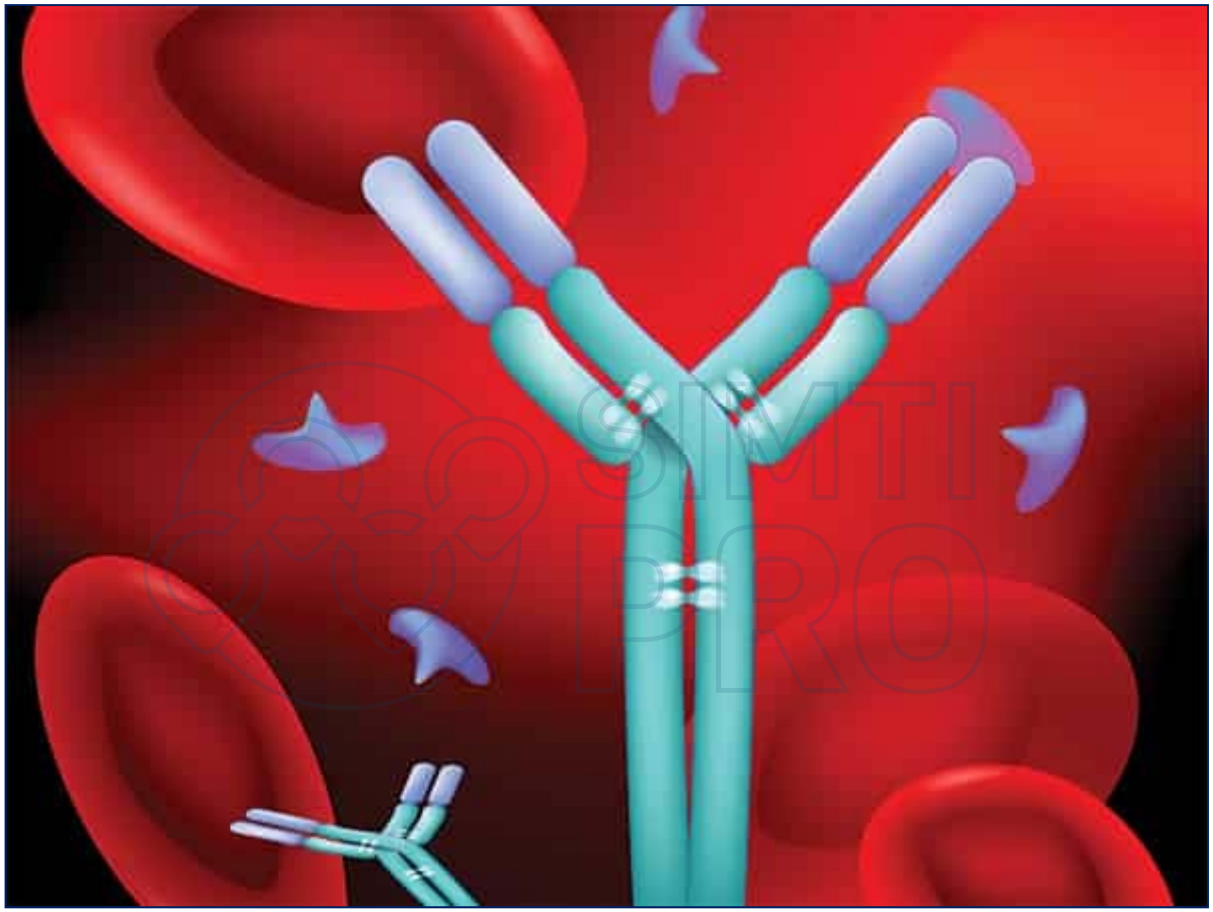
GESTIONE TRASFUSIONALE DEI PAZIENTI IN TERAPIA CON ANTICORPI MONOCLONALI



Antonella Matteocci
UOC Medicina Trasfusionale e Cellule Staminali
A.O. S. Camillo Forlanini - Roma

La sottoscritta Antonella Matteocci, in qualità di Relatore

dichiara che

nell'esercizio della Sua funzione e per l'evento in oggetto, NON È in alcun modo portatore di interessi commerciali propri o di terzi; e che gli eventuali rapporti avuti negli ultimi due anni con soggetti portatori di interessi commerciali non sono tali da permettere a tali soggetti di influenzare le mie funzioni al fine di trarne vantaggio.

Antonella Matteocci

Immunotherapy: the good, the bad, the ugly, and the really ugly

TRANSFUSION 2019;59;437–440

The "good" with immunotherapeutics is that these are often successful at ameliorating various autoimmune and malignant conditions.

The "bad" with immunotherapeutics is the potential for cross reactivity with RBCs in serologic testing.

The "ugly": Immunotherapeutics are now in clinical trials that target a highly expressed RBC antigen

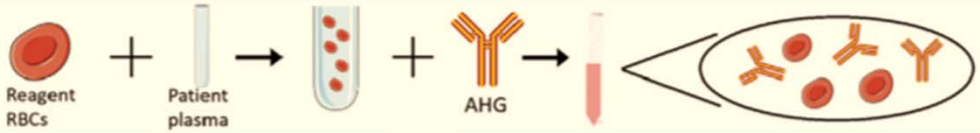
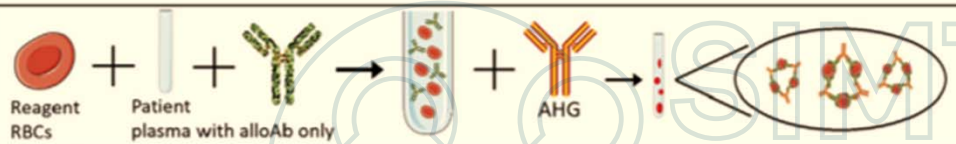
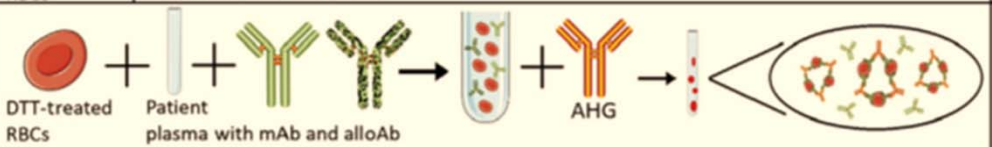
Perhaps "really ugly" scenarios will evolve as more humanized monoclonal therapeutics are developed for the treatment of various diseases, and especially for cancer therapies, where the target antigen may share cross reactivity with RBCs. Future headaches for blood bankers could involve the use of therapeutic antibodies to adhesion molecules

Impact of Novel Monoclonal Antibody Therapeutics on Blood Bank Pretransfusion Testing

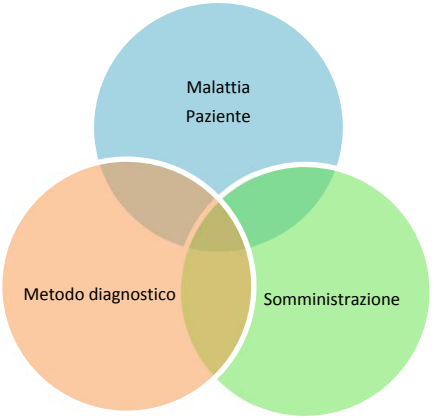
Zhen Mei, MD[a], Geoffrey D. Wool, MD, PhD[b,*]

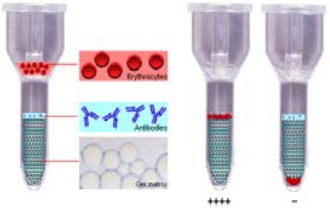
Hematol Oncol Clin N Am 33 (2019) 797–811

Anti-CD38
Daratumumab (human IgG1-kappa), MOR202 (human IgG1-lambda) and Isatuximab (chimeric IgG1-κ) and TAK-079 (human IgG1)

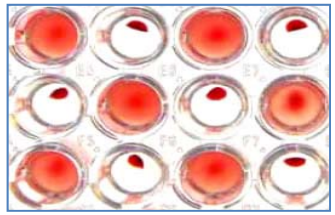
Test Situation	Elements of Test	Result	Comment
No Abs		No agglutination	
AlloAb		True positive agglutination	***These three situations cannot be resolved without further testing***
mAb		False positive agglutination	
AlloAb + mAb		Agglutination cannot be attributed to a specific antibody	
AlloAb + mAb + test modification		True positive agglutination	
mAb + test modification		No agglutination	

INTERFERENZA





Fase solida – Proteina A



SPRCA

Agglutinazione su colonna

Provetta

LISS, PEG

Test positivo in card per una mediana di 103gg (63-190)

Chari et al, 2018

Table 2
Representative pretransfusion compatibility testing results for daratumumab

	Patient Results Before Anti-CD38 Therapy	Patient Results Post Anti-CD38 Therapy	Presence of Interference?
ABO/Rh typing	A+	A+	No
Antibody screen (IAT)	Negative	Pan-reactive positive	Yes
Autocontrol	Negative	Negative/positive	Possible
Direct antiglobulin test	Negative	Negative/positive	Possible
Eluate	Not performed	Negative/pan-agglutinin	Possible

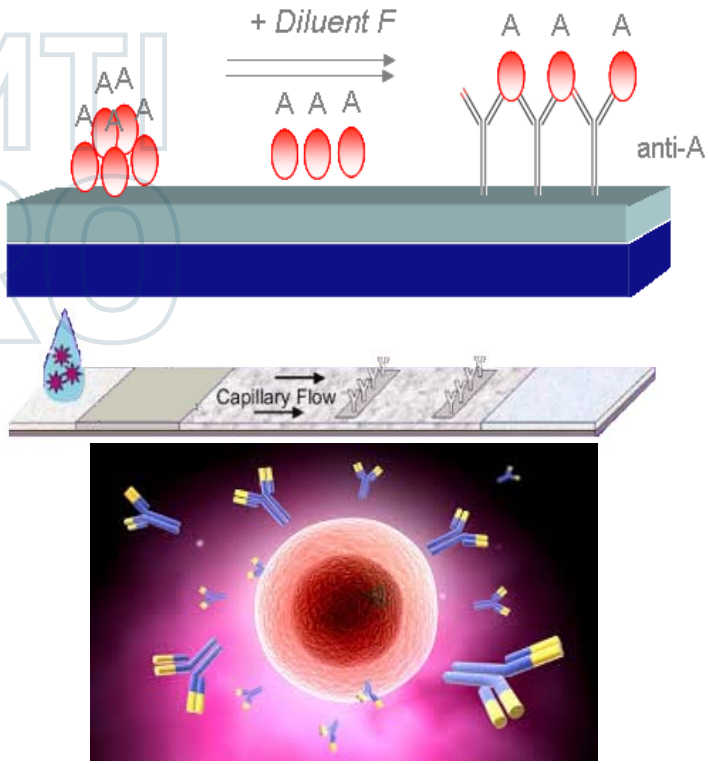
Hematol Oncol Clin N Am 33 (2019) 797–811

ANTI-CD38 e TAD

TAD

- Inizio terapia:pos
- Si Negativizza
- Perdita del CD38 dai GR (macrofagi)

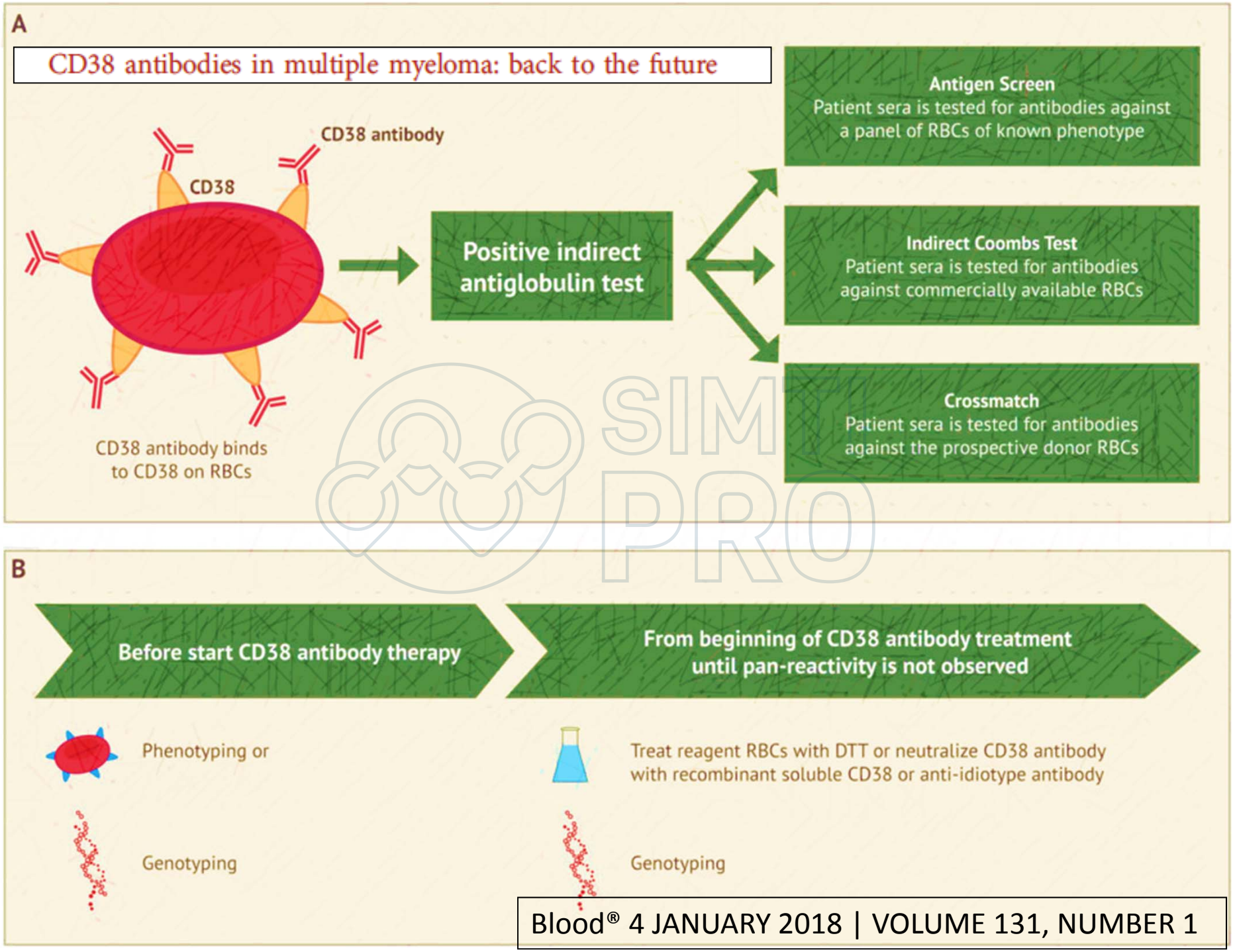
Multicard: tecnologia del flusso laterale



blood®
Prepublished online April 3, 2017;
doi:10.1182/blood-2016-11-749432

Daratumumab (anti-CD38) induces loss of CD38 on red blood cells

Harold C. Sullivan, Christian Gerner-Smidt, Ajay K. Nooka, Connie M. Arthur, Louisa Thompson, Amanda Mener, Seema R. Patel, Marianne Yee, Ross M. Fasano, Cassandra D. Josephson, Richard M. Kaufman, John D. Roback, Sagar Lonial and Sean R. Stowell



Blood® 4 JANUARY 2018 | VOLUME 131, NUMBER 1

frontiers in Immunology

Blood Transfusion Management for Patients Treated With Anti-CD38 Monoclonal Antibodies

REVIEW
published: 15 November 2018
doi: 10.3389/fimmu.2018.02616

Method	Mechanism	Advantages	Disadvantages
DTT	Denatures CD38 antigen on reagent RBCs	Cheap Easy to apply Well-validated and reliable	Denatures Kell antigen; must give K-negative RBCs (unless Kell status known) Destroys other clinically significant minor antigens (Lutheran, YT, JMH, LW, Cromer, Indian, Dombrock, and Knops systems)
Trypsin	Cleaves CD38 antigen on reagent RBCs	Cheap Easy to apply	Denatures several significant antigens (M, N, En^{a}TS, Ge2, Ge3, Ge4, Ch/Rg, and Lutheran) Not validated Less reliable than DTT at removing CD38 from reagent RBCs
Papain	Cleaves CD38 antigen on reagent RBCs	Cheap Easy to apply Reliable	Destroys many significant antigens, including MNS and Duffy systems as well as Ch/Rg, Ge2, and Ge4 Due to above, can only be used as a complementary method
RBC phenotype	Antigen profiling of patient RBCs	Only needs to be performed once Provides reliable information for future use 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 Extended-match units may be scarce and better utilized for patients with known alloantibodies
RBC genotype	Antigen profiling of patient RBCs	Only needs to be performed once Provides reliable information for future use Does not require future IAT testing if matched units available Can be performed at any time	Expensive Requires extended match to ensure no antibodies or future alloantibody formation Extended-match units may be scarce and better utilized for patients with known alloantibodies

International validation of a dithiothreitol (DTT)-based method to resolve the daratumumab interference with blood compatibility testing — Transfusion 2016

Resolving the daratumumab interference with blood compatibility testing, Transfusion. 2015.

Papain-treated panels are a simple method for the identification of alloantibodies in multiple myeloma patients treated with anti-CD38-based therapies. *Trasfus Med 2018*

Raccomandazioni per la gestione trasfusionale dei pazienti in trattamento con Daratumumab — SIMTI 2017

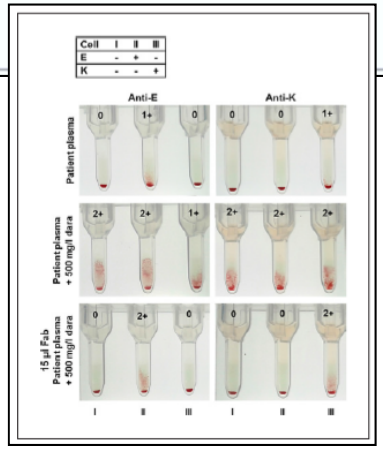
Raccomandazioni per l'impiego delle metodiche molecolari in immunoematologia — SIMTI 2018

frontiers in Immunology

Blood Transfusion Management for Patients Treated With Anti-CD38 Monoclonal Antibodies

REVIEW
published: 15 November 2018
doi: 10.3389/fimmu.2018.02616

Method	Mechanism	Advantages	Disadvantages
Anti-idiotype antibody	Neutralizes anti-CD38 antibody prior to IAT	Simple and would allow for normal blood bank testing once anti-CD38 antibody removed Commercially available (for daratumumab)	Expensive Not typically available in blood bank inventory Would require different reagent for each anti-CD38 monoclonal antibody
Soluble CD38 antigen	Neutralizes anti-CD38 antibody prior to IAT	Simple and would allow for normal blood bank testing once anti-CD38 antibody removed Applicable to any anti-CD38 monoclonal antibody Commercially available	Expensive Not typically available in blood bank inventory May be less efficacious than anti-idiotype antibody Would require large amount of soluble CD38 to neutralize therapeutic monoclonal antibodies
$F(ab')_2$ 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/In (Lu) RBCs	Reagent cells lack CD38 antigen	Easy to perform; no additional steps required	In (Lu) RBCs are rare Cord blood cell antigen expression differs from reagent RBCs; therefore, would need to be typed prior to use

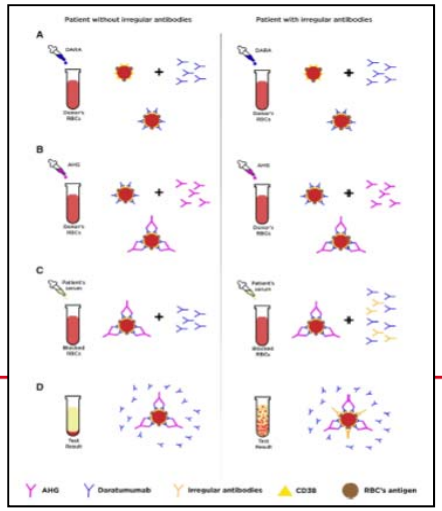


Daratumumab Interference in Pretransfusion Testing Is Overcome by Addition of Daratumumab Fab Fragments to Patients' Plasma

Transfus Med Hemother 2019;46:423–430

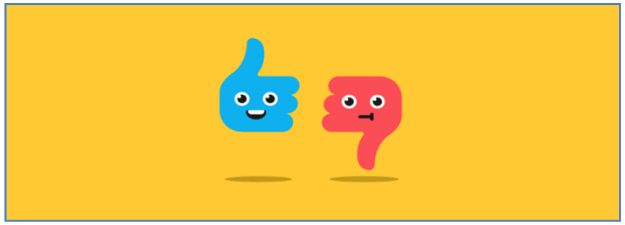
A blockage monoclonal antibody protocol as an alternative strategy to avoid anti-CD38 interference in immunohematological testing

TRANSFUSION 2019;59;1827–1835



Impact of Novel Monoclonal Antibody Therapeutics on Blood Bank Pretransfusion Testing

Hematol Oncol Clin N Am 33 (2019) 797–811



1. DTT or 2-aminoethylisothiouronium treatment of screening panel red cells

   Pros
   - Reduces a disulfide linkage in the CD38 antigen, therefore denaturing the protein and destroying the epitope recognized by the anti-CD38 antibody
   - May be used for any anti-CD38 antibody because this affects the target antigen
   - DTT is a common reagent that is already found in many blood banks

   Cons
   - Destroys and denatures RBC antigens containing a disulfide linkage, including the clinically relevant Kell antigens, along with other minor blood groups, including Dombrock, Indian, JMH, Scianna, Knops, Cromer, Landsteiner-Weiner, Lutheran, MER2, AnWj, and Cartwright antigens
   - Time intensive to DTT treat the panel, wash, and store in stabilizing storage solution

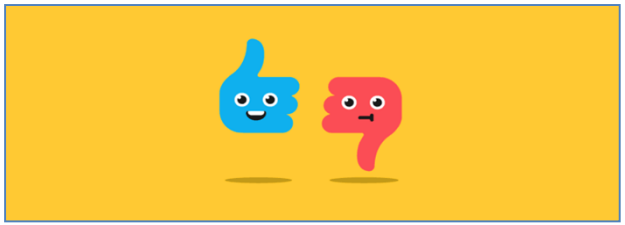
2. Soluble CD38

   Pros
   - Binds to and neutralizes free anti-CD38 antibodies, saturating the binding sites, preventing them from binding to CD38 epitopes on RBC screening cells
   - Can be quickly added to patient sample before testing

   Cons
   - Expensive reagent; not widely available
   - Must be added in excess to ensure neutralization of any remaining anti-CD38 antibodies

Impact of Novel Monoclonal Antibody Therapeutics on Blood Bank Pretransfusion Testing

Hematol Oncol Clin N Am 33 (2019) 797–811



3. Anti-idiotype antibody
   Pros
   - Binds to Fab portion of free anti-CD38 antibodies, saturating the binding sites, preventing them from binding to screening cells
   - Can be quickly added to patient sample before testing

   Cons
   - Expensive reagent; not widely available
   - A separate reagent may be required for each anti-CD38 mAb, because they may not all be recognized by the same anti-idiotype antibody

4. Use of cord blood as reagent RBCs for IAT[31]
   Pros
   - Low CD38 expression on the surface of these cells, preventing anti-CD38 antibodies from interfering
   - Can rule out all antigens expressed in normal density on neonatal RBC; this includes Kell

   Cons
   - May be used for screening, but impractical to make an antibody identification panel using only cord blood cells
   - Highly effort intensive to maintain an adequate supply of in-date and typed cord blood cells

frontiers in Immunology

Blood Transfusion Management for Patients Treated With Anti-CD38 Monoclonal Antibodies

Guido Lancman[1], Suzanne Arinsburg[2], Jeffrey Jhang[2], Hearn Jay Cho[1], Sundar Jagannath[1], Deepu Madduri[1], Samir Parekh[1], Joshua Richter[1] and Ajai Chari[1]*

REVIEW
published: 15 November 2018
doi: 10.3389/fimmu.2018.02616

Study	No. of patients	Anti-CD38 MoAb	Pre-existing alloantibodies	Positive IAT	Positive auto-control IAT	Positive DAT	Duration IAT positivity
Bub et al. (16)	5	Dara	n/a	5/5	2/5	2/5	n/a
Carreño-Tarragona et al. (17)	33	30 Dara 3 ISA	anti-D and anti-C ($n = 2$), anti-E and anti-C ($n = 1$)	33/33	n/a	5/21 for Dara 1/2 for ISA	Median 5 months (range 1–9 months)
Chapuy et al. (6)	5	Dara	n/a	5/5	3/5	3/5	n/a
Chari et al. (15)	7	Dara	anti-D and anti-E ($n = 1$), anti-E, K, Jkb, Fya, Fyb S, Knops ($n = 1$)	7/7	1/7	1/7	Median 3.4 months (range 2.1–6.3)
Deneys et al. (18)	14	Dara	None	14/14	n/a	n/a	n/a
Oostendorp et al. (7)	11	Dara	None	11/11	0/11	0/11	Range 2–6 months
Sullivan et al. (12)	13	Dara	n/a	13/13	0/13	0/13	n/a
Subramaniyan et al.(19)	1	Dara	n/a	1/1	0/1	0/1	n/a
Lin et al. (20)	1	Dara	n/a	1/1	0/1	0/1	n/a
Setia et al. (21)	1	Dara	n/a	1/1	n/a	1/1	n/a

MoAb, monoclonal antibody; IAT, indirect antiglobulin test; DAT, direct antiglobulin test; Dara, Daratumumab; ISA, isatuximab; n/a, not available.

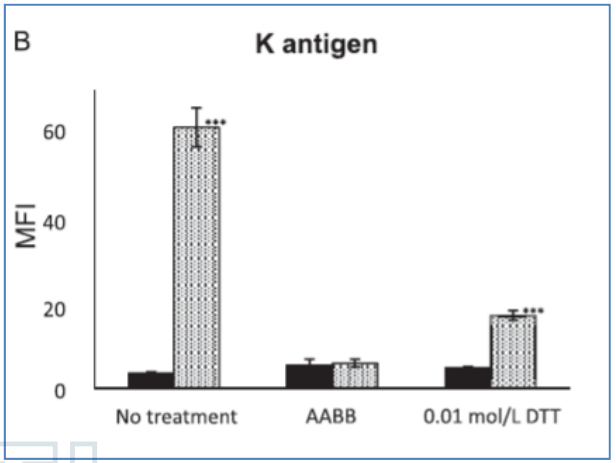
ORIGINAL RESEARCH

Distinct effects of daratumumab on indirect and direct antiglobulin tests: a new method employing 0.01 mol/L dithiothreitol for negating the daratumumab interference with preserving K antigenicity (Osaka method)

Mika Hosokawa,[1] Hirokazu Kashiwagi,[2] Kotarosumitomo Nakayama,[1] Mikiko Sakuragi,[1] Mayumi Nakao,[1] Tamayo Morikawa,[1] Tomoko Kiyokawa,[1] Hiroshi Aochi,[1] Keisuke Nagamine,[1] Hirohiko Shibayama,[2] and Yoshiaki Tomiyama[1,2]

Transfusion 2018;9999:1-11

DTT 0.01 mol/L



Extending shelf life of dithiothreitol-treated panel RBCs to 28 days.
Vox Sang. 2018 May;113(4):397-399

VoxSanguinis
The International Journal of Transfusion Medicine
ISBT
Vox Sanguinis (2018) 113, 686–693
© 2018 International Society of Blood Transfusion
DOI: 10.1111/vox.12689

ORIGINAL PAPER

Thirty-three-day storage of dithiothreitol-treated red blood cells used to eliminate daratumumab interference in serological testing

Henriette Lorenzen,[1] Nazia Lone Akhtar,[2] Maria Nielsen,[1] Lea Svendsen[1] & Pernille Andersen[2]
[1]Faculty of Health and Technology, Metropolitan University College, Copenhagen, Denmark
[2]Department of Clinical Immunology, Herlev Hospital, University of Copenhagen, Herlev, Denmark

Vox Sanguinis 2018;113:686-693

DTT 0.2 mol/L

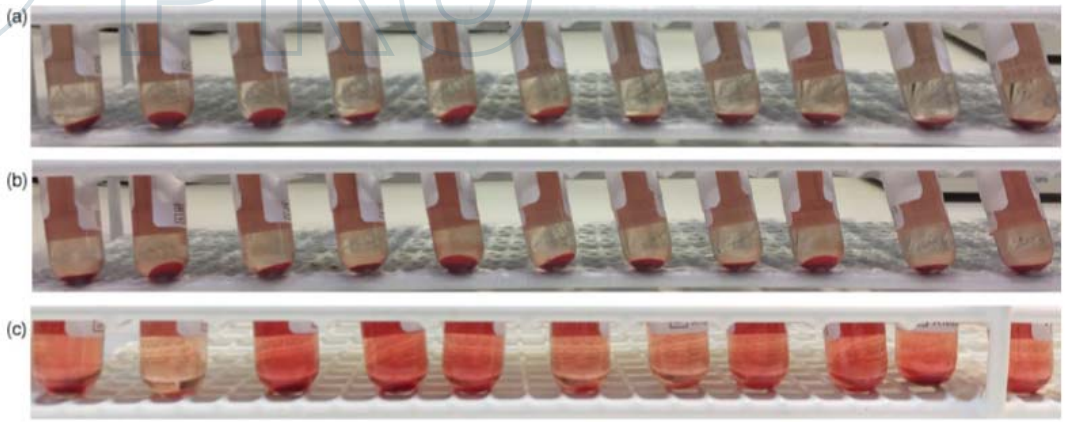
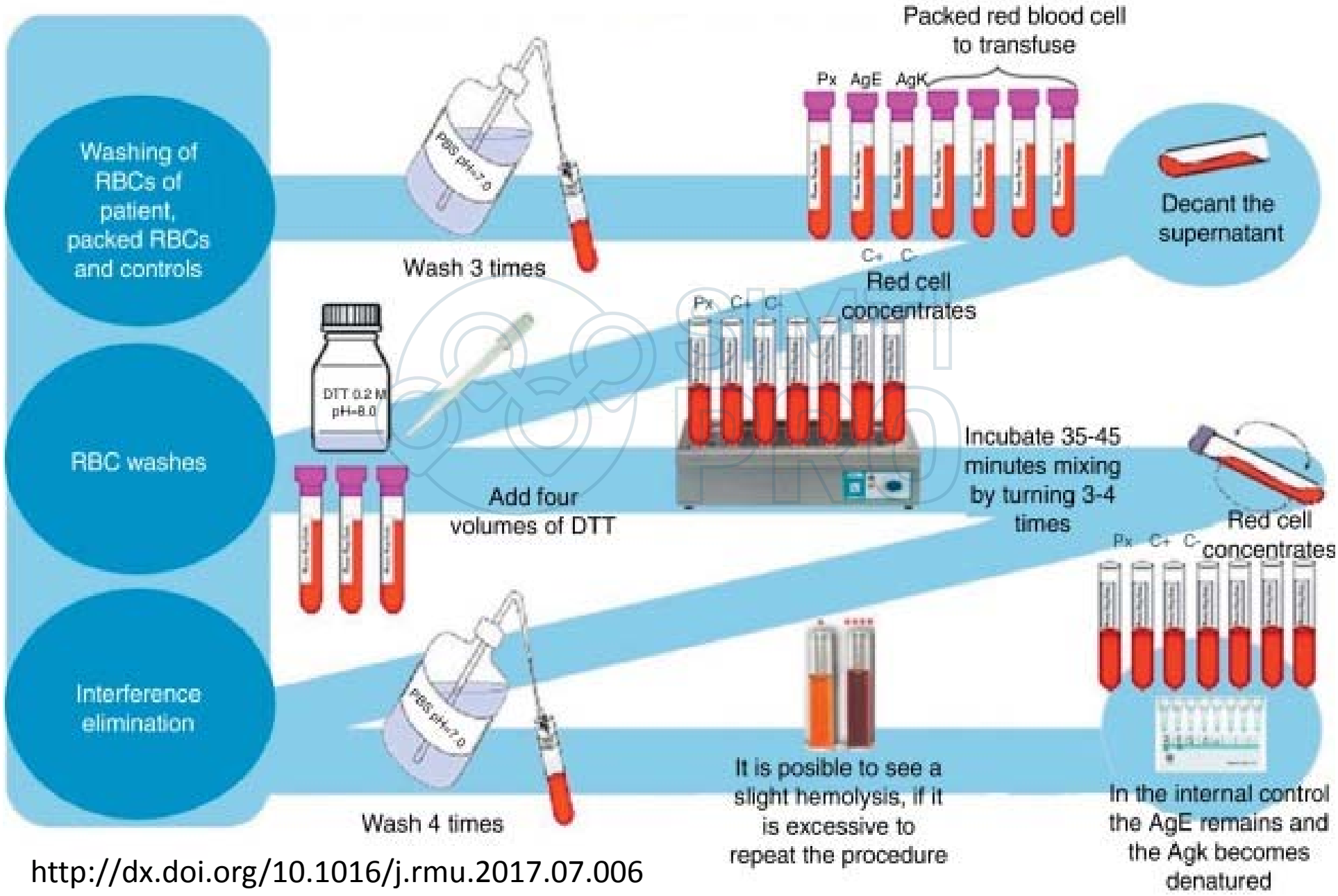


Fig. 2 Representative visible haemolysis on day 33 of (a) untreated panel cells, (b) DTT-treated panel cells (RBC:DTT ratio 30:25, Sigma-Aldrich) and (c) DTT-treated panel cells (RBC:DTT ratio 30:120, Sigma-Aldrich, AABB method).

RBCs 30µl:DTT 25µl

The use of DTT in the resolution of the interferences generated by daratumumab in the blood bank

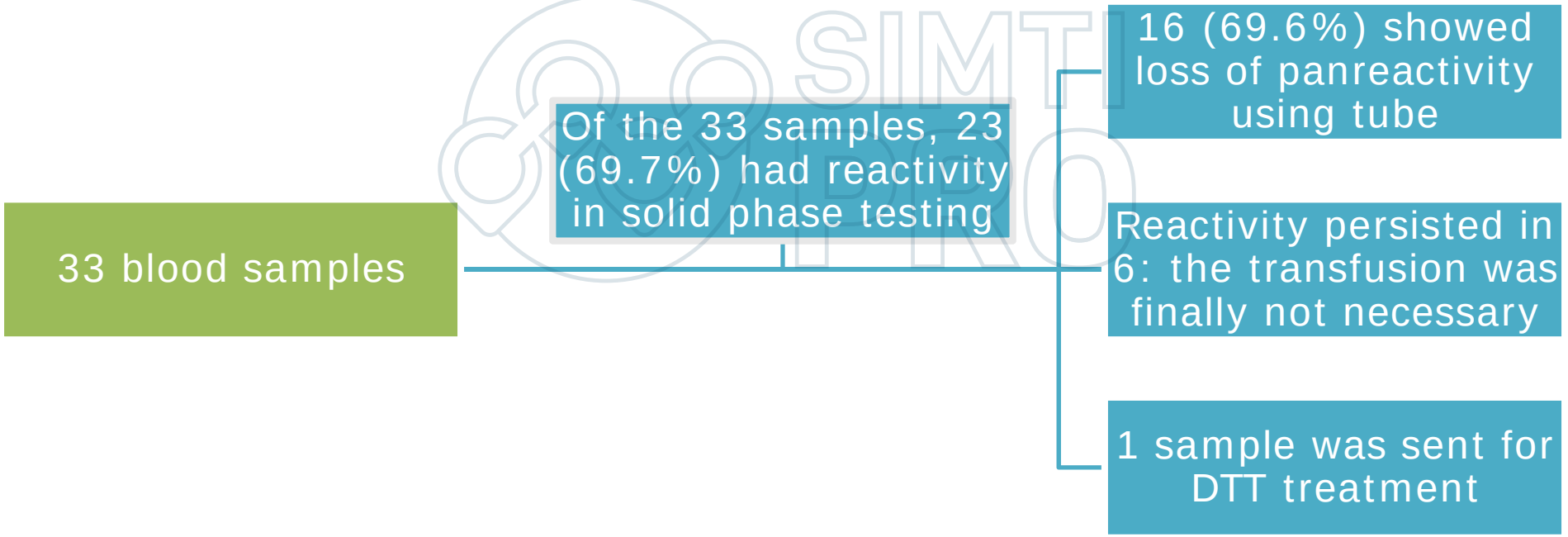


http://dx.doi.org/10.1016/j.rmu.2017.07.006

USE OF STANDARD LABORATORY METHODS TO OBVIATE ROUTINE DTT TREATMENT OF BLOOD SAMPLES WITH DARATUMUMAB INTERFERENCE

- 33 blood samples from 4 patients were initially tested by solid phase.
- DARA ranged from 1 to 14.
- Any reactivity by solid-phase testing led to additional **tube testing**:

  60 min of incubation without any enhancement (LISS)



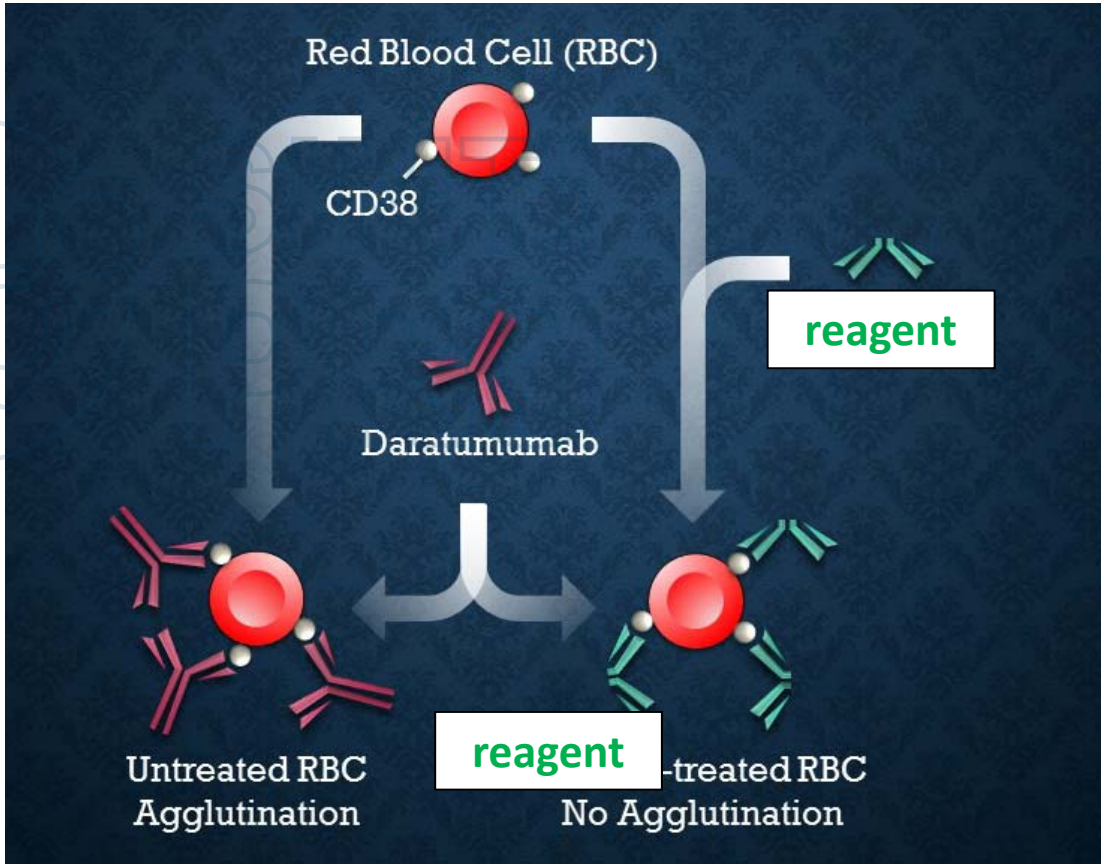
Lintel NJ et al. *Immunohematology 2017;33(1):22-26*

Transfusion Clinique et Biologique
Volume 26, Issue 3, Supplement, September 2019, Page S57

P-072

Reagent : Le graal de la transfusion ?

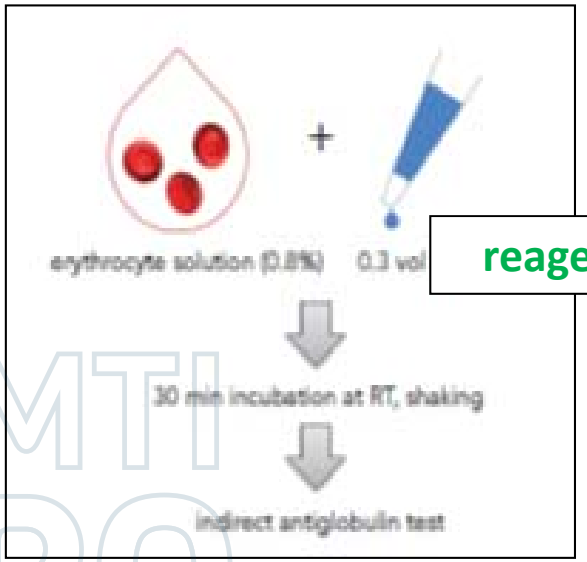
It is an anti-CD38 antibody without a human Fc region.
When treating red blood cells (RBC) with this neutralizating reagent, the antibody binds CD38, thus covering the epitope of Daratumumab, MOR 202 and isatuximab.

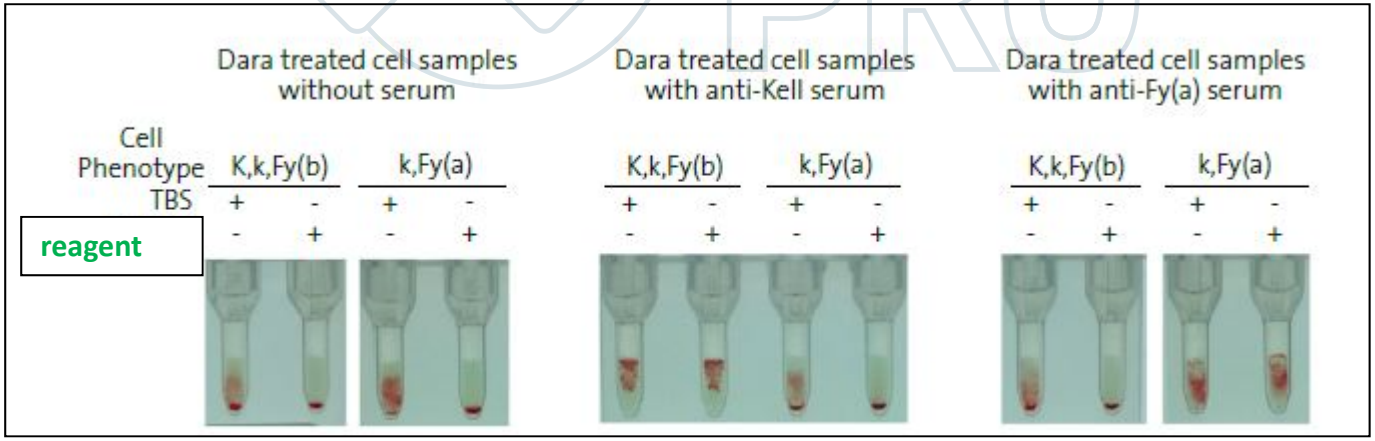


Validation of Reagent to Resolve Daratumumab-Induced Interferences in Pre-Transfusion Screen Tests

Blood (2019) 134 (Supplement_1): 4983.
https://doi.org/10.1182/blood-2019-131345

- Best alternative to Dithiothreitol (DTT).
- Ready in 30 min.
- One incubation only, without washes.
- Keeps erythrocyte antigens intact.





ABSTRACT

IGT23: Novel Soluble CD38 for Efficient Neutralization of High Titer Anti-CD38 Antibodies

Sunday, October 14, 2018 01:00 PM - 02:00 PM

Hall A - Boston Convention and Exhibition Center

Background/Case Studies: Novel anti-CD38 drugs used in treatment of multiple myeloma, such as daratumumab (DARA), interfere with diagnostic screening and identification of unexpected antibodies. They cause pan-reactivity of Reagent Red Blood Cells (RRBC), which complicates the detection of underlying allo-antibodies of potential clinical relevance. At the moment there are few strategies to overcome this problem, however with several drawbacks. The aim of this study was to evaluate the diagnostic use of a novel recombinant CD38 with particular emphasis on dilution effects of this soluble CD38 (sCD38) on detection of unexpected antibodies.

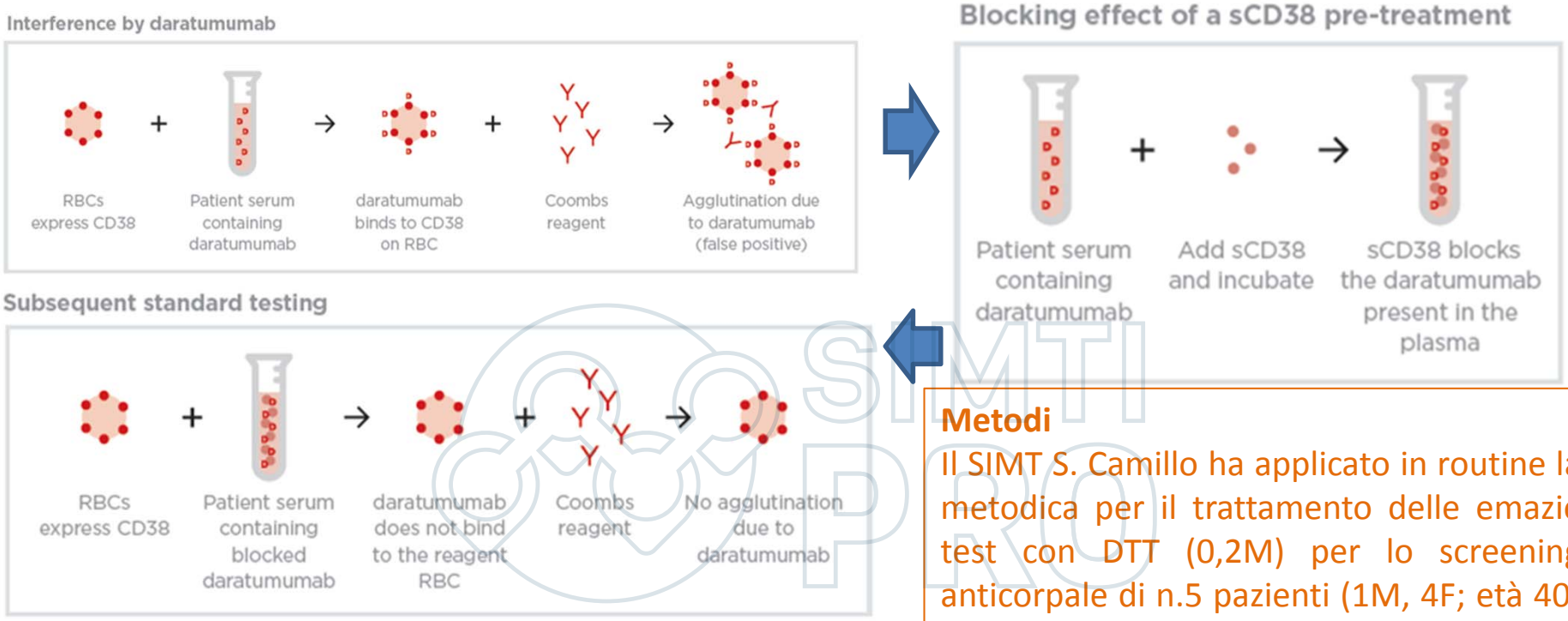
Study Design/Method: A fusion protein containing the extracellular domain of CD38 was expressed in mammalian cells and purified as sCD38. For evaluation of diagnostic functionality, anti-CD38 spiked donor plasma (containing allo-antibodies or not) were mixed with varying volumes/concentrations of sCD38 (or PBS as control) and incubated for 15 minutes at 37°C. Antibody detection was then performed by Indirect Antiglobulin Test (IAT) in conventional tube technique or DG Gel technique.

Results/Finding: A ratio of 2µl and 4µl of recombinant sCD38 at nominal concentration of ~30mg/ml per 25µl of plasma, allowed for complete inhibition of anti-CD38 (respectively 0.5mg/ml and 1mg/ml). After inhibition, spiked allo-antibodies (anti-D, -E, -c, -Cw, -K, -Fya, -Jka, -S, -s, -M, -Lua, -Cob) at barely detectable amounts into DARA-spiked donor plasma could be readily detected in 16/16 samples. In contrast, as demonstrated in DG Gel technique, after incubation with 20µl and 200µl of diluted preparations of sCD38, respectively 15/16 and 3/16 of the same simulated DARA plasma spiked with antibodies could still be detected.

Conclusion: The presented results show the inhibition of therapeutic plasma concentrations of daratumumab using a novel sCD38 at small volumes without interference in alloantibody detection. Additionally, these data confirm that successful neutralization and subsequent antibody detection requires highly concentrated sCD38. After neutralization, the plasma can be screened with available routine techniques, such as tube and gel technique. Enabling complete anti-CD38 inhibition, while minimally diluting the plasma with sCD38, the highly concentrated sCD38 presented in this work may provide, in combination with IAT, a rapid and accurate screening and identification method of even weakly reacting allo-antibodies masked by anti-CD38.

PROTEINA RICOMBINANTE CD38 SOLUBILE PER LA NEUTRALIZZAZIONE DELL' INTERFERENZA SIEROLOGICA DA DARATUMUMAB

A. Matteocci et al: Conferenza SIMTI Rimini 2019



Test	Metodo Usato	Risultati
TAI (emazie test e plasma non trattati)	Metodica in provetta / Schedine	Positivo 1+ / Positivo 2+
TAI (emazie test trattate con DTT)	Metodica in provetta / Schedine	Negativo
TAI (plasma inattivato con sCD38)	Metodica in provetta / Schedine	Negativo

Metodi

Il SIMT S. Camillo ha applicato in routine la metodica per il trattamento delle emazie test con DTT (0,2M) per lo screening anticorpale di n.5 pazienti (1M, 4F; età 40-75 anni) affetti da MM e in terapia con Daratumumab***. Il kit prevede l'incubazione di 2 uL di sCD38 con 25 uL di siero per 15 minuti a 37 ºC. Quindi, è possibile eseguire immediatamente i test pre-trasfusionali*** secondo i metodi utilizzati in laboratorio (provetta, schedine, micropiastra) senza riscontrare alcuna interferenza da anti- CD38.

150 microl di RBC 3%, o

400 microl di RBC 0,8%

400 microl DTT 0,2 M

37°C per 30', 4 lavaggi

200 microl LISS , gel card

Decision tree for the management of blood transfusion in patients treated with daratumumab

Patient eligible for treatment with daratumumab

↓

Contact of clinician with hospital blood bank

↓

Immuno-hematological assessment
- Blood group ABO-RHD
- Erythrocyte phenotyping: RH - KEL - FY - JK - Ss
- IAT "Type and Screen"

R/ daratumumab →

↓

Transfusion needs
Order of erythrocyte concentrates

↓

Pre-transfusion tests (IAT/compatibility tests)

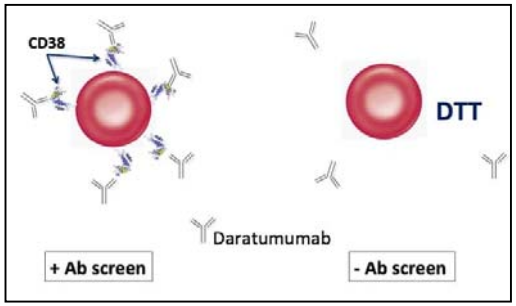
Negative results → Nominative distribution

Positive results → and/or

Treatment of RBC with DTT → IAT → (−) Nominative distribution; (+) → Phenotype of patient available

Phenotype of patient available → No → Genotyping → Phenotype-compatible erythrocyte concentrates available → No / Yes → Nominative distribution

Phenotype of patient available → Yes → Phenotype-compatible erythrocyte concentrates available → No / Yes → Nominative distribution



IAT: indirect antiglobulin test
RBC: red blood cells
DTT: dithiothreitol

Daratumumab: Therapeutic asset, biological trap!

Daratumumab : joker thérapeutique, piège biologique !

V. Deneys[a,*], C. Thiry[a], A. Frelik[a], C. Debry[a], B. Martin[b], C. Doyen[b]

TRANSFUSION CLINIQUE ET BIOLOGIQUE

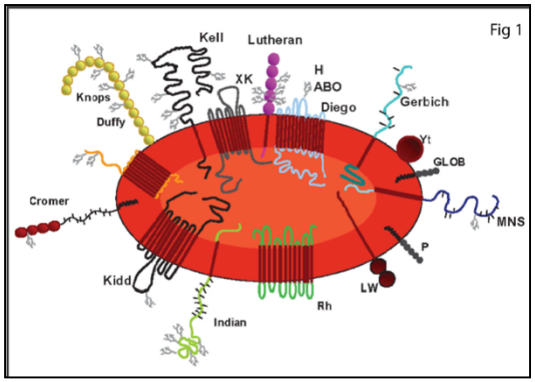
Transfusion Clinique et Biologique 25 (2018) 2–7

TIPS AND TRICKS

doi:10.1111/imj.13707

POSITION PAPER

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



Perform IAT antibody screen using DTT or trypsin treated red cells[c]

NEGATIVE

POSITIVE

NEGATIVE

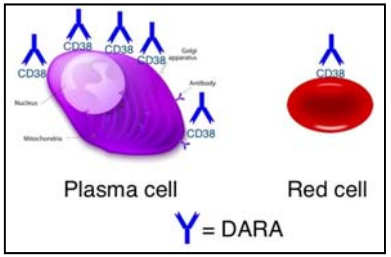
- Assume no clinically significant red cell alloantibody/ies
- Cannot exclude antibodies to antigens denatured by chosen treatment method (**see Box 1**)
- Transfuse ABO /RhD compatible blood and blood compatible for any significant antigens destroyed by the method used e.g. **Kell compatible** for DTT methods (see **Box 1**)
- Consider selecting blood matched to patient's extended **phenotype / genotype**, particularly if long-term transfusion support anticipated
- Abbreviated crossmatch (eXM or IS) and issue blood by usual protocol
- If IAT crossmatch used – will be positive unless donor cells are DTT or trypsin treated

POSITIVE

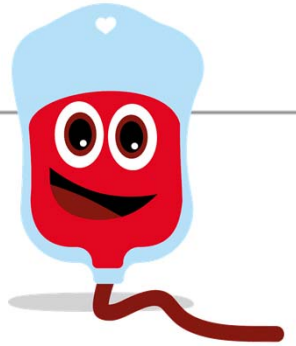
- Suggests presence of red cell alloantibody/ies.
- Identify antibody/s using DTT or trypsin treated ID antibody panel – *may require investigation by a Reference Laboratory*
- Cannot exclude alloantibodies against antigens denatured by chosen treatment method (**see Box 1**)
- Select blood that is compatible for antibody/s and antigens denatured by chosen treatment method, e.g. **Kell compatible** for DTT methods (see **Box 1**)
- If alloantibody cannot be identified for any reason, consider selecting blood matched to patient's extended **phenotype/genotype**, particularly if long-term transfusion support anticipated[d]
- Full IAT crossmatch – will be positive unless donor cells are DTT or trypsin treated

Internal Medicine Journal 48 (2018) 210–220

Azienda Ospedaliera "San Camillo-Forlanini" Roma	Gestione dei test pre-trasfusionali in pazienti trattati con daratumumab	PRO PSQST



Anno 2019: N.3/12 Pazienti trasfusi (25%), 21 U, >DRd



REDAZIONE, VERIFICA, APPROVAZIONE

Attività	Qualifica	Firma
Redazione	Responsabile area	Dott.ssa A. Matteocci
Verifica	RAQ	
Approvazione	Direttore SIMT	Prof. L. Pierelli

Anticorpo monoclonale umano IgG1k

GU n.153 del 3.7.17

GU n. 90 del 12.4.18

→ Pazienti adulti con mieloma multiplo recidivato e refrattario

→ Interferenza test pre-trasfusionali

→ Strategie di mitigazione

Algoritmi

Algoritmo n.1: INDAGINI IMMUNOEMATOLOGICHE

Paziente Pre-Daratumumab

ABO/Rh/K

DAT

IAT/PC

Neg

Pos

Neg

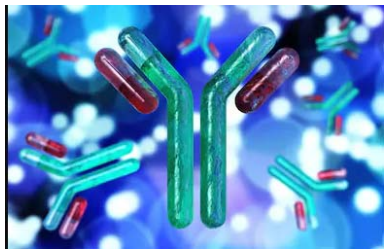
Pos

Non trasfuso

Fenotipo esteso

Genotipo esteso

Trasfuso



Paziente Post-Daratumumab

ABO/Rh/K

DAT

IAT/PC

Pos

DTT

Neg

Pos

Non trasfuso

Fenotipo esteso

Genotipo esteso

Trasfuso

UO EMATOLOGIA

A. COMUNICAZIONE PAZIENTE CON MM DA TRATTARE CON DARA

UO MEDICINA TRASFUSIONALE

NOTA EMONET

Assegnazione

ABO/Rh/K/DAT/IAT

POS

Immunoematologia EP

GENOTIPO ESTESO

STUDIO IE II LIVELLO

B. COMUNICAZIONE INIZIO TRATTAMENTO DARA E PROGRAMMA TRASFUSIONALE

Algoritmo n.2: RICHIESTA TRASFUSIONALE

UO MEDICINA TRASFUSIONALE

NOTA EMONET

Assegnazione

SELEZIONE UNITA'

Provetta

Immunoematologia EP

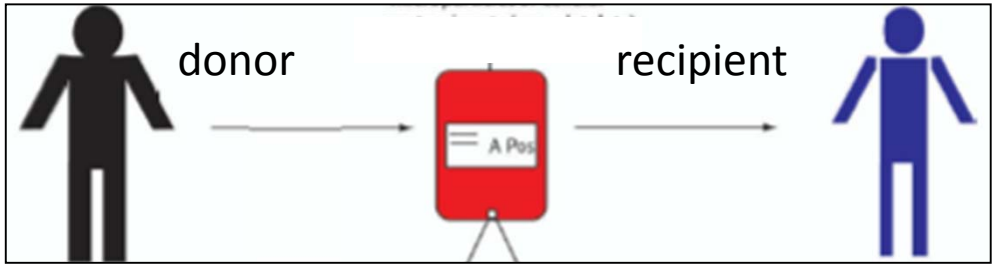
TRATTAMENTO CON DTT

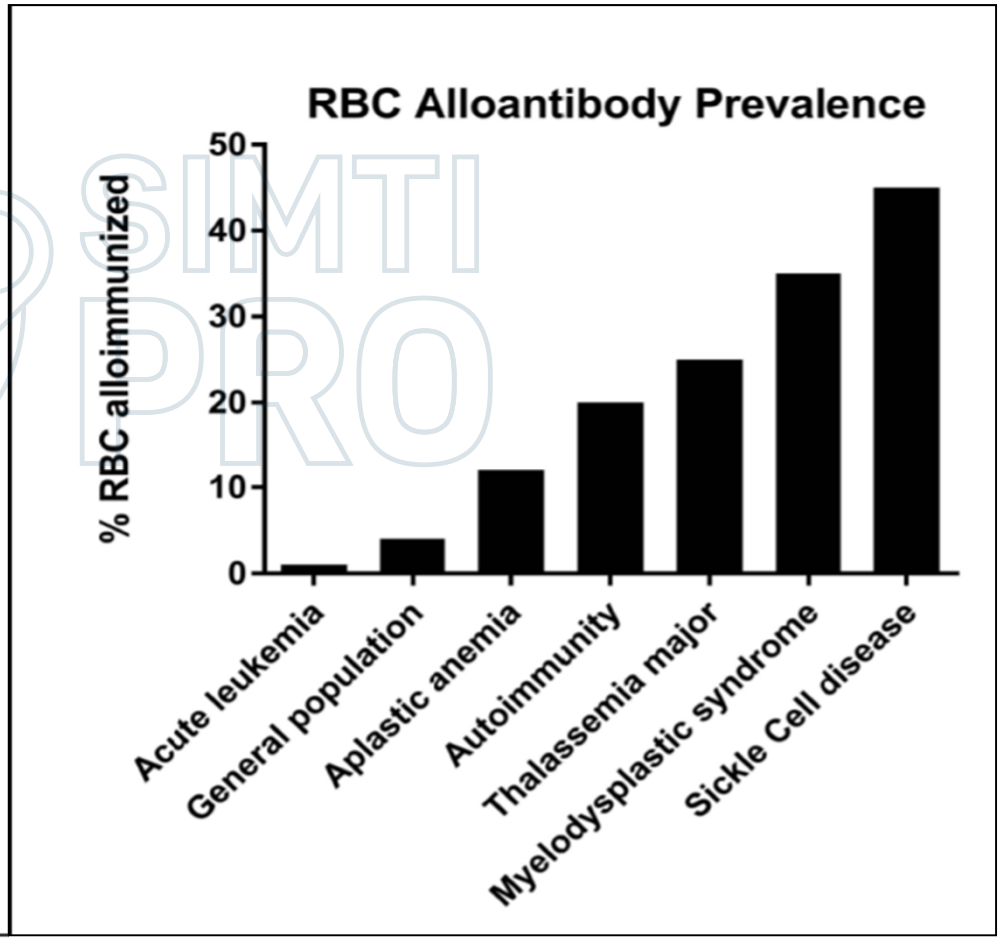
PC

Table 1. Alloimmunization rates reported in various patient populations and disease states*

Population or disease state	Reported alloimmunization rate (%)
General adult patients	
Retrospective analysis	1-3
Prospective analysis	8-10
Hemoglobin disorders	
Sickle cell disease	19-43
Thalassemia major	5-45
Inflammatory disorders	
Autoimmune disorders, general	16
Inflammatory bowel diseases	8-9
Lymphoid disorders	
Acute lymphoid leukemia	<1
Hodgkin lymphoma	<1
Non-Hodgkin lymphoma	2-3
Myeloid disorders	
Acute myeloid leukemia	3-16
Myelodysplastic syndromes (includes myelodysplastic/myeloproliferative disorders)	15-59
Solid tumors, nonhematopoietic	1-10
Transplantation	
Hematopoietic progenitor cell	1-4
Liver transplant	4-23
Other sites or multiple organ transplantation	1-10

Understanding red blood cell alloimmunization triggers, ASH 2016



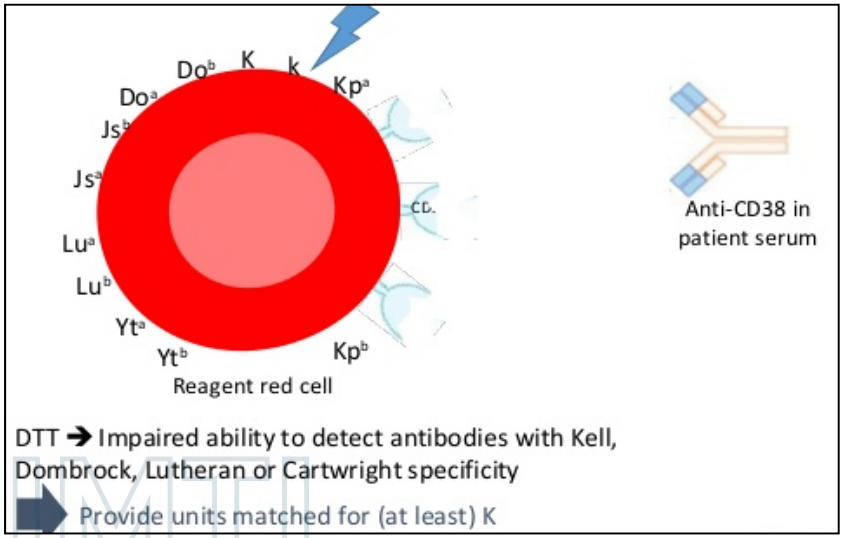


Transfusion-related red blood cell alloantibodies: induction and consequences, Blood 2019

Table 1. Mean evanescence rates by RBC alloantibody specificity.

Blood Group System	Mean Evanescence Rate in General Patient Groups*	Mean Evanescence Rate in Sickle Cell Disease Groups*
Duffy System		
Fy^a	17%	51%
Fy^b	–	78%
Kell System		
K	32%	41%
Js^a	–	80%
Kidd System		
Jka	49%	–
Jkb	54%	58%
Lewis System		
Le^a	48%	–
Le^b	52%	–
Lutheran System		
Lu^a	65%	–
MNS System		
M	30%	38%
S	30%	66%
P System		
P1	50%	–
Rh System		
D	12%	36%
C	19%	47%
c	27%	0%
E	38%	41%
C^w	61%	–
V	–	39%

Transfusion-related red blood cell alloantibodies: induction and consequences, Blood 2019



Blood Group	Clinical significance for transfusion reaction
Kell	Mild to severe/delayed hemolytic
Knops	No
Dombrock	Delayed and acute/hemolytic
Lutheran	No to mild/moderate
Cartwright	No to moderate(rare)/delayed
Lw^a	No to mild/delayed
JMH	No
Cromer	No to moderate to severe
Vel	No to severe/hemolytic

VoxSanguinis
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
Vox Sanguinis (2019)
© 2019 International Society of Blood Transfusion
DOI: 10.1111/vox.12864

ORIGINAL PAPER

Risk of RBC alloimmunization in multiple myeloma patients treated by Daratumumab

Zhan Ye, Laurie A. Wolf, Daniel Mettman & Fred V. Plapp
University of Kansas Medical Center, Kansas City, Kansas, USA

Table 2 Profile of transfused RBCs

	DARA transfusion group (246)	Non-DARA (284) transfusion group
ABO-Rh compatible RBCs only	109 units (32 patients)	284 units (All 46 patients) 2 patients with positive Ab screen received
ABO compatib phenotypicall matched RBC		
Both ABO-Rh compatible a compatible p phenotypicall matched RBC		

Table 1 Patient demographics

	Number of patients	Age	Gender ratio (female/male)	Percentage of death
DARA transfusion group	45 (145)	65·1 ± 10·6	21/24	27% (12/45)
Non-DARA	46 (328)	59 ± 10·1	24/22	20% (9/46)

Nessuna differenza tra i due gruppi per rischio immunizzazione

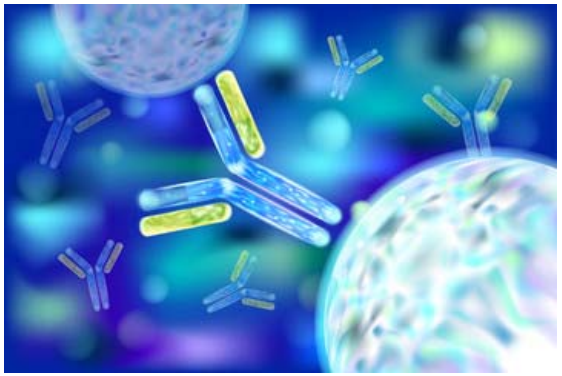
Table 3 Ab screen and Ab identification

	Ab screen before first DARA dose	Ab screen during study period: Total Ab screen performed	Positive Ab screen	Negative Ab screen	New alloantibody detected
DARA Transfusion Group (45 patients)	Negative	184	180 (42 patients)	4 (3 patients)	None (after DTT treatment)
Non-DARA Transfusion Group (46 patients)	Two patients had positive Ab screen from previous transfusion (anti-Jka and anti-K)	301	5 (2 patients)	296 (44 patients)	None (anti-Jka and anti-K persisted in those two patients)

Persistenza interferenza DARA nei test sierologici da 25 giorni a 5 mesi

VoxSanguinis
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
Vox Sanguinis (2018) 113, 492–498
INTERNATIONAL FORUM
© 2018 International Society of Blood Transfusion
DOI: 10.1111/vox.12653

Vox Sanguinis International Forum on typing and matching strategies in patients on anti-CD38 monoclonal therapy: summary



Question 1

Is dealing with patients on anti-CD38 therapy part of daily routine in your hospital or immunohaematology laboratory?

Question 2

Have you ever been dealing with a clinical relevant delay in the selection of compatible units, a missed irregular alloantibody, or haemolysis due to compatibility issues, with respect to a patient on anti-CD38 therapy?

Question 3

Are blood samples of patients on anti-CD38 therapy processed by your own immunohaematology laboratory, or by an immunohaematology reference laboratory?

Question 4

What mitigation strategy do you use to deal with the anti-CD38 interference?

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INTERNATIONAL FORUM
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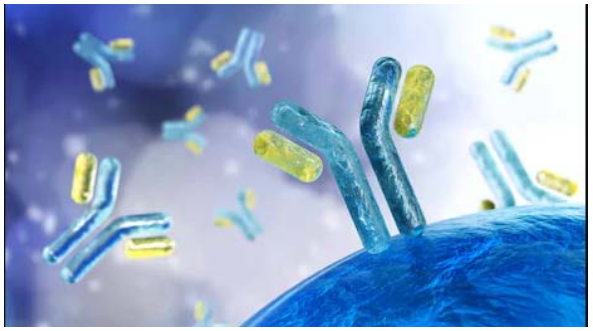


Question 5

a. In case you use DTT to denature CD38 on test RBCs, in what percentage of patient samples are irregular antibodies detected?
b. And in what percentage of samples does the DTT method fail (with conflicting controls)?

Question 6

What, if there would not be any technical, financial or reagent limitations, would be the ideal mitigation strategy to deal with the anti-CD38 interference?

Question 7

What, compared to routine type and screen selection, is the time delay you experience in selection of a compatible erythrocyte unit for a new patient on known anti-CD38 therapy?

Question 8

In your hospital, how is the putative anti-CD38 interference communicated with patient, laboratory and physicians?

VoxSanguinis
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
Vox Sanguinis (2018) **113**, 492–498

DOI: 10.1111/vox.12653

INTERNATIONAL FORUM

Vox Sanguinis International Forum on typing and matching strategies in patients on anti-CD38 monoclonal therapy: summary

K. M. K. De Vooght, M. Lozano, J-L. Bueno, A. Alarcón, I. Romera, K. Suzuki, E. Zhiburt, A. Holbro, L. Infanti, A. Buser, H. Hustinx, V. Deneys, A. Frélik, C. Thiry, M. Murphy, J. Staves, K. Selleng, A. Greinacher, J. M. Kutner, C. Bonet Bub, L. Castilho, R. Kaufman, M. E. Colling, P. Perseghin, A. Incontri & M. Dassi, D. Brilhante, A. Macêdo, C. Cserti-Gazdewich, J. M. Pendergrast, J. Hawes, M. N. Lundgren, J. R. Storry, A. Jain, N. Marwaha, R. R. Sharma

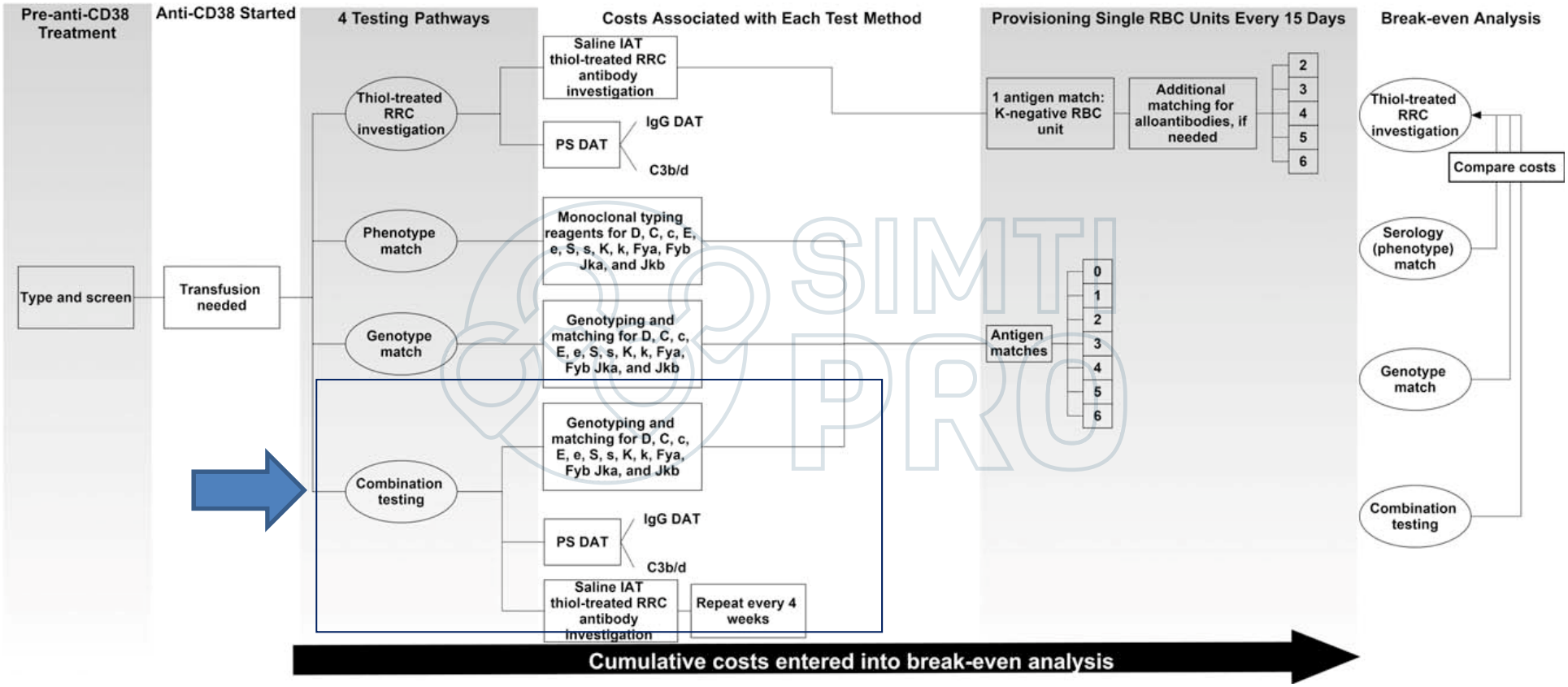
Conclusions

The interaction of anti-CD38 monoclonals with CD38 on the surface of test and donor RBCs could limit transfusion laboratories from completing routine pretransfusion testing and complicates the selection of suitable RBC units for patients on anti-CD38 therapy. As expected, institutions have different ways to deal with this interference. Most respondents use the DTT mitigation strategy, some rely on selection of extensively blood group typed RBCs for transfusion. Half of the respondents would prefer a plasma anti-CD38 neutralisation strategy, which has the advantage of being applicable in routine automation techniques. However, the development of such a technique lags behind.

Most respondents report a delay of one to a couple of hours to select compatible RBC units for a patient on therapy. The communication on the putative anti-CD38 interference with patient, laboratory and physicians is often orally and hardly automated.

From this survey, we can conclude that a lot of institutions have protocols in place to deal with the interference, however, these protocols could be tuned and there is a wish for automation in both preventing the interference as improving the communication about it between healthcare workers.

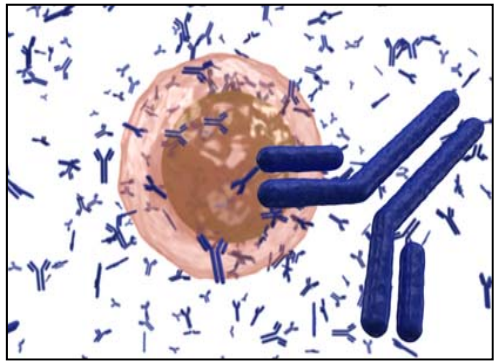
Practical approaches and costs for provisioning safe transfusions during anti-CD38 therapy



TRANSFUSION 2017;57;1470–1479

The impact of Daratumumab on transfusion service costs

Melissa M. Cushing,[1] Robert A. DeSimone,[1] Ruchika Goel,[1] Yen-Michael S. Hsu,[1] Priscilla Parra,[1] Sabrina E. Racine-Brzostek,[1] Diana Degtyaryova,[1] Dian T. Lo,[1] Meta Morrison,[1] Kathleen M. Crowley,[1] Adrianna Rossi,[2] and Ljiljana V. Vasovic[1]



TRANSFUSION 2019;59;1252–1258

A Notification and Testing and Transfusion Algorithm (NATTA)

Anti-CD38 ABSC Results	History of an Autoantibody or Alloantibody?	Is red cell genotyping available?	Is a DT ABII indi
Negative	No	Not indicated	Not ind
Negative	Yes	Available*	Not ind
Positive	Yes	Available*	Not ind
Positive	No	Available	Not ind
Positive	No	Pending	Indic

B Genotyping / Phenotypically -similar RBCs Transfusion Algorithm (GPSR)

Is red cell genotyping available?	History of an Auto or Allo Antibody?	What is the ABSC result?	Al
Always Available as per GPSR Protocol	Yes or No	Positive or Negative	Not per

Fig. 1. Comparison of two approaches to pretransfusion testing and RBC transfusi

TABLE 4. DARA patient characteristics

Patient demographics	
Number of patients (%)	91 (100%)
Age (years, mean +/– SD)	65.2 +/– 10.2
Male (%)	53 (58.2%)
Diagnoses (%)	
- Multiple myeloma	90 (98.9%)
- Diffuse large B-cell lymphoma	1 (1.1%)
Stem cell transplant (%)	61 (67.0%)
- Autologous transplant	57 (62.6%)
- Allogeneic transplant	4 (4.4%)
Patients with unexpected antibodies detected (%)	24 (26.4%)
- Alloantibodies	6 (6.6%)
- Warm autoantibodies	12 (13.2%)
- Cold autoantibodies	9 (9.9%)
- Nonspecific reactivity	5 (5.5%)
Patients with pretransfusion testing (ABSC) (%)	60 (65.9%)
Patients with positive ABSC (%)	53/60 (88.3%)
Patients transfused after NATTA (%)	31 (34.1%)
RBC units transfused per patient among the 31 patients transfused, median (IQR)	8 (3,13)

ABSC = antibody screen; DARA = daratumumab; IQR = interquartile range; NATTA = notification and testing/transfusion algorithm; SD = standard deviation.

The impact of Daratumumab on transfusion service costs

Melissa M. Cushing,[1] Robert A. DeSimone,[1] Ruchika Goel,[1] Yen-Michael S. Hsu,[1] Priscilla Parra,[1] Sabrina E. Racine-Brzostek,[1] Diana Degtyaryova,[1] Dian T. Lo,[1] Meta Morrison,[1] Kathleen M. Crowley,[1] Adrianna Rossi,[2] and Ljiljana V. Vasovic[1]

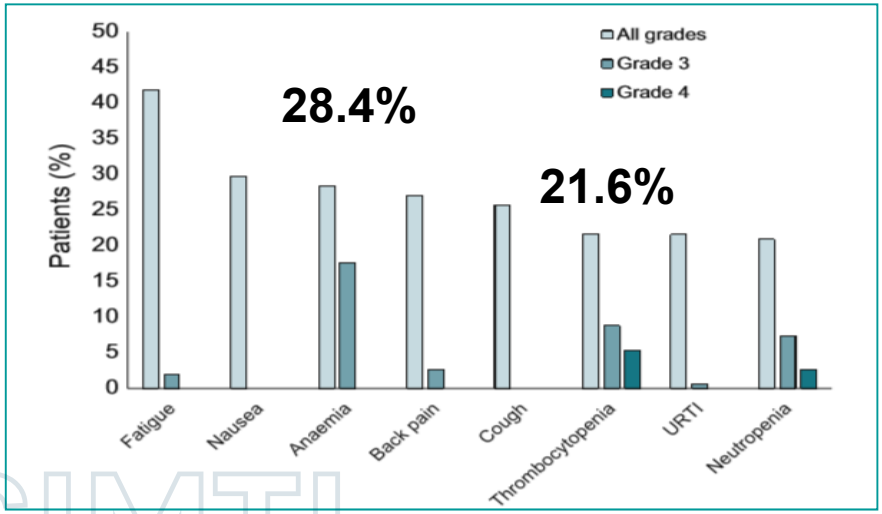
TRANSFUSION 2019;59;1252–1258

TABLE 3. Cost comparison of two approaches to pretransfusion testing and RBC transfusion for patients on daratumumab (estimated costs during year 1)

		NATTA approach		GPSR approach		GPSR/NATTA
Test/Unit	Cost per test and RBC unit	Tests/ units (N)	Total cost	Tests/ units (N)	Total cost	estimated additional cost
Pretransfusion testing			**$28,014.30**		**$35,725.41**	**27.53%**
DTT screen	$57.66	*315*	$18,162.90	*0*	$0.00	
Genotyping*	$295.00	*24*	$7,080.00	*91*	$26,845.00	
IAT XM	$29.80	*93*	$2,771.40	*298*	8,880.41	
EXM	$0	*205*	$0.00	*0*	$0.00	
RBC transfusions			**$20,739.8**		**$49,344.00**	**137.92%**
Phenotyping for K-negative RBC units	$32.20	*179*	$5,763.80	*0*	$0.00	
Phenotypically similar RBC units†	$192.00	*78*	$14,976.00	*257*	$49,344.00	
Total cost per year 1			**$48,754.10**		**$85,069.41**	**74.48%**
Average cost per patient per year (N = 91)			**$535.76**		**$934.83**	

* For NATTA approach, genotyping only performed for recipients with autoantibodies or alloantibodies.
† A four-antigen match requirement was used.
DTT = dithiothreitol; EXM = electronic crossmatch; GPSR = genotyping with a provision of phenotypically similar RBC transfusions; IAT XM = indirect antiglobulin test crossmatch; NATTA = notification and testing/transfusion algorithm.

Dopo l'infusione di Daratumumab è stata osservata una lieve e clinicamente non-significativa riduzione dei livelli di Hb di circa 1.6 g/dL ed un incremento compensatorio nella conta reticolocitaria.
Test in vitro non hanno evidenziato alcuna lisi eritrocitaria complemento-mediata.
La frazione di emazie legate al farmaco scompare dal circolo per un sequestro splenico mediato dal legame con Fc-receptor.

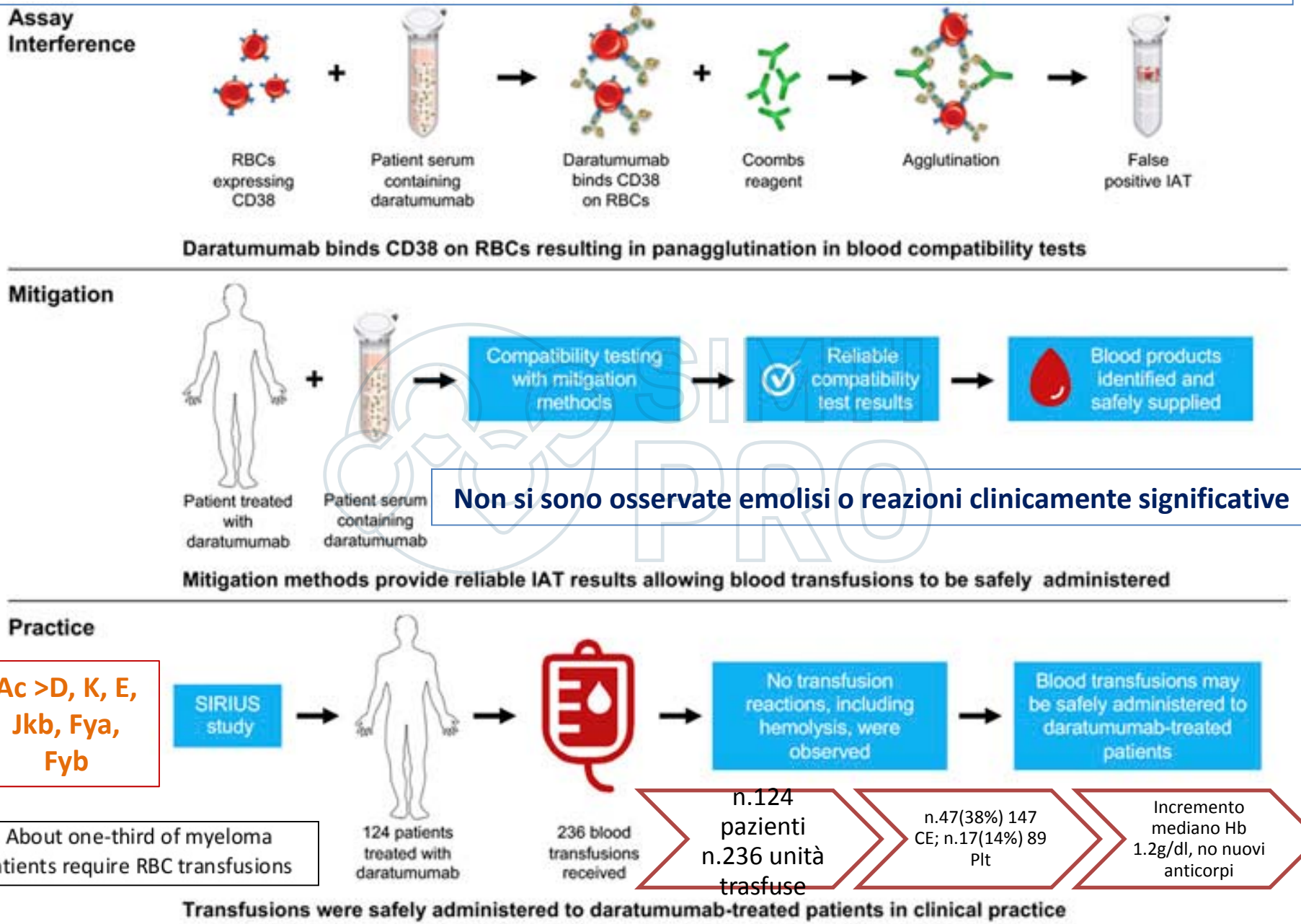


Dara + Lenalidomide + Desametasone
Studio POLLUX

Dara + Bortezomib + Desametasone
Studio CASTOR

	Gruppo Daratumumab		Gruppo Controllo	
	Tutti i Gradi	Gradi 3-4	Tutti i gradi	Gradi 3-4
POLLUX Anemia	31,1%	12,4%	34,9%	19,6%
POLLUX Piastrinopenia	26,9%	12,7%	27,4%	13,5%
CASTOR Anemia	26,3%	14,4%	31,2%	16%
CASTOR Piastrinopenia	58,8%	45,3%	43,9%	32,9%

Blood Transfusion Management and Transfusion-Related Outcomes in Daratumumab-Treated Patients With Relapsed or Refractory Multiple Myeloma - Clinical Lymphoma, Myeloma & Leukemia 2018



RBC, red blood cell; IAT, indirect antiglobulin test.

Transfusion management in multiple myeloma patients receiving daratumumab: Experience of a single tertiary care centre

Transfusion and Apheresis Science, 2019

In order to optimize and to make the DTT technique available for 24 h, DTT-treated RBCs were suspended in a RBC storage solution that extended the shelf life until 30 days.

Four volumes of 0.2M DTT were added to 1 vol of washed packed RBCs. The mixture was gently shaken and incubated at 37 °C for 30–45 min in a dry heat block. After incubation, the DTT treated RBCs were washed four times manually with normal saline. DTT treated RBC were reconstituted at 3–5% in an RBC preservative solution.
The DTT treated RBC were stable for a month, as checked by RBC phenotyping for Rh, K, Fy, JK, M and S antigens.

Disbro WL. Stability guidelines for dithiothreitol-treated red blood cell reagents used for antibody detection methods in patients treated with Daratumumab. Immunohematology 2017;33:105–10.

Pazienti	N.
Maschi	21
Femmine	23
Totale	44

Periodo osservazione: 30 mesi

Età mediana 70 anni

Mediana di 2 (range 1–7) linee di terapia and mediana di 16 dosi di Dara (range 1–39)

N.53 RAI eseguite a 13 pazienti (29.5%) che hanno trasfuso una mediana di 6 unità di GR (Rh/K) (range 1–22)
N. 99 unità trasfuse senza ritardi o eventi avversi. Nessuna nuova alloimmunizzazione.

Daratumumab in monoterapia (mediana di 3 linee di terapia, range 2 - 8):

- 2 casi di anemia grado I
- 13 casi di anemia grado II
- 7 casi di anemia grado III
- 13 casi di piastrinopenia di vario grado

12/50 pz (24%) hanno necessitato di trasfusioni per anemia e piastrinopenia

Daratumumab in combinazione con Lenalidomide (DRd) o Bortezomib (DVd) e Desametasone (mediana di 1 linea di terapia, range 1 - 6):

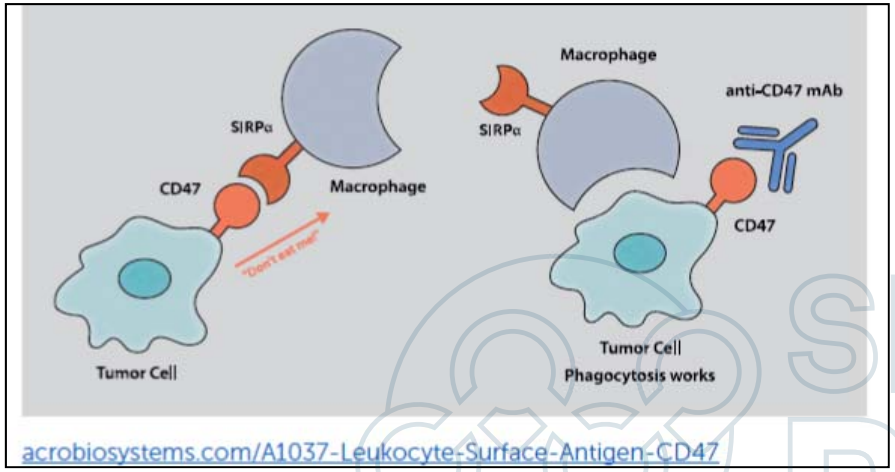
- 3 casi di anemia grado I
- 4 casi di anemia grado II
- 7 casi di anemia grado III
- 12 casi di piastrinopenia di vario grado (10 nel braccio DVd)

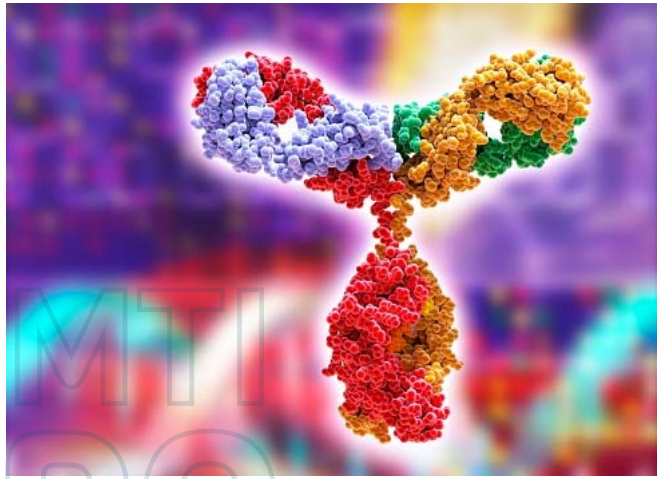
10/70 pz (14%) hanno necessitato di trasfusioni per anemia e piastrinopenia

Gruppo GIMEMA LAZIO - Federico Vozella, 2020

The effects of monoclonal anti-CD47 on RBCs, compatibility testing, and transfusion requirements in refractory acute myeloid leukemia

Transfusion 2019





Anti CD47 (Hu5F9-G4) è un anticorpo monoclonale umanizzato IgG4

E' una glicoproteina transmembrana espressa su tessuti e cellule

Agisce come segnale self, «Don't eat me»

Ha un ruolo nella fagocitosi per la regolazione dei processi cellulari attraverso i macrofagi (legame CD47-SIRPα)

Sono in corso diversi trial clinici (malattie ematologiche e tumori solidi)

Impact of Novel Monoclonal Antibody Therapeutics on Blood Bank Pretransfusion Testing

Hematol Oncol Clin N Am 33 (2019) 797–811

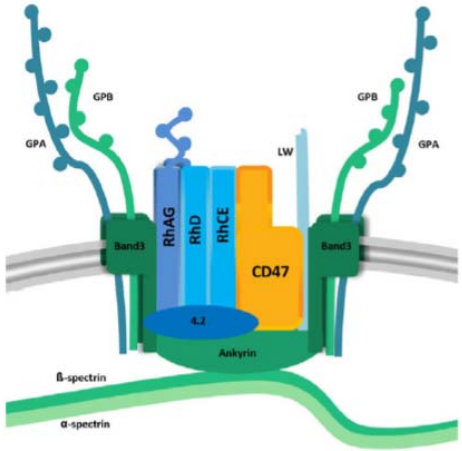


Table 5
Representative pretransfusion testing results for one of the anti-CD47 agents

	Patient Results Before Anti-47 Therapy	Patient Results Post Anti-47 Therapy	Presence of Interference?
ABO/Rh typing	A+	Forward type: A+ Reverse type: O	Yes
Antibody Screen	Negative	Pan-reactive +	Yes
Autocontrol	Negative	Positive	Yes
Direct antiglobulin test	Negative	Negative/positive	Possible
Eluate	Not performed	Negative/pan-agglutinin	Possible

Noticeable interference is present during ABO/Rh typing for A, B, and AB patients. The antibody screen is always positive and can be resolved using a reagent that does not bind to IgG4 Fc regions.

Monoclonal anti-CD47 interference in red cell and platelet testing

TRANSFUSION 2019;59;730–737

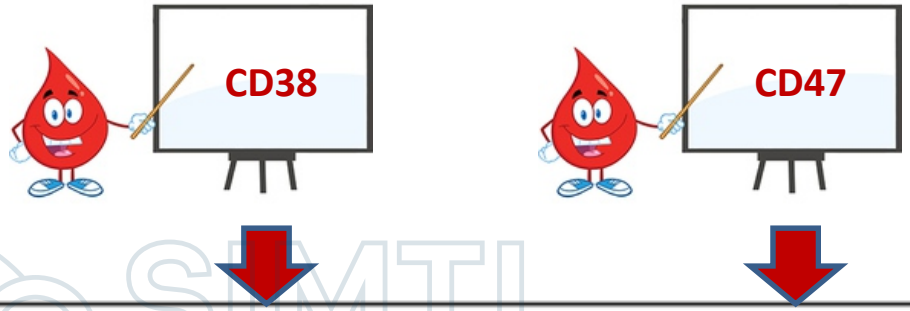


TABLE 3. Comparison between the RBC expression and the characteristics of pretransfusion interference observed with anti-CD38 (Daratumumab/DARA or Isatuximab) and anti-CD47 (Hu5F9-G4) therapy

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 antiglobulin without IgG4
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+)

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

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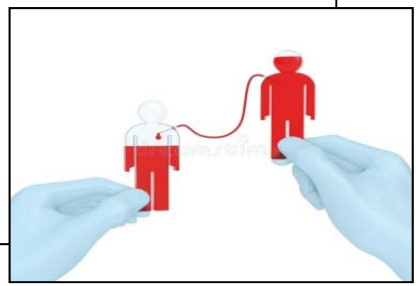
Overall the strategies to mitigate interference by these mAb agents can be divided into 3 different categories:

- Remove the presence of the target antigen on reagent RBC
- Neutralize the offending antibody
- Use different reagents that are less sensitive to the mAb effect



POSITION PAPER

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

Internal Medicine Journal 48 (2018) 210–220

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).

Before starting daratumumab my blood test results collected on

_____ / _____ / _____ were:
DD MM YYYY

Blood type: ▫ A ▫ B ▫ AB ▫ O ▫ RhD+ ▫ RhD-

Antibody screen was:

▫ Negative ▫ Positive for the following antibodies:

_____

Other: _____

Contact details of institution where the blood tests were performed:

_____

CONCLUSIONI

Circa il 25-30% dei pazienti trattati con daratumumab vengono trasfusi (DARA>DRd>DVd), diversi fattori legati a malattia/terapia

Procedura DTT necessaria nel 30% dei casi se non utilizzata la provetta.

<u>Problematiche</u>
Molarità DTT, ratio GR/DTT, tempo incubazione, conservazione, tecnologie, automazione, nuovi reagenti, algoritmi trasfusionali e costi, **altri farmaci???**

GRAZIE

Some patients visit multiple hospitals and relevant history may be obtained from another facility, if known. Complete patient demographics and transfusion and medication history orders will improve efficiency and the costs of testing.