CME/SAM

Monoclonal anti-CD47 interference in red cell and platelet testing

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BACKGROUND: Anti-CD47 (Hu5F9-G4) is a human monoclonal immunoglobulin G (IgG)4 antibody that is in clinical trials to treat hematologic or solid malignancies. CD47, a glycoprotein expressed on all cells, binds to signal-regulatory protein α on macrophages and regulates phagocytosis. Blocking CD47 is thought to enhance phagocytosis and promote antitumor responses. Here, we evaluate drug interference in pretransfusion testing, determine mitigation strategies, and compare interference with anti-CD38 (Daratumumab).

STUDY DESIGN AND METHODS: Samples from four patients were tested by standard methods. Anti-IgG (Immucor monoclonal Gamma-clone and Ortho BioClone) were used, and dithiothreitol and enzyme-treated RBCs were tested. Allo-adsorption was performed with papain treated RBCs, pooled platelets, or with commercial human platelet concentrate. Platelet antibody testing was performed according to manufacturer's instructions.

RESULTS: All plasma samples reacted 3+ to 4+ in all phases with all red blood cells (RBCs) by all methods including immediate spin. Stronger reactivity was observed with D– RBCs with titers as high as 16,384 at indirect antiglobulin testing. Reactivity at indirect antiglobulin testing using Gamma-clone anti-IgG (which does not detect IgG4) was only weakly positive and confirmed to be carryover agglutination. Plasma reacted with dithiothreitol, trypsin, papain, α-chymotrypsin, or warm autoantibody removal medium (W.A.R.M., Immucor) treated RBCs. Direct antiglobulin testing and autocontrol were negative or weak with 3+ reactive eluates. Reactivity was removed by multiple alloadsorptions with papain-treated cells or pooled platelets. Polyethylene glycol adsorption was invalid due to precipitation of antibody.

CONCLUSION: Anti-CD47 (Hu5F9-G4) interferes with all phases of pretransfusion testing, including ABO reverse typing. To remove interference requires multiple RBC alloadsorptions and/or the use of monoclonal Gamma-clone anti-IgG in the indirect antiglobulin testing.

The use of monoclonal antibodies directed to molecules expressed on tumor cells is an active area of drug development with the goal of delivering targeted treatment for hematologic malignancies and solid tumors.[1] When the target of the monoclonal antibody is also expressed on red blood cells (RBCs) and/or platelets, interference with pretransfusion testing can result. Interference has been well described in samples from patients receiving monoclonal anti-CD38 (daratumumab, DARA)[2,3] approved in 2015, for treatment of relapsed multiple myeloma which targets the high expression of CD38 on plasma cells.

More recently, CD47 has gained attention as a target for treatment of hematologic malignancies and solid tumors and several therapeutic anti-CD47 monoclonal and CD47-blocking fusion proteins have entered clinical trials.[4–6] CD47 is a cell surface glycoprotein expressed on all cell types, including RBCs and platelets.[7,8] CD47 is an attractive target for therapy because it is involved in regulation of cell survival and cell death by acting as a ligand for signal-regulatory protein alpha (SIRPα) expressed on macrophages.[9] Observations in mice revealed that upon binding CD47, the macrophage receives a "do not eat me" signal, preventing phagocytosis of CD47-expressing

ABBREVIATIONS: DAT = direct antiglobulin testing; DTT = dithiothreitol; EGA = ethylenediaminetetraacetate glycine acid; EDTA = ethylene diamine tetraacetate; HPC = human platelet concentrate; IAT = indirect antiglobulin testing; IS = immediate spin; PEG = polyethylene glycol; SIRPα = signal-regulatory protein alpha

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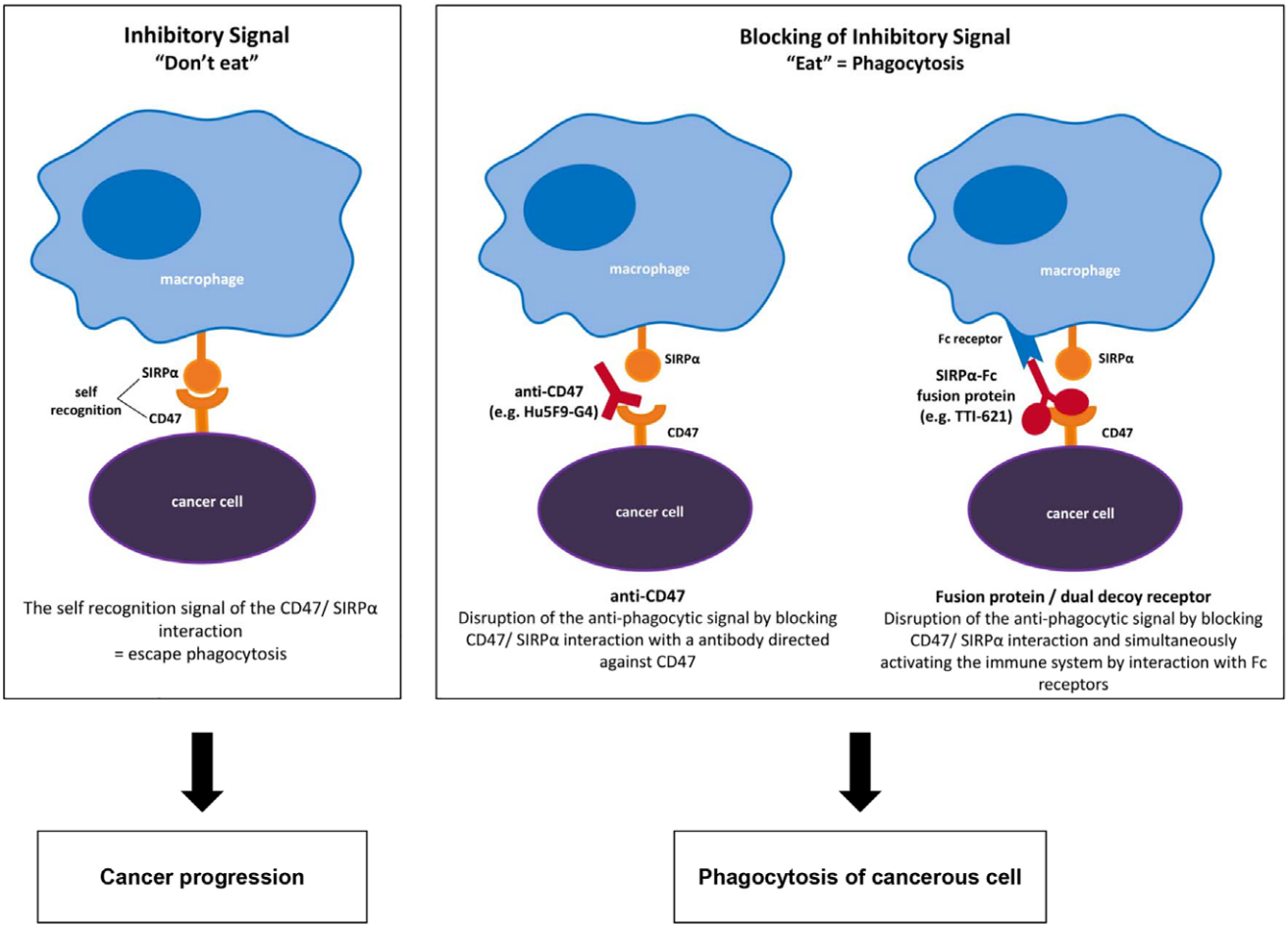

Fig. 1. Proposed mechanism of action of CD47-targeted anti-tumor therapy. Left panel. CD47 binding to SIRPα on the macrophage sends an inhibitory signal preventing phagocytosis and removal of the tumor cell. Right panel. Illustrates the difference between the mechanism of action of anti-CD47 directly targeting the CD47 protein (shown on the left), and the mechanism of action of CD47 antagonist represented by TTI-621 (shown on the right).

cells. CD47 decreases with cell age, and the decrease in expression, accompanied by a change in conformation, is thought to be a mechanism for recognition and clearance of aging RBCs.[7,9] On cancer cells, several studies indicated that expression of CD47 is sustained or even increased, suggesting one mechanism by which tumor cells may escape clearance.[10,11] Elevated expression levels of CD47 have been shown to correlate with poor clinical outcome in various cancer types.[10,12] As a mechanism of action, binding of anti-CD47 to tumor cells would mask CD47 and disrupt the CD47-SIRPα "do not eat me" signal, resulting in tumor cell phagocytosis,[4–6,13,14] and is illustrated in Fig. 1.

Of relevance for pretransfusion testing, CD47 is highly expressed on RBCs as a member of the Rh complex in the membrane (Fig. 2). Expression levels of CD47 differ according to the Rh phenotype[15] and correlate with levels of RhCE protein. D– (ce/ce) RBCs have the highest expression of CD47 compared to D+ (DcE/DcE), rare cells that lack RhCE (D – –) have significantly reduced CD47 (by approximately 75%),[16] and Rh_{null} have nearly undetectable CD47.[17] In preclinical studies, transient hemolytic anemia was associated with anti-CD47 therapy due to elevated RBC clearance[6] and the potential for possible drug interference in pretransfusion compatibility testing was suggested.[5]

Here, we report characteristics of drug interference in pretransfusion RBC and platelet compatibility testing in samples from patients treated with anti-CD47 (Hu5F9-G4), discuss approaches to mitigate interference, compare and contrast to interference with anti-CD38, and discuss other CD38- and CD47-targeting drugs in clinical trials.

MATERIALS AND METHODS

Patient samples

Samples were obtained from four patients enrolled in two local hospital phase I escalating-dose trials of Hu5F9-G4, a

humanized immunoglobulin G (IgG)4 monoclonal anti-CD47. Patients received Hu5F9-G4 (1 mg/kg) with escalated dosing to 20 mg/kg per week IV infusion over the course of 4 to 5 weeks. Pretreatment EDTA samples were tested by

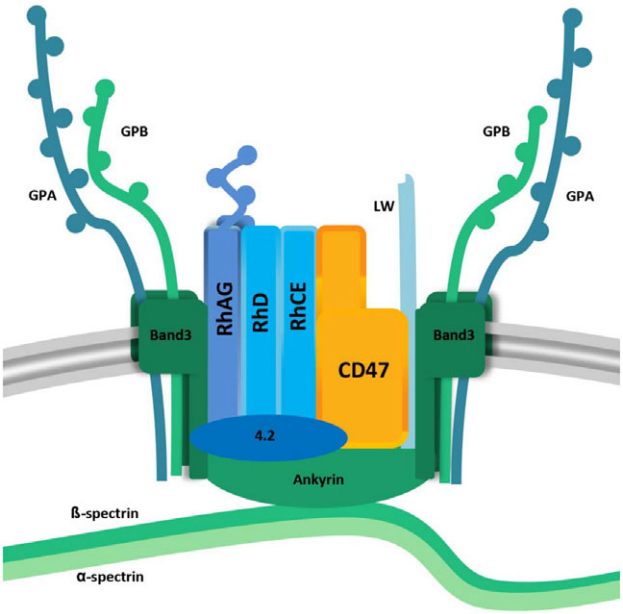

Fig. 2. CD47 is a member of the Rh complex in the RBC membrane. The diagram shows CD47 associates with RhCE/D and Rh-associated glycoprotein (RhAG), which associates with the Band 3 complex. Known points of interaction are between protein 4.2 and CD47 and between RhAG and band 3 with attachment to the spectrin skeleton via ankyrin. Other members of the complex include glycophorin A (GPA), glycophorin B (GPB), and LW.

the hospital and multiple samples from each patient were referred to the reference laboratory after interference was observed in routine pretransfusion testing. The reference laboratory was not part of the clinical trial, and samples were referred by the hospital blood bank when attempting to determine RBC compatibility for transfusion orders, as per standard protocol.

Testing

ABO typing, direct antiglobulin testing (DAT), antigen typing, and serologic detection of antibodies to RBCs were performed by standard tube methods.[18] Plasma samples were tested at immediate spin (IS), 37 °C when appropriate, and by indirect antiglobulin testing (IAT) using 22% albumin, low-ionic-strength solution (LISS) and polyethylene glycol (PEG). Gel card testing (ID-MTS, Ortho Clinical Diagnostics) was also performed.

Platelet antibody testing was performed according to manufacturers' instructions using solid-phase adherence with an IgG platelet antibody detection and crossmatch system (Capture-P, Immucor), which utilizes fresh platelets bound to microplate wells from single group O donors, and an antibody screening system (Capture-P Ready-Screen, Immucor), which utilizes dried immobilized platelet membranes of 13 group O donors phenotyped for selected HLA-A, HLA-B, and HPA antigens. A qualitative solid-phase enzyme linked immunosorbent assay (PakPlus, Immucor), which utilizes monoclonal captured platelet glycoproteins IIb/IIIa and Ia/IIa and affinity purified HLA class I and platelet glycoprotein Ib/IX and IV, was also tested according to the manufacturer's instructions.

Test RBCs were pretreated with papain, trypsin, α-chymotrypsin, 0.2 M dithiothreitol (DTT), or warm autoantibody removal medium reagent (W.A.R.M., Immucor). Commercial ficin-treated panel cells (Immucor), cord RBCs, and rare phenotype RBCs were also tested. RBCs were treated with ethylenediaminetetraacetate glycine acid (EGA) (Gamma EGA Kit, Immucor) to remove coating IgG according to manufacturer instructions. Anti-IgG monoclonal Gamma-clone (Immucor), which lacks reactivity with human subclass IgG4, and Ortho anti-IgG, which detects all human IgG subclasses, were used. For titration studies, serial dilutions of patient plasma were made in phosphate-buffered saline at pH 7.0–7.2. The plasma dilutions were tested in saline at IS and in the IAT. Eluates were made using Gamma ELU-KIT II (Immucor). Additional washes were sometimes required to remove unbound plasma antibody prior to performing elution from the patients' RBCs to avoid positive reactivity in the last wash control.

Adsorptions

Plasma samples were adsorbed with allogeneic R_1R_1(DCe/DCe) R_2R_2 (DcE/DcE) and rr (ce/ce) RBCs that had been papain treated to maximize antibody uptake, or with pooled single-donor outdated apheresis platelets, or with commercial human platelet concentrate (HPC) (Immucor). For allogeneic adsorptions, equivalent volumes of RBCs or platelets and patient plasma were incubated for 15 minutes at 37 °C and multiple (3×–4×) adsorptions were performed by transferring the original plasma aliquot to a fresh aliquot of RBCs, pooled platelets or commercial HPC product. PEG allogeneic RBC adsorption was performed as described.[18] Briefly, plasma was adsorbed once at 37 °C for 15 minutes with rr (ce/ce) RBCs in the presence of an equal volume of PEG to plasma. The plasma/PEG mixture was harvested after centrifugation for 5 minutes and used for testing. Autoadsorptions were not attempted, as three of the four patients had been recently transfused and the RBC volume for the remaining was insufficient.

RESULTS

Nearly all patients receiving Hu5F9-G4 therapy show an initial drop in hemoglobin, as previously observed in preclinical studies in primates,[6] with thrombocytopenia occurring infrequently.[19] Hemoglobin values and platelet counts

began to resolve after approximately 21 days. These observations are the basis for using an initial low priming dose followed by higher maintenance doses to allow time for compensated reticulocytosis.[6]

Plasma testing

Patient samples submitted for ABO, Rh, antibody screen, and extended typing/genotyping prior to start of therapy (as suggested by the drug manufacturer) were negative for atypical antibodies. All plasma samples taken over the course of treatment reacted 3+ to 4+ in all phases including saline IS, room temperature, 37 °C, and IAT with or without enhancement (LISS, albumin, PEG) using Ortho anti-IgG (Table 1). Cord RBCs were also reactive in all phases. Reactivity with D– rr (ce/ce) RBCs was approximately one grade stronger than with D+ R_2R_2 (DcE/DcE). RBCs with rare D– –, Rh_{mod}, or Rh_{null} phenotypes, which are known to have significantly reduced CD47 expression, were nonreactive with the patient plasma in saline IS, room temperature, and 37 °C, but 2+ to 3+ in IAT. Reactivity was obtained with A and/or B cells in ABO reverse typing, causing ABO typing discrepancies to be observed in non–group O patients. Serial dilutions of patient plasma tested against R_2R_2 cells indicated the Hu5F9-G4 anti-CD47 plasma titer ranged from 4096 up to 16,384 in the IAT (with Ortho anti-IgG) in the samples tested. In contrast, weak microscopic to $1+^S$ reactivity in IAT was observed using monoclonal Gamma-clone anti-IgG, which does not detect human subclass IgG4, and this reactivity was shown to be carryover agglutination using phosphate-buffered saline in control tests (summarized in Table 1).

RBC testing

DATs performed on patient RBCs over the course of treatment were negative or weakly positive (microscopic IgG), due to blocking or steric hindrance, regardless of the anti-IgG reagent used. However, eluates were strongly reactive (3+ to 4+) in IAT with all cells tested using Ortho anti-IgG. Consistent with plasma IAT testing and the lack of detection of IgG4, eluates were nonreactive (or showed weak false positive reactivity due to carryover agglutination) using Immucor Gamma-clone anti-IgG.

Most patients had at least one RBC sample tested over the course of treatment that demonstrated weak spontaneous agglutination causing weak false-positive reactivity in ABO forward typing and in Rh (DCEce) antigen typing. Attempts to avoid spontaneous agglutination using warm washing or EGA treatment of the patients' RBCs was not successful. Interestingly, EGA treatment also did not remove sufficient antibody from the patients' RBCs to overcome antibody blocking and/or steric interference by anti-CD47 responsible for the negative or weak positive DAT in the face of the strongly positive eluate.

TABLE 1. Reactivity of plasma samples (n = 4) from patients receiving anti-CD47 (Hu5F9-G4)

Test RBCs	IS	IAT* Ortho anti-IgG	IAT Carryover from IS Gamma-clone anti-IgG
D– rr (ce/ce)	4+	4+	mi-1+
D+ R_2R_2 (DcE/DcE)	3+	4+	mi-1+
D– –	0	4+	0/0
Rh_{mod}	0	3+	0/0
Rh_{null}	0	2+	0/0
D+ cord	3+	2+	mi-1+
Reverse (A or B)	3+		
Auto/DAT	0	0/mi	0/mi
Eluate	NT	4+	mi-1+

* Albumin, LISS, or PEG.
DAT = direct antiglobulin testing; IAT = indirect antiglobulin testing; IS = immediate spin; mi = microscopic.

Mitigation of interference

Plasma samples were tested against RBCs pretreated with ficin, papain, trypsin, α-chymotrypsin, 0.2M DTT or W.A.R.M. reagent. The plasma continued to demonstrate 3 + to 4+ reactivity in all phases of testing with the treated RBCs. In an attempt to remove the anti-CD47 interference from the patients' plasma, adsorption studies were carried out with 1) papain-treated allogeneic RBCs, 2) pooled single-donor apheresis platelets, or 3) commercial HPC product. Patient plasma samples were adsorbed four times with papain-treated R_1R_1, R_2R_2 or rr RBC samples. The majority of the plasma samples adsorbed with papain-treated RBCs were nonreactive in saline at IS and in PEG IAT using both Immucor Gamma-clone and Ortho anti-IgG. Occasional weak microscopic reactivity remained in the IAT in some samples, which presumably was due to very high drug titer.

Plasma adsorbed four times with pooled single-donor platelets no longer showed reactivity at IS and in IAT with Immucor Gamma-clone, but reactivity remained in IAT with Ortho anti-IgG. Plasma samples that were adsorbed three times with Immucor HPC showed reduced reactivity, but moderate 2+ reactivity remained at IS and IAT using Ortho anti-IgG and in the IgG gel test.

In communications to study sites, the drug manufacturer suggests that PEG adsorption and/or a PEG crossmatch might mitigate interference. We attempted to remove anti-CD47 reactivity using PEG adsorption. Four or six drops of the PEG adsorbed plasma tested against rr RBCs were nonreactive in the IAT. However, the dilution control consisting of PEG and plasma incubated together at 37 °C in the absence of RBCs was also nonreactive, consistent with precipitation of antibody and invalidating the results.

TABLE 2. Reactivity of plasma samples from patients receiving anti-CD47 (Hu5F9-G4) or anti-CD38 (DARA) in platelet antibody testing

Sample	Sex	Monoclonal antibody therapy	Capture-P (% positive wells of 8)	Capture-P ready-screen (% positive wells of 13)	PakPlus
1	Male	Hu5F9-G4	Pos (100%)	Pos (100%)	Neg
2	Female	Hu5F9-G4	Pos (100%)	Pos (100%)	Neg
3	Male	DARA	Neg	Neg	Neg
4	Male	DARA	Pos (75%)	Pos (8%)	GP Ib/IX, IV HLA
5	Male	DARA	Pos (100%)	Pos (30%)	Neg
6	Male	DARA	Pos (100%)	Pos (30%)	Neg
7	Male	DARA	Pos (100%)	Neg	GP IIb/IIIa
8	Male	DARA	Pos (100%)	Pos (23%)	Neg

Platelet antibody testing

As CD47 is highly expressed on platelets, samples available from two patients receiving Hu5F9-G4 were tested to detect potential interference in platelet antibody screening (Table 2). Pretherapy samples were not available for testing, but neither had previously received platelet transfusions. Sera from both patients gave positive results with both Capture-P methods, but were negative by PakPlus assay. We also tested samples from patients receiving daratumumab (DARA) anti-CD38 (n = 6). Five of six samples had positive screens by Capture-P, and four were positive by Capture-P Ready-Screen. Two tested positive for antibody to GPIb/IX,-IV and HLA, or GPIIb/IIIa alone by PakPlus.

DISCUSSION

Therapy with Hu5F9-G4, an IgG4 monoclonal antibody targeting CD47, is associated with plasma panreactivity in all phases of pretransfusion antibody screening and crossmatch, including ABO reverse typing. The reactivity with test RBCs in IS is reminiscent of an IgM antibody, although the drug is IgG, and this observation is due to the high level of CD47 on RBCs. Spontaneous agglutination of the patient's RBCs may also be seen in some samples. Plasma interference and strong panreactive eluates are observed as soon as 1 hour after infusion.

CD47 is not cleaved from test RBCs by treatment with papain, ficin, trypsin, α-chymotrypsin, 0.2M DTT or W.A.R.M. reagent, and therefore interference cannot be mitigated in this way. Importantly, Immucor monoclonal Gamma-clone anti-IgG, which does not detect IgG4 subclass antibodies, avoids interference in the IAT, although weak reactivity may be observed due to carryover agglutination. Multiple alloadsorptions of the plasma with papain-treated allogeneic RBCs or with pooled platelets can remove the reactivity to allow valid reverse ABO typing and antibody screening and crossmatch. Efficacy and number of adsorptions required will depend on the circulating drug plasma concentration. Pooled outdated platelets are an attractive option for adsorption to avoid removal of underlying RBC antibodies, but were less efficient than allogeneic RBCs for drug removal in our experience. Adsorptions performed with commercial HPC only moderately reduced Hu5F9-G4 plasma reactivity and was not an efficient approach to mitigate interference. Although adsorptions in the presence of PEG may appear to remove reactivity, we found that PEG adsorptions can cause precipitation of plasma antibody and false-negative test results, as has been reported previously.[20,21] In addition to precipitation of the anti-CD47 drug, underlying clinically significant blood group alloantibodies can also be precipitated and therefore not be detected. Autologous adsorption using W.A.R.M. reagent was not attempted, as either patients had been recently transfused or the volume of autologous RBCs was insufficient. Multiple autologous adsorptions could be attempted but would be anticipated to be challenging, as EGA treatment did not remove sufficient antibody from the patients' RBCs to overcome blocking in the DAT. In summary, our preferred approach to remove Hu5F9-G4 interference in all phases of testing is to perform multiple alloadsorptions of the plasma. Use of monoclonal Gamma-clone anti-IgG avoids interference in the IAT.

In RBC testing, bound anti-CD47 drug on the patient's RBCs causes blocking or steric interference or hindrance in the auto control and DAT, resulting in a negative or weak positive result in the face of a strong panreactive eluate. Similar to interference in RBC antibody detection, anti-CD47 therapy can interfere with the detection of platelet specific and HLA Class I antibodies and platelet crossmatching due to reactivity with CD47 expressed on intact platelets or platelet membranes. In contrast, PakPlus, which consists of monoclonal captured or affinity purified glycoprotein molecules rather than intact platelet membranes, avoids anti-CD47 interference.

Comparison of anti-CD38 and anti-CD47 interference

Table 3 shows a comparison between RBC expression of CD38 and CD47, and the characteristics of interference observed with anti-CD38 (daratumumab/DARA and isatuximab) or anti-CD47 (Hu5F9-G4) therapy. The low level of CD38 on RBCs is in direct contrast to the high level expression of CD47, which varies with Rh phenotype.[15] CD38 levels on RBCs do not differ among individuals, with the

TABLE 3. Comparison between the RBC expression and the characteristics of pretransfusion interference observed with anti-CD38 (Daratumumab/DARA or Isatuximab) and anti-CD47 (Hu5F9-G4) therapy

Differences in	CD38	CD47
RBC expression	Low	High
Epitope/antigen shedding	Yes	No
Testing	*__Anti-CD38__	†__Anti-CD47__
Subclass	IgG1	IgG4
ABO interference	No	Yes
D and extended antigen typing problems	No	Possible
Antibody screen and crossmatch interference	IAT only (1+)	All phases (3+ to 4+)
Mitigation	Treat test RBCs with 0.2 M DTT or Trypsin	Use Immucor/Gamma monoclonal anti-IgG for IAT
Alloadsorption onto RBCs or platelets	No	Yes – multiple 3× to 4×
DAT/auto control (cause)	Negative or w+ (antigen loss)	Negative or w+ (blocking)
Eluate	Negative or w+	Strongly positive (3+ to 4+)
Platelet Capture-P antibody screen interference	Variable	Yes
Platelet PakPlus antibody detection interference	No	No

* Anti-CD38 (daratumumab, DARA, and isatuximab).
† Anti-CD47 (Hu5F9-G4).
DTT = dithiothreitol; IAT = indirect antiglobulin testing.

exception of RBCs with the dominant In(Lu) phenotype and cord cells, which lack serologically detectable CD38.[23,24] Observations of absence or reduced serologically detectable CD38 on some Fy(a–b–) RBCs from black individuals has also been reported.[25] Following administration of anti-CD38, trogocytosis, that is, antigen shedding of CD38 molecules from the patient's own RBCs, occurs within 6 hours,[26] whereas loss of CD47 is not observed with anti-CD47 therapy. This may be because the majority of CD47 in the RBC membrane is linked to the underlying cytoskeleton.[15]

Anti-CD38 and anti-CD47 differ in IgG subclass (Table 3). Anti-CD38 does not interfere in ABO, Rh, or extended antigen typing, whereas anti-CD47 causes robust interference in reverse ABO typing, which is revealed in non–group O individuals. In addition, spontaneous agglutination of the patient's own RBCs heavily coated with anti-CD47 can occasionally result in false positive ABO, Rh (DCEce) or extended antigen (e.g., MN/FY/JK etc) typing. In the antibody screen and crossmatch, anti-CD38 causes interference in the IAT phase only, showing characteristic 1+ or weaker reactivity. In contrast, anti-CD47 causes interference in all phases of testing including IS, resulting in interference in the computer crossmatch as well, and demonstrating 3 + to 4+ reactivity. This difference in the reactivity of anti-CD38 and anti-CD47 is a reflection of the differences in the level of antigen expression on test RBCs. Treating test RBCs with reducing agents, 0.2M DTT or trypsin, removes CD38 epitopes to mitigate inference, whereas CD47 cannot be removed or denatured by enzymes or reducing agents. However, in contrast to anti-CD38, anti-CD47 can be removed by adsorption onto allogeneic RBCs or platelets, although multiple rounds are required. Interestingly, both drug therapies are associated with false-negative or weak positive DAT or auto controls but due to different mechanisms (e.g. antigen loss vs. blocking). Anti-CD38 therapy is associated with negative or weak positive reactions in an eluate, the result of the length of time on therapy with antigen shedding from the patient cells,[26] whereas robust reactivity is observed in eluates from the RBCs of patients receiving anti-CD47. Finally, both therapies appear to interfere in platelet antibody detection using fresh platelets or platelet membranes but do not interfere when using monoclonal captured platelet glycoproteins.

Monoclonal therapy and pretransfusion testing

Clinical trials testing the efficacy of monoclonal antibody therapy are expanding, and in particular the use of anti-CD38. In addition to the use in multiple myeloma, current clinical trials include use in amyloidosis, chronic lymphocytic leukemia, recurrent acute myeloid leukemia, acute lymphoblastic leukemia, and Hodgkin and non-Hodgkin lymphoma, among others. Additional anti-CD38 drug therapeutics in current phase I or II clinical trials in addition to DARA (Janssen Biotech) include MOR202 (MorphoSys), Isatuximab (Sanofi-Aventis), and TAK-079 (Takeda) for treatment of systemic lupus erythematosus or other autoimmune diseases.[27]

Additional CD47 drug therapies are also in phase I clinical trials and include the CD47 targeting antibodies CC9002 (Celgene), which like Hu5F9 is also an IgG4 antibody and the human monoclonal SRF231 (Surface Oncology).[28–30] CD47 agonists are also in clinical trials. These include TT1-621 (Trillium)[31] and ALX148 (ALX Oncology), which are fusion proteins with the Fc region of IgG1 antibody fused to the CD47-binding domain of SIRPα with the goal of interrupting the CD47-SIRPα survival signal (Fig. 1). These "dual functioning decoy receptors" block the suppressive CD47 signal and at the same time engage Fc receptors activating the innate and adaptive immune system to enhance antigen presentation and T-cell proliferation. Unlike CD47-targeting antibodies, TT1-621 appears to bind only

minimally to human RBCs[5] and interference in pretransfusion testing has not been observed (personal observations) or reported to date.

It seems plausible that monoclonal antibody interference in pretransfusion testing may become more commonplace. Interference will be drug specific for agents targeting CD47, as those in the agonist/antagonist category differ from antibodies directly targeting CD47 due to differences in RBC-binding profiles. The fact that patients have some degree of anemia and thrombocytopenia following therapies directly targeting CD47 increases the possibility that they may require repeat transfusions, further complicating serologic studies. This makes extended phenotyping/genotyping imperative before initiating therapy, with consideration of extended matched RBCs for transfusion, particularly if the number of clinical conditions that benefit expands and includes patients whose underlying disease often requires transfusion support. Communication with the blood bank laboratory, obtaining a pretherapy sample for baseline ABO and antibody screening, and RBC antigen extended phenotype or genotype before the start of therapy is key to transfusion safety.[32] We would suggest that the inability to determine crossmatch compatibility and the labor involved in workup of samples from these patients will drive the need for cost-effective extended antigen-matched units for optimal patient care.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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