Myeloma in Focus:
An information sheet for healthcare providers

Coombs testing in multiple myeloma

This document, created by Sanofi, aims to provide education to healthcare providers on relevant topics in multiple myeloma (MM). It will focus on Coombs testing, including an overview of Coombs tests, the impact of anti-CD38 antibody interference on Coombs testing, alternative methods to mitigate anti-CD38 interference, and the clinical management of anti-CD38 interference in pre-transfusion testing.

Coombs testing is an immunology laboratory procedure used for a variety of diagnoses, including pre-transfusion testing, and autoimmune or drug-induced hemolytic anemias. Anti-CD38 interference in pre-transfusion Coombs tests is a particular problem in patients being treated with anti-CD38 monoclonal antibodies for MM. A number of recommendations have been established to mitigate anti-CD38 interference, but further investigation is warranted to solve problems relating to crossmatching and antibody screening prior to and after the use of anti-CD38 monoclonal antibodies.

In this document

The role of transfusion in MM

Patients with MM frequently experience anemia due to accumulation of plasma cells in the bone marrow, which can crowd out normal red blood cells (RBCs). Myelosuppressive treatments may also reduce RBCs in the blood and bone marrow and contribute to anemia.[1] Anemia is commonly associated with fatigue due to decreased oxygen-carrying capacity. If left untreated, it can cause end organ failure and death.[2]

Common treatment options for anemia in MM include RBC transfusions that have been proven to restore hemoglobin levels in the blood and provide rapid, short-term relief of symptoms associated with anemia.[3]

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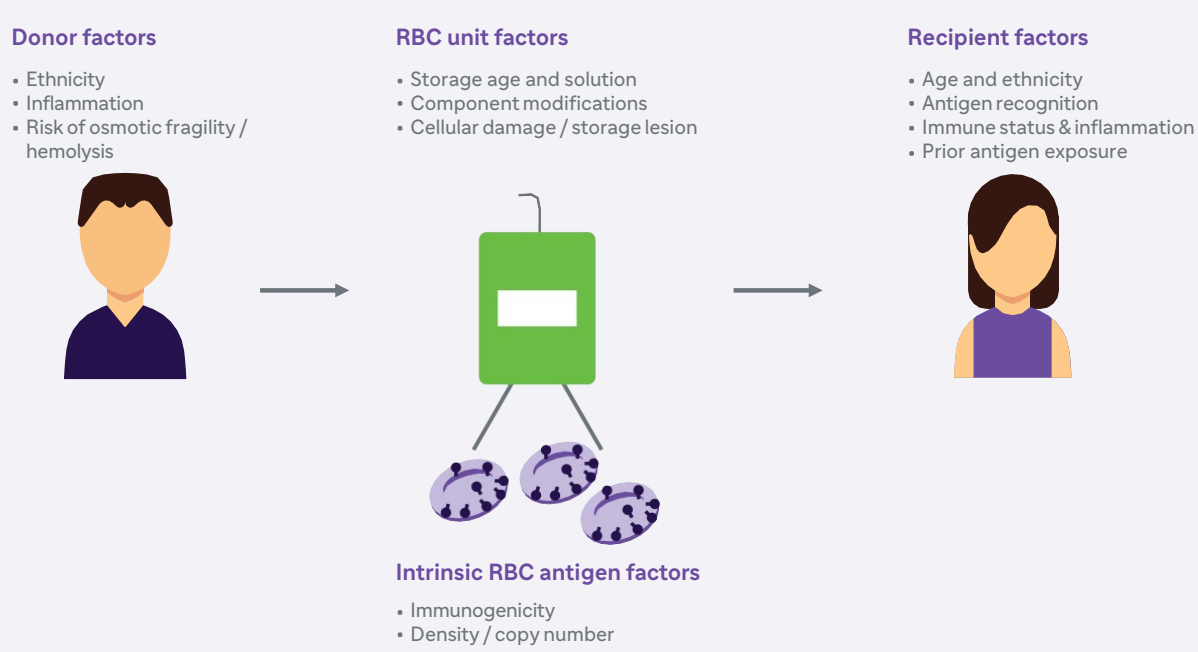
However, there is increasing clinical evidence suggesting that RBC transfusions may be associated with poor clinical outcomes due to high disease recurrence rates and the lack of clear guidelines on transfusion protocols. One of the main factors that contribute to complications of RBC transfusions is the development of alloantibodies (Figure 1). The presence of alloantibodies in the blood may be clinically important for future transfusions, possibly leading to hemolytic transfusion reactions and difficulties identifying compatible RBCs for future transfusion.[4]

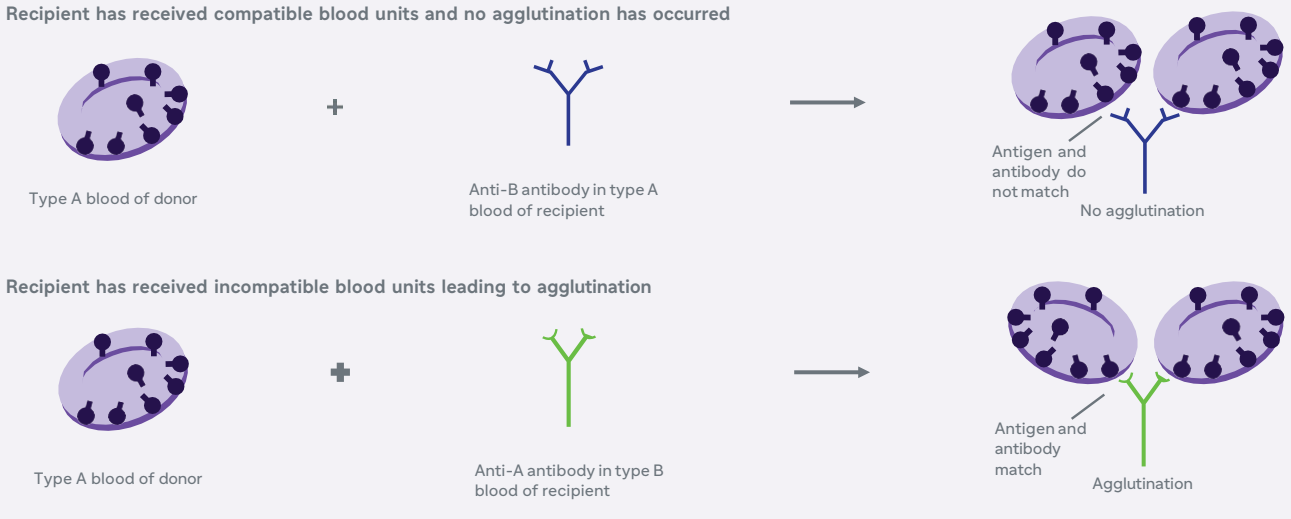
If incompatible blood units are given through a transfusion, the recipient's antibodies will attach to the donor's RBCs and cause them to clump together (known as agglutination; Figure 2). Agglutination of RBCs obstruct blood vessels and triggers oxygen and nutrient deprivation of tissues in the body.[5] To prevent agglutination, three standard pre-transfusion tests are required: blood type identification, antibody screening to detect any unexpected RBC alloantibodies in plasma, and the determination of compatibility between donor and recipient RBCs by RBC crossmatching. The presence of hemagglutination is considered a positive test result, regardless of the method or technology used.[6]

Figure 1: Factors influencing blood group alloimmunization



RBC, red blood cell
Adapted from: Tormey CA & Hendrickson JE. Blood 2019;133:1821–1830

Figure 2: Agglutination reaction[1]



Transfusion strategies and thresholds are poorly described in the clinical setting, and large-scale, well-designed prospective studies are warranted to provide recommendations and minimize risks associated with transfusions.[7]

Overview of Coombs testing

Coombs testing, also known as antiglobulin testing, is a laboratory method used in immunohematology. It is used to detect antibodies that interact with RBCs in the blood.[8]

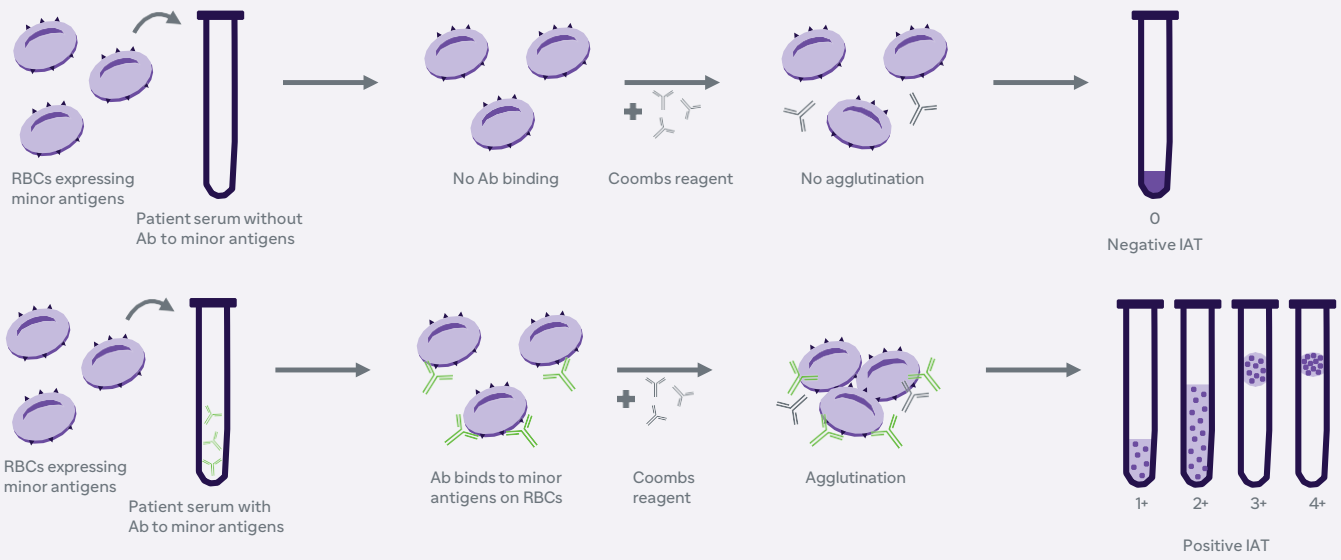
Mechanism of Coombs testing

There are two types of Coombs test: direct and indirect. Direct antiglobulin testing (also known as DAT) is used to detect the presence of antibodies or complement factors adhered directly to RBCs. This multi-step test begins with the collection of an ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood sample, followed by washing the blood sample with a saline solution to isolate RBCs. The sample is incubated and centrifuged with the Coombs reagent containing monospecific or polyspecific anti-IgG, anti-IgM and / or anti-C3 antibodies that will bind to the antibodies adhered to RBCs. The RBCs are examined for agglutination; a positive DAT result may be followed with further monospecific testing.

DAT is generally used in a clinical setting for the diagnosis of autoimmune hemolytic anemia, hemolytic transfusion reactions due to alloantibodies and hemolytic disease of the fetus and newborn.[8]

By contrast, indirect antiglobulin testing (IAT) detects unbound antibodies. For this form of testing, native RBCs from the patient's serum are removed, leaving behind the unbound antibodies, then foreign RBCs with known antigens are added to the isolated serum. The Coombs reagent is added to the antigen-antibody complex. Similar to DAT, RBC agglutination indicates a positive result (Figure 3). IAT is mainly used to test blood for compatibility screening in preparation for an RBC transfusion to ensure the blood donor's antibodies match the recipient's antibodies. IAT is also commonly performed in prenatal testing to screen for IgG antibodies that may cross the placenta and cause hemolytic disease of the newborn.[8]

Figure 3: Interpretation of an indirect Coombs test



Ab, antibody; IAT, indirect antiglobulin testing; RBC, red blood cell
Adapted from: Song Z, et al. American Association for Cancer Research 2021; Presentation 1888

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Anti-CD38 interference

CD38 is a transmembrane multifunctional glycoprotein abundantly expressed on MM cells. This high expression profile in MM cells led to the development of anti-CD38 monoclonal antibodies (mAbs) that have been proven to show potent activity against myeloma cells.[9,10]

Clinical impact of anti-CD38 interference in Coombs testing

CD38 is also expressed at low levels on RBCs. Due to this expression, anti-CD38 mAbs may interfere with indirect Coombs tests by binding to endogenous CD38 molecules on RBCs instead of on MM cells, causing a false positive IAT.[1] In the treatment setting of MM, many patients require RBC transfusions due to anemia developed during disease progression or ongoing disease therapy. False positive results of IATs may lead to delays in issuing RBC units to patients and incompatible crossmatches between donor and recipient prior to blood transfusions. Incompatibility in blood transfusions can be unsafe and dangerous, potentially resulting in an acute or delayed hemolytic transfusion reaction.[9] Laboratory studies have shown that there are differences in the strength of RBC agglutination caused by distinct anti-CD38 mAbs, with some causing a higher degree of interference than others.[11]

Although the rates of anti-CD38 interference with IATs differ across therapeutic interventions due to different epitope binding or the requirement of a cofactor, they remain high across drugs targeting CD38. This suggests that this interference is not drug-specific but connected to the anti-CD38-targeting characteristics of the drugs.[1,12]

In contrast, DATs are usually negative in patients treated with anti-CD38 mAbs due to the downregulation of CD38 on RBCs following the administration of anti-CD38 mAbs, meaning fewer antigen-antibody complexes are present on RBCs. It was shown that few patients develop a hemolytic reaction following treatment with anti-CD38 mAbs, further suggesting a low expression of CD38 on RBC surfaces.[9]

Alternative methods to eliminate anti-CD38 interference

Overcoming anti-CD38 interference is of vital importance to eliminate incompatible crossmatches and unnecessary risks related to blood transfusions. A variety of alternative methods have been established to neutralize this interference (Table 1); however, each approach should be chosen on a case-by-case basis, as there is no universal solution that is applicable to all scenarios. The mechanism, applicability, cost, and supporting clinical data should all be taken into consideration when selecting an alternative approach to combat anti-CD38 interference.[1,9,13]

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Table 1: Alternative approaches for overcoming anti-CD38 interference with IAT

Method	Mechanism	Advantages	Disadvantages
DTT	Denatures CD38 antigen on RBCs	Cheap, easy to apply, reliable	Denatures Kell antigen, must give K-negative RBCs, destroys other clinically significant minor antigens
Trypsin	Cleaves CD38 antigen on RBCs	Cheap, easy to apply	Denatures several significant antigens, not validated, less reliable than DTT
Papain	Cleaves CD38 antigen on RBCs	Cheap, easy to apply, reliable	Destroys many significant antigens, can only be used as a complementary method to other approaches
RBC phenotype	Antigen profiling of patient RBCs	Only needs to be performed once, reliable, does not require future IAT testing if matched units available	Cannot be done if already started anti-CD38 therapy or blood transfusion within 3 months, requires extended match to ensure no antibodies or future alloantibody formation
RBC genotype	Antigen profiling of patient RBCs	Only needs to be performed once and can be performed at any time, reliable, does not require future IAT testing if matched units available	Expensive, requires extended match to ensure no antibodies or future alloantibody formation
Anti-idiotype antibody	Neutralizes anti-CD38 antibody prior to	Simple and would allow for normal blood bank testing once anti-CD38 mAb removed	Expensive, not typically available in blood bank inventory, would require different reagent for each anti-CD38 mAb
Soluble CD38	Neutralizes anti-CD38 antibody prior to IAT	Simple and would allow for normal blood bank testing once anti-CD38 mAb removed, applicable to any anti-CD38 mAb, commercially available	Denatures several significant antigens, not validated, less reliable than DTT
F('ab') fragments	Fragments preferentially bind CD38 and do not cause IAT positivity	Simple and would allow for routine blood bank testing after application	Not validated, not commercially available
Cord blood	Reagent cells lack CD38 antige	Easy to perform, no additional steps required	Not commercially available, cord blood cell antigen expression differs from reagent RBC, therefore, would need to be typed prior to use

DTT, dithiothreitol; IAT, indirect antiglobulin test; mAb, monoclonal antibody; RBC, red blood cell
Adapted from: Lancman G, et al. Front Immunol. 2018;9:2616

The most common and effective method currently available to eliminate anti-CD38 interference is treatment with dithiothreitol (DTT), a thiol-reducing agent. DTT disrupts anti-CD38 mAb binding to RBCs by denaturing the CD38 antigen. The efficacy of DTT has been proven by an international Biomedical Excellence for Safer Transfusion consortium study that demonstrated a lack of anti-CD38 mAb binding on RBCs in all patients treated with DTT. One drawback of DTT treatment is that it also denatures other RBC antigens along with CD38, such as Kell-antigens, that may lead to a hemolytic transfusion reaction, therefore transfusion of Kell-negative units is required, unless the patient is known to be Kell-positive.[1,9,13]

Other commonly used methods include proteolytic enzymes, such as trypsin or papain, that do not degrade Kell antigens like DTT does, although these methods denature a number of other antigens that can interfere with pre-transfusion testing. However, trypsin and papain have not been investigated to the same extent as DTT. Phenotyping and genotyping of patient's RBCs prior to treatment with anti-CD38 mAbs are likely the most effective and safe methods to provide compatible blood transfusions; however, it is one of the most expensive approaches as well. Information collected from phenotyping and genotyping can be stored for future transfusions to avoid incompatibility.[1,9]

Another approach is to neutralize the effects of anti-CD38 mAbs prior to conducting an IAT with an anti-idiotype antibody; however, this method is currently not widely available. Soluble CD38 antigens work through the same principle as anti-idiotype antibodies, but it is costly and large quantities would be required to negate the effects of anti-CD38 mAbs in the serum. Finally, F('ab') fragments act by preferentially binding CD38, thereby preventing anti-CD38 mAb binding, and avoiding a false positive IAT result. The use of F('ab') fragments requires further investigation and is currently not commercially available.[1,9,13]

Clinical management of anti-CD38 interference in pre-transfusion testing

As treatment with anti-CD38 mAbs is becoming increasingly popular in the MM setting, it is crucial to acknowledge potential interference in common laboratory tests. Any mAb that targets antigens present on RBC has the potential to interfere with pre-transfusion Coombs tests; therefore, it is vital to have uniform procedures in place to bypass this interference and ensure blood compatibility pre-transfusion.[13,14] Specific recommendations for physicians and blood banks have been provided by the American Association of Blood Banks (AABB; Figure 4) that comprise an extensive baseline screen prior to treatment to identify if there are any alloantibodies present, as well as phenotyping or genotyping, if resources are available. Baseline testing prior to anti-CD38 treatment should be conducted to identify any alloantibodies and the compatibility information for the recipient. This information should be made readily available for other healthcare professionals in the event of travel or an emergency requiring care away from a cancer center. If the patient is already receiving anti-CD38 therapy prior to transfusion, the clinic should be immediately notified that the patient is receiving anti-CD38 treatment. DTT treatment may be considered, if available, to eliminate IAT interference, and Kell-negative blood transfusion should be provided.

However, clinicians should remain cautious as there is a small risk of transfusion reaction due to the non-specific elimination of other antigens by the DTT treatment. Crossmatch may be considered with ABO / rhesus (RhD)-compatible, K-matched units for patients with a negative antibody screen (as per standard requirements).

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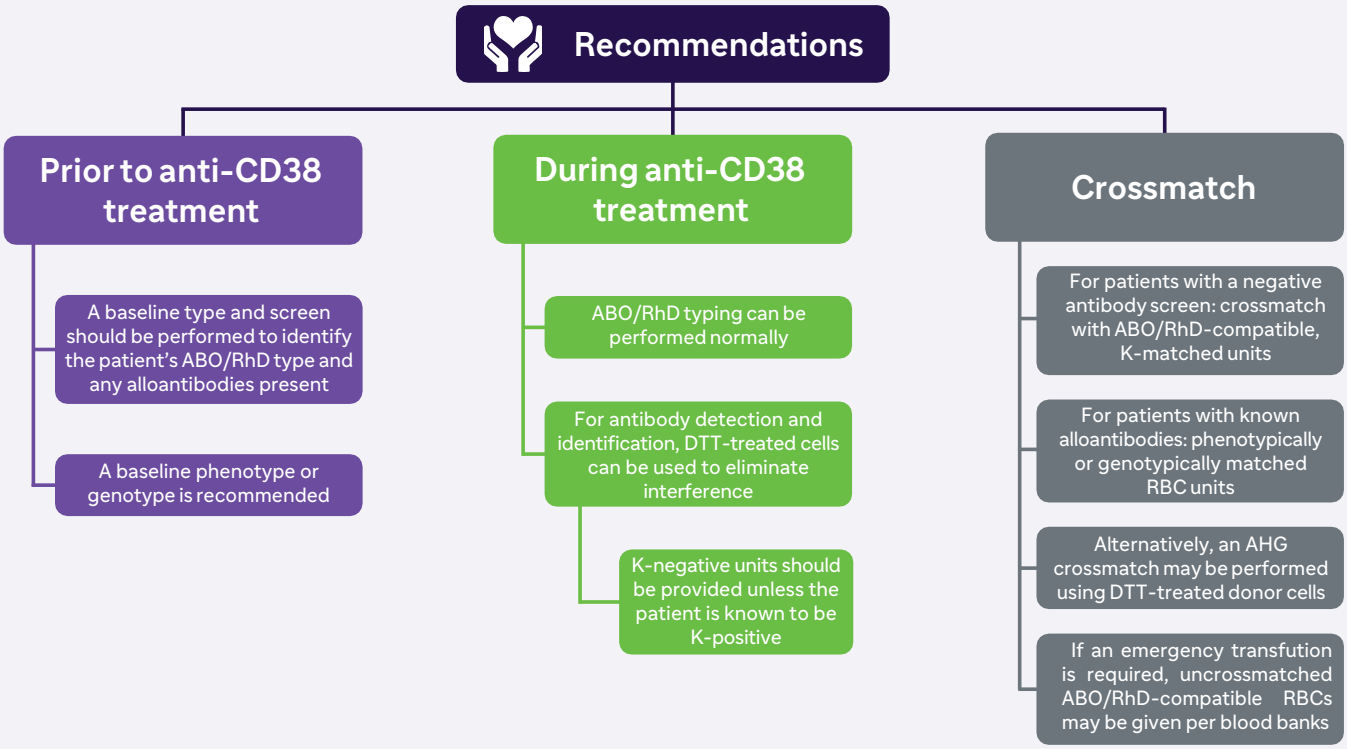
An anti-human globulin (AHG) crossmatch may be carried out as an alternative approach using DTT-treated donor cells. If an emergency transfusion is needed, un-crossmatched ABO/RhD-compatible RBCs may be given per blood bank practices. It should also be noted that positive IAT results can persist for up to 6 months following discontinuation of anti-CD38 treatment.[1,14]

Figure 4: Recommendations for pre-transfusion testing



AHG, anti-human globulin; DTT, dithiothreitol; K, Kell; RBC, red blood cell; RhD, rhesus
Adapted from: Mitigating the anti-CD38 interference with serologic testing. 2016 http://www.aabb.org/programs/publications/bulletins/Documents/ab16-02.pdf

Summary

The Coombs test, also known as antiglobulin test, is used to detect antibodies on red blood cells. The direct Coombs test (DAT) detects antibodies directly attached to red blood cells, while the indirect Coombs test (IAT) is used for the identification of circulating antibodies in the serum. DAT is valuable in the diagnosis of autoimmune hemolytic anemia and hemolytic infusion reactions due to alloantibodies, while IAT is mostly used for compatibility screening ahead of blood transfusions, and prenatal testing. In recent years, anti-CD38 monoclonal antibodies have been approved by the FDA as efficacious for the treatment of MM; however, it has been recognized that the drug class interferes with Coombs tests by binding to CD38 on red blood cells and causing false positive IAT results.

False positive results of IATs may lead to incompatible crossmatches between donor and recipient prior to blood transfusions. A number of alternative approaches have been established to overcome anti-CD38 interference. The most prevalent method currently available is treatment with DTT. Other methods include proteolytic enzymes, genotyping and phenotyping, anti-idiotype antibody, soluble CD38 antibody, and F('ab') fragments. In order to further eliminate anti-CD38 interference and facilitate safer blood transfusions, the AABB has provided specific recommendations for blood banks and physicians.

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Abbreviations:

AABB, American Association of Blood Banks; AHG, anti-human globulin; DAT, direct antiglobulin testing; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; IAT, indirect antiglobulin testing; mAb, monoclonal antibody; MM, multiple myeloma; RBC, red blood cell; RhD, rhesus

References:

1. Lancman G, et al. Front Immunol 2018;9:2616
2. Vincent JL, et al. JAMA 2002;288:1499–1507
3. St. Lezin E, et al. Transfusion 2019; 59:1934–43
4. Tormey CA, et al. Blood 2019;133:1821–30
5. Dean L, et al. Bethesda (MD): National Center for Biotechnology Information (US); Chapter 3, Blood transfusions and the immune system 2005 https://www.ncbi.nlm.nih.gov/books/NBK2265
6. Alquist C, Harm S. Transfusion-service-related activities: Pretransfusion testing and storage, monitoring, processing, distribution, and inventory management of blood components. In: Cohn CSD, M; Johnson Katz LM, ed. AABB Technical manual, 20th edition. Digital 2020:816
7. Goubran HA, et al. Cancer Growth Metastasis 2016;9:1–8
8. Theis SR, et al. 2021 https://www.ncbi.nlm.nih.gov/books/NBK547707
9. Song J, et al. J Clin Lab Anal 2021;35:e23832
10. van de Donk NWCJ, et al. Immunol Rev 2016;270:95–112
11. Chami B, et al. Transfusion 2022 [Accepted]
12. Song Z, et al. AACR 2021; Presentation 1888
13. Chari A, et al. Clin Lymphoma Myeloma Leuk 2018;18:44–51
14. Mitigating the anti-CD38 interference with serologic testing 2016 http://www.aabb.org/programs/publications/bulletins/Documents/ab16-02.pdf