused by the anti-CD38 drug.

Cord RBCs lack CD38, so these may be used as an alternative RBC reagent for pretransfusion alloantibody testing. However, although a bank of cord cells may be available to certain immunohematology reference laboratories (IRLs), it is difficult and impractical to maintain these in most transfusion service laboratories.

There are a variety of methods described in the literature that can be used to interfere with anti-CD38 in order to determine the presence of allo-antibodies in patients undergoing DARA therapy but with a lack of consensus and guidelines. We wanted to know what the current practice was with regard to testing samples from patients being treated with DARA. Thus, this study involved surveying those who work in transfusion laboratories in order to determine how DARA-treated patient samples are being tested for allo-antibodies in pretransfusion testing.

MATERIALS AND METHODS

The study design included a nonexperimental survey consisting of 16 questions, (Tables 1–3) regarding the laboratory's processes and procedures in handling workups for patients undergoing DARA therapy. The questions collected data on how laboratory personnel are notified of impending or current DARA therapy, the technical procedures for preparing and testing these specimens, what type of blood products are selected for transfusion, and how crossmatches are performed and reported. The survey was designed so that certain questions were automatically omitted if the respondent's previous answers made further questioning irrelevant. We distributed the survey to transfusion services and IRLs across the United States using SurveyMonkey Inc. and an electronic contact database of graduates of a Specialist in Blood Bank Technology Program. Responses were electronically received and reviewed. Participation was voluntary, anonymous, and uncompensated.

RESULTS

Forty-two responses were collected, with 38 being complete and four partially complete. Selected responses to our survey questions are presented in Tables 1–3. We have condensed the survey responses to highlight the most important information. The additional survey items not included in the tables are described in the discussion section. It is of note that response numbers (*n* value) differ because of two factors. First, some of the questions were engineered to be bypassed by the survey when previous responses made further questions irrelevant. Second, four of the respondents began but did not complete the survey in its entirety.

Respondents were composed of the following groups: hospitals without an onsite IRL (69.1%), IRLs (21.4%), and blood centers (9.5%). Our findings indicate that the majority (66.7%) of laboratories without an IRL onsite are working up DARA specimens in-house, either with DTT treatment or with another method, and the remaining 33.3% refer these specimens to an IRL for workup. The majority of facilities working up DARA samples in-house indicated they are using DTT-treated cells. However, methods concerning the preparation, storage, and quality control (QC) of DTT-treated cells vary widely. We observed that respondents use different protocols for the type of blood

selected for transfusion and how the crossmatch is defined (Table 2). The majority of the respondents report that antigen-confirmed K (K1)-negative blood is selected for transfusion (44.7%) and that this crossmatch is defined as "crossmatch incompatible due to DARA interference" (39.5%). Only one respondent indicated the use of cord blood cells in the workup of DARA samples, and none of the respondents indicated the use of trypsin for the removal of CD38 from the RBCs.

DISCUSSION

DARA specimens are being submitted to the laboratory with increasing frequency, and with a lack of regulation or standardization in this area, it is the laboratory's responsibility to develop and validate procedures and protocols for mitigating the interference of DARA with laboratory testing. These challenges are expected to escalate as various other mAbs for the treatment of malignancies (eg, additional CD38 mAbs, anti-CD47, and so forth) whose primary targets are also expressed on the erythrocyte membrane in varying degrees are currently under development. It is anticipated that, like DARA, any mAb that is capable of sensitizing to antigens on RBCs will be detected in an IAT and may have similar negative effects upon various other laboratory tests like those described above. The responses to our administered survey display a wide variability of systems, methods, and procedures currently being employed to counter the interference of DARA.

Of the responding facilities that perform their own DARA workups ($n = 28$), 23 utilize DTT (82.1%), showing it to be by far the most widely used method (Table 1). However, facilities must determine their own procedures for preparing and performing QC testing on the reagent RBCs treated with DTT. According to the results of the survey, the process of treating the screening cells varies between different facilities. The variations include the pH of the DTT, the concentration of cells used, the method of measuring cells, the incubation period, and QC procedures. The majority of respondents indicated they discard any leftover DTT-treated cells after testing, whereas others store the treated cells for various intervals as validated by each individual laboratory. A pH of 7.3 was most commonly used along with packed red cells when preparing screening cells, but other variations, such as a pH of 8.0, not checking the pH, and using a 2%–5% red cell suspension, were also utilized. QC testing of the treated RBCs also varies greatly, with k (K2) antigen being the most commonly used as a control. Cellano, or k (K2) antigen, is a high-incidence antigen within the Kell blood group system. The expression of k on an RBC after DTT-treatment indicates an incomplete disulfide reduction by DTT. The respondents reported inconsistent QC results regarding the preservation of antigens that should remain intact after DTT-treatment (eg, Rh, Kidd, Duffy, etc). Preservation of these antigens is necessary for the detection of alloantibodies to antigens within these blood group systems.

In most protocols, following the preparation and QC testing of DTT-treated cells, the workup may begin by performing an antibody screen (IAT) with the treated cells. If the result of the antibody screen with DTT-treated cells is negative, the transfusion service may proceed with an electronic or IS crossmatch. If the antibody screen is positive, antibody identification with DTT-treated panel cells is performed. Due to the inability to screen for Kell system antibodies with the DTT method and because of the relatively common prevalence of anti-K (K1) alloantibodies within the previously transfused patient population, K (K1)-negative blood will often be selected for transfusion. This is especially pertinent if the patient's transfusion history is unknown, if phenotyping or genotyping results are unavailable, and when blood is imminently required. The highest percentage (44.7%) of our respondents indicated they select antigen-confirmed K (K1)-negative blood for transfusion, whereas others select blood to match the patient's extended RBC phenotype based upon genotype (18.4%) or previously tested phenotype (7.9%). Patients with MM often receive multiple RBC transfusions, which increases the risk of alloantibody stimulation.

Cord blood cells can be used as an alternative to DTT-treated cells for antibody screening because they lack CD38 and are thus not affected by DARA. This method allows for the detection of Kell system antibodies that would not be detected with DTT-treated cells. However, because the collection and maintenance of a library of cord cells sufficient for antibody testing is so arduous, the majority of transfusion facilities appear to be issuing K (K1)-negative blood in lieu of cord testing. This was made evident in our survey results, with only one respondent indicating they utilize cord blood in their testing protocol.

Of the total number of respondents ($n = 42$), five (11.9%) indicated the specimens are worked up in-house using a method other than DTT. However, the survey did not include specific follow-up questions to this response, which limits our ability to fully evaluate which alternative methods are being employed at these facilities.

To minimize confusion and possible delays in procuring transfusion services, it is recommended that the blood bank be notified of a patient beginning DARA therapy. This may be achieved via an automated process built into the laboratory information system (LIS). Respondents were asked how often and in what ways they are being alerted of DARA treatment. Both the frequency and method of notification varied greatly from facility to facility. This may be a result of inadequate training of the provider or lack of communication between clinical staff and the transfusion service prior to initiating the therapy.

The DARA package insert recommends that patients are ABO- and (Rh)D-typed and that an antibody screen be performed before initiating therapy. This helps to facilitate the collection of baseline results and to notify the laboratory in advance of potential problems that may be

encountered with future specimens. Testing conducted prior to DARA therapy may indicate a need to order additional tests, such as extended phenotype or genotype testing. Blood centers may be informed so that donors with matching phenotypes may be recruited if necessary. One facility recently developed a computer algorithm that automatically places an order for a type and screen and adds a flag for DARA when the drug is ordered via the hospital's electronic medical record system. Unfortunately, as shown in Table 3, a significant percentage of respondents indicated that they rarely or infrequently receive notification when a patient begins DARA therapy. For those who are notified, the notification originates from various sources, including clinic liaisons, specimen requisitions, electronic medical records, calls from the floor, and the pharmacy. However, 7.9% responded that they are not alerted prior to receipt of the patient's sample, which indicates opportunities for improvement in this area of patient care. The reactivity displayed by a DARA specimen can mimic a warm autoantibody, an alloantibody toward a high-incidence antigen, or a "high titer/low avidity"–like antibody, among others, each of which requires extensive, time-consuming, and completely unnecessary testing that could be avoided if the laboratory is alerted to the presence of the drug in the specimen prior to testing.

We propose that facilities implement specialized computer algorithms in order to avoid some of the obstacles we have described. First, we recommend that the hospital's computer system reflexes a type and screen order as soon as the order for DARA is placed. Second, we recommend that a popup window or other form of electronic notification be sent to the provider that details the drug's interference with laboratory testing and requests a consultation with the blood bank. Lastly, we recommend that a DARA therapy comment be added to the patient's transfusion history in the LIS.

CONCLUSION

DARA is the first anti-CD38 mAb used in the treatment of MM that commonly interferes with pretransfusion testing; however, it is anticipated that several other mAbs that target various specific antigens also expressed on RBCs will pose similar challenges in procuring accurate and timely laboratory results. Because many of the patients with these malignancies require blood transfusions to treat the symptoms of anemia associated with their condition, it is anticipated that these drugs will continue to interfere with blood bank testing. An emphasis on the development of efficient lines of communication between the ordering provider and laboratory and the standardization of laboratory protocols to mitigate the effects of these drugs in laboratory testing are crucial to provide excellent patient care.

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