EDUCATIONAL COMMENTARY: MONOCLONAL ANTIBODIES AND THEIR EFFECTS ON BLOOD BANK TESTING

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Learning Objectives

On completion of this exercise, the participant should be able to

- discuss the effects of anti-CD38 monoclonal antibodies on common blood bank tests;
- discuss the effects of anti-CD47 agent Hu5F9-G4 on common blood bank tests; and
- discuss several available options for overcoming therapeutic monoclonal antibody interferences in blood bank testing.

Introduction

In recent years, daratumumab has become a commonly seen interference in blood bank testing. Additional monoclonal antibodies that interfere in blood bank testing are in clinical trials now. A few examples of anti-CD38 antibodies now in clinical trials are isatuximab (SAR650984), MOR03087 (MOR202), and TAK-079. Anti-CD47 antibodies now in clinical trials include Hu5F9-G4, CC-90002, SRF231, and IBI188. Because both CD38 and CD47 are typically expressed on red blood cells (RBCs), both anti-CD38 and anti-CD47 agents affect blood bank tests. This brief overview will summarize recent literature describing the effects of anti-CD38 and anti-CD47 on blood bank testing.

Daratumumab and Other Anti-CD38 Medications

Daratumumab (proprietary name DARZALEX, Janssen Pharmaceuticals) is an IgG1κ anti-CD38 monoclonal antibody first approved by the US Food and Drug Administration (FDA), in 2015, for use in a subset of patients with multiple myeloma. Since then, the FDA has approved its use in additional populations of patients with multiple myeloma (as recently as September 2019, when the FDA approved use in patients with newly diagnosed multiple myeloma eligible for autologous stem cell transplant in combination with bortezomib, thalidomide, and dexamethasone). CD38 is a transmembrane protein present on both the therapeutic target and on RBCs. The expression of CD38 on RBCs results in daratumumab (and other anti-CD38) interference in blood bank testing.

In a 2016 bulletin, AABB recommended that before a patient begins taking an anti-CD38 agent, blood banks perform a baseline type and screen, as well as a baseline phenotype or genotype. In addition, the
DARZALEX package insert recommends baseline type and screen before initiation of daratumumab therapy.³

Patients receiving daratumumab treatment typically show panreactivity on indirect antiglobulin test (IAT) antibody screens and panels.⁵⁻⁷ The autocontrols and direct antiglobulin test (DAT) may be positive or negative,⁵ ABO typing is not altered, and crossmatches will often be incompatible.¹ As early as 6 hours after daratumumab exposure, trogocytosis (antigen shedding or downregulation) may occur, such that CD38 is no longer detectable on the patient’s RBCs.⁵,⁸ This change is reversible after treatment ceases.⁹ Daratumumab interference with testing may be observed up to 6 months following treatment.³,⁷,⁹

The use of dithiothreitol (DTT) to remove daratumumab interference has been well described.⁶,¹⁰ Dithiothreitol acts to cleave disulfide bonds, thereby denaturing CD38 on panel RBCs, resolving the panreactivity, and allowing alloantibody identification. Unfortunately, DTT denatures multiple RBC antigens in addition to CD38. For example, the Kell blood group antigens are also denatured by DTT, so an underlying anti-K antibody would not be detected using DTT-treated reagent RBCs. Thus, K antigen-negative RBCs are provided to individuals who do not express K, or whose K phenotype is unknown, when DTT-treated reagent RBC panels are used for antibody identification.³,⁷,¹⁰

Multiple alternative methods of avoiding daratumumab interference have been described, including use of trypsin, anti-idiotype antibody, soluble recombinant CD38, cord blood panels, In(Lu) panels, and DARA cells, panels of RBCs from daratumumab-treated patients, whose cells are serologically CD38 negative with known phenotypes and no recent transfusions.⁹⁻¹³ Trypsin is a proteolytic enzyme that has been described as decreasing daratumumab binding to CD38-expressing cells, although to a lesser degree than DTT. Trypsin does not cleave Kell antigens, but it does degrade M, N, Lutheran, and other antigens.¹⁴ Anti-idiotype antibody and soluble recombinant CD38 have been reported to prevent daratumumab binding with CD38-expressing reagent RBCs but are expensive and not easily obtained.¹,¹² Cord blood, In(Lu), and DARA cell panels have been suggested due to their decreased expression of CD38; however, these panels are not commonly found in hospital blood banks.¹,⁹

Several other agents that target CD38 are currently in clinical trials, as stated above.¹ Oostendorp et al found that anti-CD38 antibodies 38SB19 (a surrogate for SAR650984), MOR03087 (a surrogate for MOR202), and Ab79 reacted in a similar way to daratumumab, and they concluded that anti-CD38 agents as a class will cause false-positive IAT results.¹²
Hu5F9-G4 and Other Anti-CD47 Medications

CD47 is a glycoprotein found on all cells throughout the body. It binds signal-regulatory protein alpha (SIRPα) on macrophages to inhibit phagocytosis. Of note for Blood Bank technologists, both RBCs and platelets express CD47.8 Multiple anti-CD47 agents are now in clinical trials for the treatment of solid tumors and hematologic cancers, although none has received FDA approval yet.1,8,15

One particular anti-CD47 agent, Hu5F9-G4, has been reported in the literature to interfere with reverse typing of non–group O patients, show panreactivity in IAT antibody screens, panreactivity in eluates, and DATs that may or may not be positive.1,8,15 Autocontrols and DATs are thought to be negative to weakly positive owing to steric hindrance. Alloadsorptions of patient plasma with papain-treated reagent RBCs or pooled platelets have been described to allow for successful reverse ABO type, antibody screen, and crossmatch. Utilization of anti-IgG monoclonal Gamma-clone (Immucor, Inc.) escapes interference with IAT and allows for antibody identification. Specifically, Hu5F9-G4 is an IgG4 monoclonal antibody, and anti-IgG monoclonal Gamma-clone (Immucor, Inc.) may not detect IgG4 antibodies. However, weak reactivity may be seen if carryover agglutination occurs. Papain, ficin, trypsin, α-chymotrypsin, 0.2M DTT, and WARM (warm autoantibody removal medium) reagent will not cleave CD47. Velliquette et al warn that although some have suggested polyethylene glycol (PEG) adsorption as a possible method to escape this drug’s interference, they have found that PEG adsorptions result in precipitation of antibodies and therefore false-negative results.8

A baseline type and screen, as well as RBC phenotyping or genotyping are recommended before starting Hu5F9-G4 therapy.8,15

Because of the expression of CD47 on platelets, anti-CD47 agents also have the ability to alter the results of testing for antiplatelet and anti-HLA class I antibodies, as well as platelet crossmatching. Test methods that use platelet membranes or platelets will experience interference owing to their expression of CD47. However, methods that use monoclonal captured or affinity-purified glycoprotein molecules will be unaffected by Hu5F9-G4.8

It has been reported that other anti-CD47/SIRPα therapeutic agents show different reactivity in blood bank testing as compared to that of Hu5F9-G41.

Conclusion

Multiple monoclonal antibody therapeutic agents are now in use or development. The transfusion medicine community will need to continue to identify agents that may yield spurious results and work
toward alternative testing methods to best care for the transfusion needs of patients receiving these antibodies.

References


