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POSITION PAPER

Considerations for pre-transfusion immunohaematology testing in patients receiving the anti-CD38 monoclonal antibody daratumumab for the treatment of multiple myeloma

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Abstract

In recent years, the anti-CD38 monoclonal antibody daratumumab (Darzalex; Janssen-Cilag Pty Ltd) has been shown to be highly efficacious in relapsed and refractory multiple myeloma, with the final results of treatment in newly diagnosed patients awaited. Despite awareness of the potential interference of daratumumab in pre-transfusion immunohaematology testing during phase I and II clinical studies, there was a degree of unpreparedness in the community upon the introduction of this drug into the clinics, particularly the impact that it has on the operational processes in hospital transfusion laboratories and timely issue of red blood cells (RBCs). Anti-CD38 interference in pre-transfusion immunohaematology tests is a particular problem in patients being treated with daratumumab for multiple myeloma as many will require RBC transfusions during their disease treatment. Panagglutination caused by anti-CD38 monoclonal antibody during the indirect antiglobulin test may mask the presence of a clinically significant RBC alloantibody in the patient's plasma during the antibody screen and identification process, which may be overlooked, particularly in urgent situations, subsequently resulting in a delayed or acute haemolytic transfusion reaction. Here, we summarise daratumumab's effects on pre-transfusion immunohaematology testing and its impact on clinical practice and make practical recommendations based on a consensus from medical and scientific transfusion experts and myeloma specialists on behalf of the Australian and New Zealand Society of Blood Transfusion and Myeloma Scientific Advisory Group to Myeloma Australia, respectively.

Introduction

In recent years, the anti-CD38 monoclonal antibody (mAb) daratumumab (Darzalex; Janssen-Cilag Pty Ltd) has been shown to be highly efficacious in relapsed and refractory multiple myeloma (MM). In 2015, daratumumab was granted accelerated approval by the Food and Drug Administration in the United States for the treatment of relapsed/refractory MM, with Australia's Therapeutic Goods Administration (TGA) following suit in 2017. These decisions were based on results only from early phase I/II clinical studies, in which heavily pretreated patients with MM were shown to have an overall

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survival improvement of approximately 11 months from single-agent daratumumab.[1] As a result of this early move into the clinics, there was an underappreciation of the impact of daratumumab's interference with pre-transfusion immunohaematology testing and, therefore, on hospital/pathology transfusion laboratory operational processes, timely issuing of blood, potential blood transfusion reactions and, ultimately, patient safety.

CD38 is an integral transmembrane glycoprotein that is expressed on many cell types and highly expressed on plasma cells. It has diverse functions, including enzyme activity, intracellular calcium regulation and receptor-mediated adhesion.[2] It is also variably expressed on the surface of red blood cells (RBCs). Anti-myeloma activity from daratumumab occurs though anti-CD38-mediated immune mechanisms, including complement-dependent cellular cytotoxicity (CDCC), antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and immunoregulatory depletion of immune suppressive regulatory T cells.[3–5] In addition, direct tumouricidal activity occurs through pro-apoptotic signalling pathways upon cross-linking of surface CD38. As an off-target side-effect, when bound to CD38 on RBC, daratumumab interferes with the indirect antiglobulin tests (IAT), a technique routinely used in pre-transfusion testing. Anti-CD38 interference in immunohaematology tests is a particular problem in patients being treated for MM as many will require blood transfusions as part of their supportive care during ongoing disease treatment. Panagglutination caused by anti-CD38 may mask the presence of a clinically significant RBC antibody (Ab) in the patient's plasma, which may be overlooked, particularly in urgent situations, and subsequently result in an acute or delayed haemolytic transfusion reaction.

Here, we summarise daratumumab's impact on pre-transfusion immunohaematology testing, its impact on clinical practice and provide practical recommendations based on a consensus from medical and scientific transfusion experts and myeloma specialists on behalf of the Australian and New Zealand Society of Blood Transfusion (ANZSBT) and Myeloma Scientific Advisory Group to Myeloma Australia (MSAG), respectively.

The nature of daratumumab's interference with pre-transfusion tests

The binding of daratumumab to CD38 on human RBC is detected using the IAT (or indirect Coomb's test) carried out at 37°C, which is the primary antibody screening method used to detect the presence of clinically significant alloantibodies. Secondary testing methods that may be used in antibody investigations, such as room temperature testing or immediate spin tests to check for ABO compatibility, do not detect the effects of daratumumab. There is some variability of expression of CD38 on RBC, and the presence of daratumumab in the patient's plasma typically causes weak panagglutination in IAT used for pre-transfusion immunohaematology testing. In contrast, daratumumab does not interfere with ABO or RhD typing.[6,7]

In the antibody screen and antibody identification panel, the plasma of patients treated with daratumumab exhibits weak (1+ or 2+, using 0–4 scoring) panagglutination. This panagglutination occurs in all IAT tests, for example, saline, low ionic-strength saline (LISS) and polyethylene glycol (PEG), and all IAT methods, including column agglutination technology (CAT) and tube and solid phase.[6] Positive IAT may persist for up to 6 months after discontinuation of daratumumab therapy.[7–9] The presence of panagglutination must be investigated at each testing episode as the reactivity may mask the presence of a clinically significant alloantibody or the presence of autoimmune haemolytic anaemia.

Interestingly, while daratumumab in the patient's plasma will cause agglutination in IAT with all reagent RBC and donor RBCs, reactivity with the patient's own RBC is not consistent, and the auto-control in the antibody identification panel is frequently negative, as is the direct antiglobulin test (DAT). This suggests that the patient's RBCs with high levels of CD38 may be cleared from the circulation and/or be subject to anti-CD38-mediated antigen downregulation,[10] which may explain why, to date, clinical manifestations of daratumumab-related, immune-mediated haemolysis have not been reported in daratumumab-treated patients. That observation notwithstanding, interference by daratumumab has a serious impact on the ability of transfusion laboratories to perform timely pre-transfusion testing.[11] The resolution of the interference requires time-consuming specialist investigations that inevitably lead to delays in the provision of blood for transfusion, especially if it is not know that the patient is being or has been treated with daratumumab. In addition, clinically significant RBC alloantibodies may be masked and overlooked, potentially resulting in an acute or delayed haemolytic transfusion reaction. For urgent or emergency transfusions, however, it should be possible to determine the patient's ABO and RhD blood group and provide ABO-compatible blood, but provision of this without further investigation is not without risks.[12,13]

Overcoming the interference of anti-CD38 therapy

Several methods have been proposed to overcome anti-CD38 interference in immunohaematology testing and to facilitate alloantibody screening, thus reducing the risk of

incompatible transfusions and the possibility of transfusion reactions. These include testing the patient's plasma against a panel of reagent RBC treated with dithiothreitol (DTT) or trypsin. In addition, extended RBC phenotyping or genotyping of the patient prior to the first dose of daratumumab enables transfusion laboratories to provide RBC with a phenotype that matches the patient's RBC phenotype, with the aim of preventing or at least minimising the risk of incompatibility, particularly when daratumumab interference cannot be immediately resolved and/or the RBC transfusion is urgent.[6,14] Transfusion of phenotype- or genotype-matched RBC will also reduce the risk of sensitisation and future alloantibody formation.

DTT is a thiol-reducing agent that denatures RBC surface CD38 by disrupting the disulfide bonds in the molecule's extracellular domain, therefore preventing anti-CD38 from binding to the RBC.[6] The use of DTT treatment is a recognised immunohaematological method. The test is robust and reproducible[6] but not automated, and it is primarily used by specialist or reference laboratories.

Trypsin is a proteolytic enzyme not routinely used in Australian laboratories and is less efficient than DTT treatment at cleaving cell-surface CD38.[7] Other more commonly used proteolytic enzymes, such as papain, bromelin or ficin, are used in immunohaematology testing as part of antibody identification protocols, to enhance weak antibody activity or aid in the resolution of multiple antibody specificities. These enzymes may also be used as part of the immunohaematology laboratory tool kit for daratumumab interference investigations, but no validation studies of the use of these enzymes in the resolution of daratumumab interference have been published.

It must be noted that DTT and trypsin (along with other proteolytic enzymes) also denature or weaken the reactivity of some RBC antigens (see Box 1), and this should be taken into consideration when assessing results from tests where these agents are used. In particular, DTT is known to denature the Kell system antigens, and therefore, when used to resolve daratumumab interference, patients should be transfused with K-negative RBC unless they have been shown to be K-positive on previous testing.[6] At present, reagent RBC pre-treated with DTT or trypsin are not available from reagent manufacturers. Australian laboratories may not have access to sufficient quantities of reagent RBC to prepare and maintain DTT- or trypsin-treated antibody screening or identification panels cells for regular routine use.

An alternative and the optimal approach to managing the interference of the anti-CD38 antibody would be to neutralise the anti-CD38 antibody in the patient's plasma using soluble CD38 antigen or anti-CD38 idiotype antibody. However, both are expensive and not currently routinely available.

Box 1 Antigens denatured or weakened by treatment with DTT or proteolytic enzymes[15,16]

DTT	Trypsin	Papain/ Bromelin
Kell (K, k, Kp^a, Kp^b, Js^a, Js^b, Ku)	Cartwright (Yt^a)	Duffy
Cartwright (Yt^a)	Indian	MNSs, 'N'
Indian	JMH	Indian
JMH	Ge2, Ge3, Ge4	JMH
Scianna	Dombrock	Bp^a
LW	Bp^a	Ch/Rg
Lutheran	Ch/Rg	Xg^a
MER2	Xg^a	En^aFS En^aTS
Ge3	MN	Ge2, Ge4
Dombrock	En^aTS	Fy^a, Fy^b, Fy6
Some Diego	Lutheran	Yt^a
Cromer	Mer2	
	Knops	

Cord blood cells do not bind anti-CD38 mAb. A suggestion has been made that these cells could be used, but manufacturers of reagent RBC are constrained by limited supply. In a routine transfusion laboratory, other sources of suitable cord blood samples would not typically be available and would require registration as an in-house in vitro diagnostic (IVD). In addition, cord cells have altered expression of some antigens, and this method is unlikely to be routinely offered by hospital or pathology laboratories.[14,17]

Obtaining an extended RBC phenotype for the patient prior to commencement of daratumumab therapy is important in the provision of phenotype-matched RBC for future transfusions. Knowledge of the phenotype means that donor RBCs negative for the common clinically significant RBC antigens that the patient lacks can be selected for transfusion, thereby reducing the possibility of RBC antibody formation.[14] Patient RBC phenotyping should be performed by the transfusion laboratory prior to the patient commencing daratumumab and at least 3 months after any recent blood transfusion (which otherwise may lead to misleading results). The patient sample could be sent for genotyping where samples are unsuitable for phenotyping at any point pre- or post-commencement on daratumumab, but typing prior to treatment is recommended. The results are not received immediately, and this, in addition to antibody investigation confounded by the presence of daratumumab, might add to the delay in provision of safe blood for transfusion. Ideally, this information should be sought prior to commencement

of treatment. As a minimum, the patient should be typed for Rh antigens, K, Jk^a, Jk^b, Fy^a, Fy^b and Ss.[18] To manage workload and preserve reagents used, phenotyping may be performed in regular, for example, weekly batches. Genotyping is currently only offered in Australia by the Australian Red Cross Blood Service in Brisbane. Rapid genotyping testing may be available, but routinely, a 1-week turnaround time should be taken into consideration.

A practical approach for immunohaematology testing of RBCs in myeloma patients receiving treatment with the anti-CD38 mAb, daratumumab, is detailed in the following section. The real-world constraints are discussed, recognising that investigations to resolve anti-CD38 interference are time consuming and labour intensive and may not be available to all laboratories, especially regional or rural laboratories.

Pre-transfusion testing requirements

A: Prior to anti-CD38 therapy

Clear and timely communication between the treating clinician, patient and transfusion laboratory is absolutely vital when anti-CD38 therapy is planned. Patients and healthcare providers must be made aware of the potential interference of anti-CD38 in pre-transfusion testing and of the potential sequelae if appropriate immunohaematological testing is not performed.

The transfusion laboratory can be provided with a request for phenotype if there has been no recent transfusion or RBC genotyping if the patient has been recently transfused or has a positive DAT, noting that the patient will receive anti-CD38 therapy. The clinician should provide the transfusion laboratory with a full and accurate transfusion, obstetric and drug history for the patient, and this may also require review of both hospital and laboratory records.

Routine pre-transfusion testing includes a blood group (ABO/RhD) and antibody screen and will establish pre-treatment baseline results. An RBC phenotype (or genotype) is most valuable and, as a minimum, should include: Rh (C, c, E, e), K, Jk^a, Jk^b, Fy^a, Fy^b and Ss antigens. Genotyping will be informative when phenotyping is not possible due to recent transfusion (i.e. in the last 3 months) or if the patient has a positive DAT or if suitable phenotyping reagents are not available. The RBC phenotype and genotype can assist the laboratory not only by suggesting what RBC alloantibodies the patient may potentially form but also by enabling transfusion of phenotype- or genotype-matched RBC, which will minimise the risk of RBC incompatibility in situations where underlying unexpected alloantibodies cannot be excluded in the presence of daratumumab. Furthermore, phenotype- or genotype-matched RBC transfusions will minimise the potential for sensitisation and future alloantibody formation. A clinical decision may be required on whether to limit or prioritise chosen phenotypes based on the urgency of the request and the difficulty of providing matched units for transfusion.[13]

Information relating to the immunohaematology testing should be maintained in the patient's clinical and laboratory files, and the patient should be provided with a 'patient alert card', which can inform healthcare providers that they are receiving anti-CD38 therapy. It is important to consider that patients may attend several hospitals and be tested at several transfusion laboratories, and it is also important to remember that in the absence of a jurisdictional or national alloantibody register, information about the patient's treatment with daratumumab and RBC phenotype and prior RBC alloantibody history may not be accessible by the transfusion laboratory or hospital at which the patient currently presents.

Prior to treatment with daratumumab:
1 Communications from treating professional and transfusion laboratory to document that the patient is to start anti-CD38 mAb.
2 Provide a full transfusion, obstetric and drug history.
3 Perform a blood group (ABO, RhD).
4 Perform an antibody screen and DAT.
5 Perform an extended RBC phenotype (or genotype, where indicated).
6 Provide patient with an alert card (see Fig. 3).

B: Following commencement of anti-CD38 therapy

It is extremely important for the transfusion laboratory to know that a potential transfusion recipient is receiving anti-CD38 therapy. The treating clinician needs to understand the impact on pre-transfusion testing and to consider the timeframes for testing and provision of blood. Specimens from patients on anti-CD38 may need to be referred to a reference laboratory for the more complex investigations necessary in these cases. The resource impacts on specialised reference services would be mitigated if the neutralising antibody was listed on the *Australian Register of Therapeutic Goods* (ARTG) and available. This would also simplify and expedite pre-transfusion testing and improve the relative safety of transfusion.

The ABO/RhD typing is unaffected by the presence of anti-CD38 and can be reported normally. The anti-CD38 panagglutination typically results in a universally weak (1+ or 2+; using 0–4 scoring) positive antibody

screen.[18,19] If one or more of the screening cells are strongly reactive (3+ or 4+), this suggests the potential presence of an antibody, possibly an alloantibody, other than anti-CD38 (Fig. 1).

To overcome anti-CD38 interference, the antibody screen can be repeated using DTT- or trypsin-treated reagent screening RBC. If this is negative, it may be assumed that no clinically significant RBC alloantibodies are present, with the caveat that specificities directed against antigens denatured by the chosen enzyme cannot be excluded. In the case where DTT-treated cells are used, the laboratory can select donor RBC that are ABO, RhD and K compatible, and these might be issued using the standard institutional cross-match (XM) protocol for a negative antibody screen, for example, electronic (computer) or immediate spin (IS) XM. In the absence of an identified RBC alloantibody using DTT-treated screening cells, the decision to provide more extended phenotype- or genotype-matched RBC beyond RhD and K (including Rh Cc, Ee, Jk^a, Jk^b, Fy^a, Fy^b and Ss) will be influenced by the availability of suitable units, clinical urgency of transfusion, anticipated current and future transfusion requirements and local policy. If the patient is revealed to have an unexpected genotype with potential antibody formation, this could be considered in planning.

Note that apart from DTT and trypsin, no validation studies have been published for other enzymes or methods for the purpose of resolving daratumumab interference. Thus, if other enzymes or methods are used, our consensus is that blood matched to the patient's phenotype/genotype should be given, particularly if long-term transfusion support is anticipated.

A positive antibody screen using DTT- or trypsin-treated reagent RBC suggests that the patient has an additional RBC alloantibody. The antibody specificity will need to be determined using a DTT- or trypsin-treated RBC. Antigen-negative blood may then be selected for XM. RBC that match the patient's extended RBC phenotype/genotype should be selected for transfusion, with the degree of matching determined by clinical urgency and the practicable availability of the desired phenotyped donor blood. A full IAT XM is required, but this will be incompatible unless DTT- or trypsin-treated donor cells are used for the XM.

The flowchart (Fig. 2) represents the expert group's recommendation for pre-transfusion testing in the presence of anti-CD38. It is recognised that not all transfusion laboratories in Australia and New Zealand will either routinely use or have access to DTT- or trypsin-treated reagent cells. The scope of testing will depend on institutional policy, clinical urgency and availability of appropriately phenotyped (or genotyped) donor RBC. Antibodies developed by patients to antigens such as Dombrock, which are destroyed by DTT and without routine typing sera for donors or patients, will be missed. Clinicians need to pay careful attention for signs of acute or delayed haemolytic transfusion reactions in patients on daratumumab after any transfusion; the genotype might provide a clue where the phenotype is not available.

Pre-transfusion testing following commencement of daratumumab:
1 Provide laboratory with a full transfusion, obstetric and drug history.
2 Order a blood group (ABO/RhD) and DAT.
3 Perform antibody screen panel.
4 If panagglutination is indicative of interference with anti-CD38 mAb on the antibody screen (see Fig. 1), perform an antibody screen using DTT- or trypsin-treated screening cells. (Other enzymes, *e.g.* papain, bromelain, ficin, may be used as an adjunct to help identify or exclude particular alloantibodies to RBC (*Note: Methods other than DTT or trypsin have been used but might not be validated for the purpose of resolving daratumumab interference. We suggest that if enzyme methods other than DTT or trypsin are used, then extended phenotype-/genotype-matched donor RBC should be given (Rh Cc, Ee, Jk^a, Jk^b, Fy^a, Fy^b and Ss).*))
5 Perform an extended RBC phenotype (or genotype, where indicated).
6 Issue donor RBC.

C: Life-threatening bleeding and emergency transfusions

For patients experiencing life-threatening bleeding or in emergency situations where transfusion is required within 2 h, there may not be time for the recommended routine pre-transfusion testing. Previous antibody history, phenotype and genotype results are invaluable in this circumstance.

There is a need to balance the clinical risks of transfusion versus those of not transfusing the patient, but under no circumstances should transfusion be delayed in the setting of a bleeding emergency.

The greatest risk to the patient is transfusion of ABO-incompatible blood. In emergency situations, the risk is normally mitigated by transfusion of group O RhD-negative blood; however, it should be noted that RhD-negative blood is *not* necessarily the most appropriate in all cases, especially in patients that are Rh c-negative and or Rh e-negative.

ABO and RhD typing are *not* affected by the presence of anti-CD38 antibody in the patient's plasma.

Transfusions should be in accordance with institutional critical bleeding or emergency transfusion policies. Further information on transfusion in emergency situations

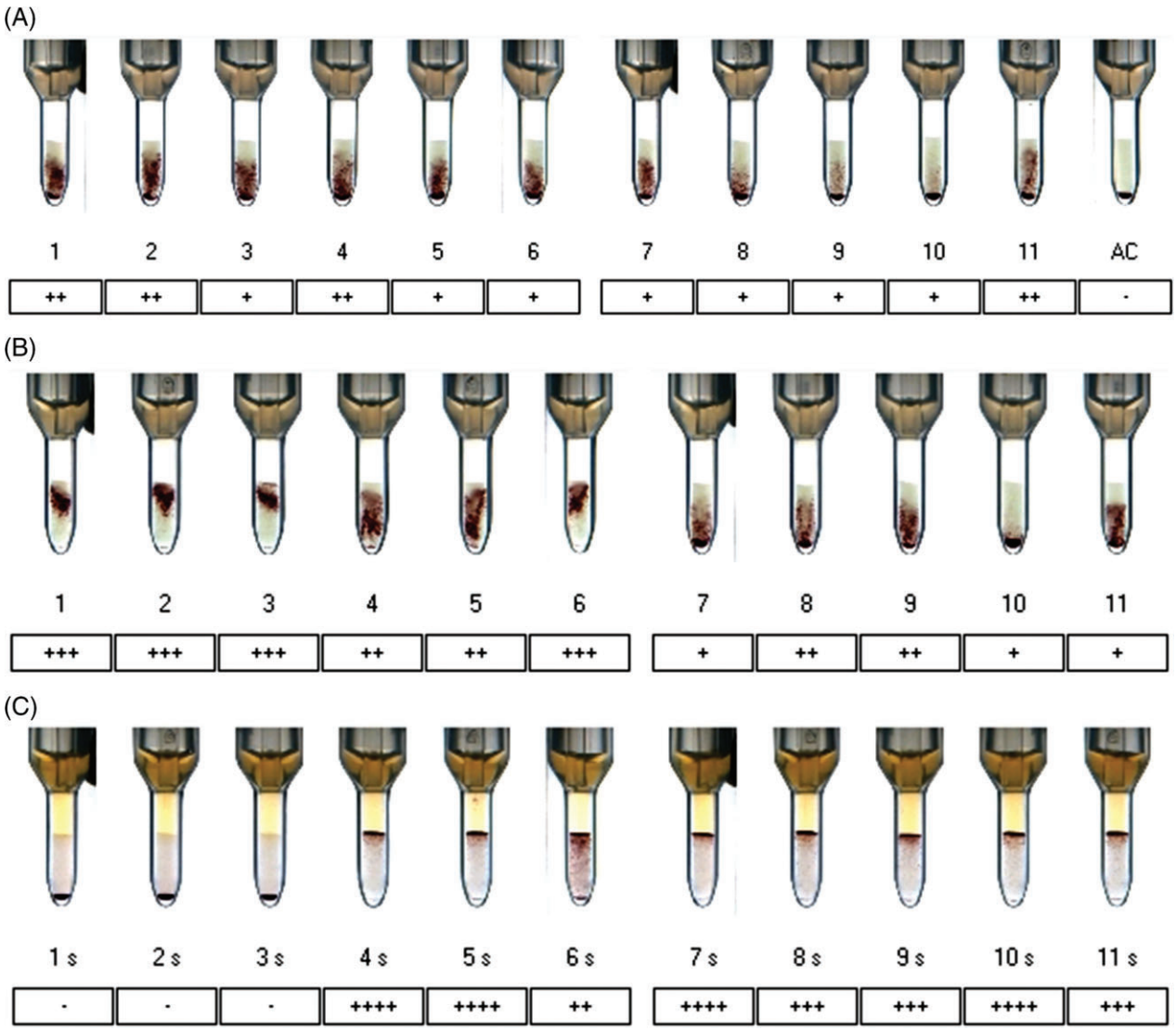

Figure 1 Typical red blood cell reactivity due to anti-CD38 in a patient's plasma. (A) Indirect antiglobulin test (IAT) panel where all cells display reactions consistent with anti-CD38 therapy; (B) IAT panel with some reactions consistent with anti-CD38 therapy; however, the pattern suggests that an alloantibody is present; (C) Saline panel where there is no interference by anti-CD38 therapy in cells 1–3, while cells 4–11 are positive for alloantibody.

can be found in the ANZSBT's 'Guidelines for Transfusion and Immunohaematology Laboratory Practice'.[20]

Clinical considerations

Daratumumab is the first anti-CD38 mAb that received clinic approval by the FDA in 2015 and subsequent TGA approval in Australia in 2017. Its use in combination with current therapeutics, such as lenalidomide or bortezomib, increases the frequency of minimal residual disease negative remissions in MM, which may translate to improvement in survival outcome.[21,22] Healthcare providers have not been adequately prepared for the critical interference of this drug in laboratory tests, particularly pre-transfusion testing. The problem will increase if daratumumab's use expands to early-phase disease treatment.

A crucial aspect in risk mitigation is education to increase awareness and a robust procedure to enable timely and routine communication with the blood transfusion laboratory. The patient and family members need to be aware of daratumumab's interference in pre-transfusion tests and the potential impact this may have on any blood transfusions. A patient alert card (see Fig. 3) is also useful for this purpose. All levels of medical care – from nursing staff to doctors and transfusion laboratory

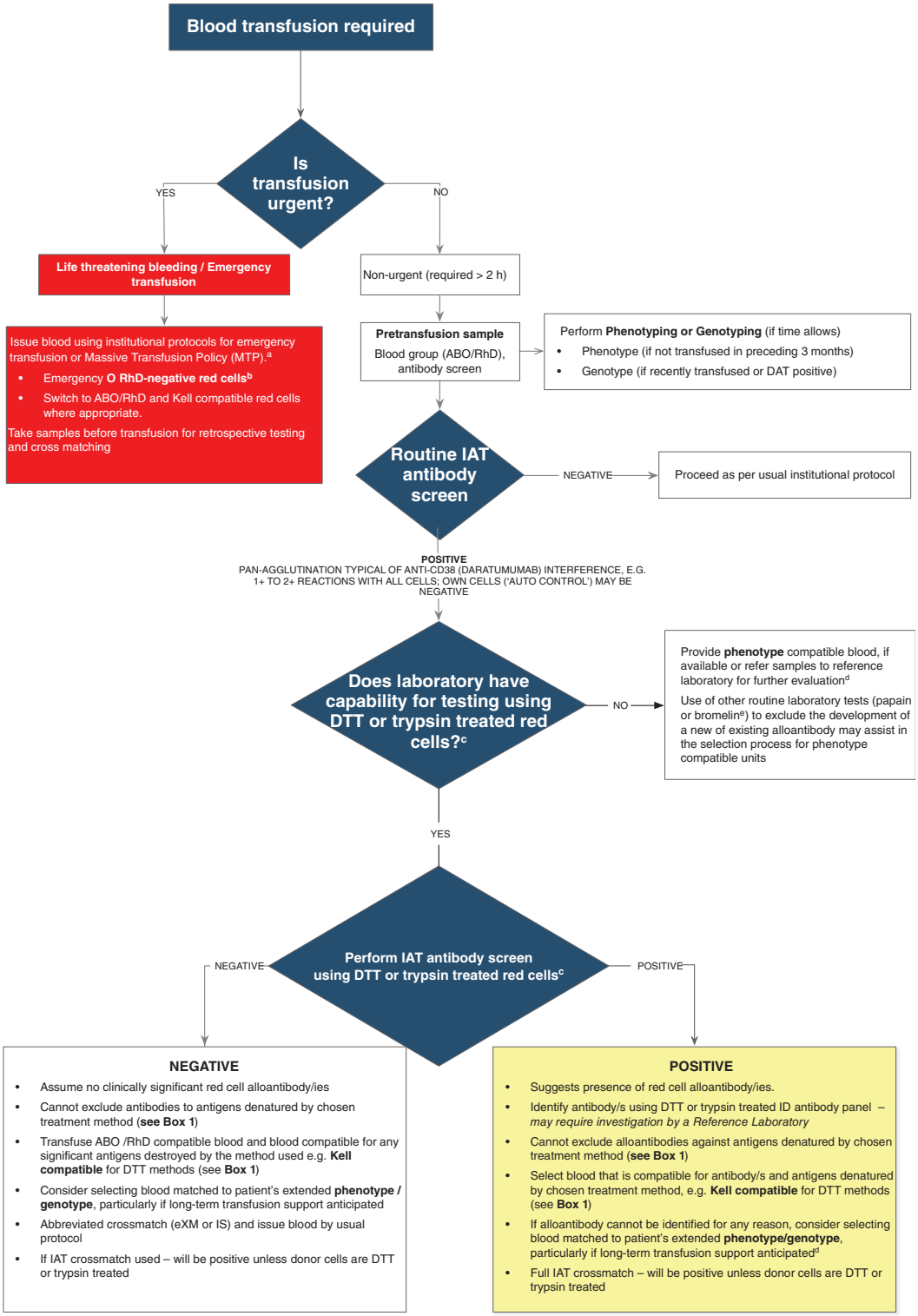

Figure 2 Pre-transfusion testing recommendations. [a]Refer to ANZSBT *Guidelines for Transfusion and Immunohaematology Laboratory Practice*; [b]O-negative blood is not without risk and may not be suitable in all circumstances, e.g. patient has anti-c or anti-e antibodies; [c]Tests using DTT or trypsin treated red cells are published methods for resolving anti-CD38 (daratumumab) interference; however, testing may not be available in all laboratories and/or subject to regulatory restrictions; [d]Extended phenotype/genotype including as a minimum: Rh (C, c, D, E, e), K, Jka, Jkb, Fya, Fyb and Ss; [e]Papain and bromeliad are not IAT methods for crossmatching purposes. DAT, direct antiglobulin test; DTT, dithiothreitol; IAT, indirect antiglobulin test.

ANTI-CD38 THERAPY OR PRETRANSFUSION (NON-URGENT CASES	Prior to commencing anti-CD38 therapy, all patients should undergo blood group (ABO/RhD), antibody screen. If time allows, perform **phenotyping or genotyping.** Phenotyping should be performed if the patient has not been transfused in the preceding 3 months. Genotyping is recommended if the patient has been recently transfused or is DAT positive.
URGENT TRANSFUSIONS (Blood required < 2 h)	Issue blood using institutional protocols for emergency transfusion of Massive Transfusion Policy (MTP). • Emergency **O RhD-negative RBCs.** Refer to ANZSBT *Guidelines for Transfusion and Immunohaematology Laboratory Practice.* Transfusing O RhD negative blood is not without risk, and may not be suitable in all circumstances, e.g. if the patient has anti-c or anti-e antibodies. • Switch to ABO/RhD and Kell compatible RBCs where appropriate. Take samples before transfusion for retrospective testing and cross matching.
NON-NON-URGENT TRANSFUSIONS	Complete blood group (ABO/RhD) and antibody screen, phenotyping or genotyping as per " *Prior to commencing anti-CD38 therapy*" box. **Perform a routine IAT antibody screen.** If negative, proceed as per usual institutional protocol. If positive, and the sample shows panagglutination typical of anti-CD38 interference, e.g. 1+ or 2+ reactions within all cells, then the sample should undergo testing using DTT or trypsin treated RBCs. **Perform IAT antibody screen using trypsin treated RBCs.** Tests using DTT or trypsin treated RBCs are published methods for resolving anti-CD38 interference, however, testing may not be available in all laboratories and/or subject to regulatory restrictions. If DTT or trypsin treated RBC testing is not available, provide phenotype compatible blood if available, or refer to a reference laboratory for further evaluation. Use of other routine laboratory tests, such as papain or bromelin, to exclude the development of a new existing alloantibody may assist in the selection process for phenotype compatible units. If DTT or trypsin treated RBC testing is possible, if negative, assume there are no clinically significant RBC antibodies, however, you cannot exclude antibodies to antigens denature by the chosen treatment method. Transfuse ABO/RhD compatible blood and blood compatible for any significant antigens destroyed by the method used, e.g. Kell compatible for DTT methods. Consider selecting blood matched to the patient's extended phenotype or genotype, and particularly if long-term transfusion support is anticipated. Perform an abbreviated cross match (eXM or IS) and issue blood using the usual protocol. If an IAT crossmatch is used, note it will be positive unless donor cells are DTT or trypsin treated. If positive, this suggests that RBC alloantibodies are present. Identify these antibodies using DTT or trypsin treated ID antibody panel. This method cannot exclude alloantibodies against antigens denatured by the chosen treatment method. Select blood that is compatible for antibodies and antigens that are denatured by the chosen treatment method. If an alloantibody cannot be identified for any reason, consider selecting blood matched to the patient's extended phenotype or genotype particularly if long-term transfusion support is anticipated. The extended phenotype and genotype should include at a minimum Rh (C, c, D, E, e), K, Jk^a, Jk^b, Fy^a Fy^b and Ss. A full IAT crossmatch will be positive unless the donor cells are DTT or trypsin treated.

Figure 2 (Continued)

scientists – need to be educated to ensure effective communication and adequate documentation in the patient record and the transfusion laboratory information system (LIS). Every public and private haematology/oncology facility should have a procedure to automatically notify the relevant transfusion laboratory when a patient is about to commence daratumumab and provide the appropriate specimens for testing. This will allow for baseline extended RBC phenotype (regardless of the immediate need for blood transfusion). The transfusions laboratory requires ongoing notification of daratumumab treatment when RBC transfusion is requested for up to 6 months post-treatment cessation. Updating blood transfusion requisition forms to include questions about antiCD38 mAb might be considered, as well as suitable alert notifications in electronic alert/chemotherapy

Name: _____

I am taking the following medication:

- <<*insert anti-CD38 antibody*>> product for the treatment of multiple myeloma

Dear Healthcare Provider,

Indirect antiglobulin test [IAT; Indirect Coomb's test] may show positive results in patients taking daratumumab, even in the absence of other clinically significant RBC antibodies in the patient's plasma. The determination of a patient's ABO and RhD blood type are not affected.

If an emergency transfusion is required, uncrossmatched, ABO/RhD compatible RBC's can be given as per local institutional policies. As dithiothreitol (DTT) treatment also denatures Kell antigens, K-negative units must be provided unless the patient is known to be K-positive.

For more information, please contact <<*insert company name, telephone number and email address*>>

Additional information on interference with blood compatibility testing can be found in the <<*insert anti-CD38 antibody*>> product information leaflet at <<*insert website*>>.

Before starting <<*insert anti-CD38 antibody*>>, my blood test results collected on (date) _____were:
Blood type:☐ A ☐ B ☐ AB ☐ O ☐ Rh+ ☐ Rh-

Indirect Antiglobulin Test IAT (Coomb's) antibody screen was:
☐ Negative ☐ Positive for the following antibodies:

Other: _____

Contact details of institution where the blood tests were performed:

_____

Figure 3 Example of patient alert card.

prescribing systems and alerts in the transfusion LIS to state that the patient is receiving daratumumab.

In the transfusion laboratory, while both DTT and trypsin are widely recommended, these methods are not always practical when laboratories rely heavily on automation. These methods are manual and laborious and incur additional costs. Although robust and reproducible,[6] in Australia, both DTT and trypsin used in these methods have not been approved for use as *in vitro* diagnostics by the TGA. There are no commercially available DTT or trypsin reagents listed on the ARTG, nor are DTT- or trypsin-treated reagent RBC screening or extended panels available. Thus, both methods would be considered 'in-house' methods and may not meet Australian IVD device regulations, despite being fully validated by laboratories before introduction. There is no current prospect of commercial availability of ATRG-listed soluble CD38 or anti-idiotype antibodies to neutralise the effect of daratumumab.

In the face of these constraints, the default contingency for many laboratories will be to issue extended phenotype- or genotype-matched blood where available. The ensuing impact of increased demands on the ARCBS and the increasing need for relevant immunohaematology expertise outside of large metropolitan laboratories will need to be considered. The establishment of a national RBC alloantibody register has been under consideration and might reasonably include relevant documentation for these circumstances.

With respect to the impact on patients, the risk pertains not only to possibility of missing a significant alloantibody that may cause acute or delayed haematolytic transfusion reactions but also to the delay in issuing of blood products. The potential for delay is present both when transfusion laboratories are unaware that patients are receiving daratumumab and when, if aware, are required to undertake increased testing. Haemolytic transfusion reactions because of daratumumab interference with pre-transfusion testing were not reported in the two pivotal phase III CASTOR[23,24] and POLLUX[25,26] studies. The patients in these trials were in the relatively

early course of their disease (with a median of 1–2 prior lines of treatment) and were not commonly transfusion-dependent. Conversely, in the clinic, daratumumab is currently also FDA- and TGA-approved as monotherapy for heavily pretreated patients who have had at least three prior lines of therapy. It is, therefore, expected that higher transfusion requirements will be seen in these end-stage patients, and we cannot be certain of the notion that no haemolytic transfusion reactions have been observed in daratumumab-treated patients before. Clinicians and laboratories should be aware of the potential for acute and delayed haemolytic transfusion reactions and should investigate, document and report any such reactions, or adverse events through their local haemovigilance programme.

Future directions

As the use of mAb is becoming increasingly prevalent for therapy of cancers and other medical conditions, the concept of potential interference in critical laboratory tests needs to be recognised and appropriate antibody neutralising solutions developed, preferably prior to the widespread introduction of these agents into the community. The introduction of daratumumab into clinical use in MM has indeed created a predicament in the transfusion laboratory that is without precedent, but should serve as a case in point to gain experience and prepare for similar scenarios in the future. Any mAb that targets common antigens present on RBC have the potential to interfere with pre-transfusion testing. Currently, these include the other anti CD38 mAb, such as isatuximab and MOR202,[27,28] both of which are undergoing clinical studies for the treatment of MM. While the nature of interference of these monoclonal antibodies is anticipated to be similar to that of daratumumab, this may not become clear until the drugs are more widely used. It is unclear whether there is concurrent development of an antidote to neutralise any of their interference in critical tests within the core laboratory. For daratumumab, neutralisation methods (soluble anti-CD38 mAb or anti-CD38 idiotype antibody) have been used successfully and are a fast and uniform way to deal with the interference.[11] Such kits could attain IVD approval and reduce the need for labour-intensive testing within the transfusion laboratory. Cost has been a barrier, and currently, the only commercial kit available (DIRA; Sebia, Evry Cedex, France) is in use to resolve daratumumab's interference in serum protein electrophoresis and immunofixation assays, which are methods to quantitate and type monoclonal immunoglobulins (M-proteins), respectively, in the serum or urine. In the absence of such a kit for pre-transfusion testing, other ways to resolve the problem, to minimise workflow disruption to transfusion laboratories and mitigate risks to patients must be considered.

If a transfusion laboratory is not aware that a patient is receiving daratumumab, protracted investigation and delays are likely to occur when unexpected panagglutination is found in the routine antibody screen. A national database (or register) of patients treated with daratumumab or any other mAb that interferes with pre-transfusion tests could provide an easily accessible source of information for patients who may demonstrate interference in immunohaematology testing. Such a database, if incorporated in an antibody register or database, could also potentially alert the local laboratory service when a patient is known to have RBC allo- or autoantibodies. This might reduce delays in immunohaematology testing and time to appropriate transfusion. Such databases have been recommended in other jurisdictions.[14]

At the hospital level, routine and automatic notification to the transfusion laboratory about a patient's treatment status could be mandated. Automated alerts, through electronic medical record systems to the transfusion laboratory, for every patient on treatment that may interfere with immunohaematology tests or require selection of specialised blood products could be implemented. Investment in the development of this infrastructure needs to happen now to prepare adequately for the surge of mAb in clinical use in the near future. For future targeted therapies, we emphasise the need to explore fully any potential interference with critical laboratory assays that may impact the other areas of clinical practice prior to their introduction into the clinics.

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