STRATEGIE TERAPEUTICHE ATTUALI E FUTURE NEL MIELOMA MULTIPLO:
LA CHEMIOTERAPIA E GLI ANTICORPI MONOCLONALI

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Interferenza degli anticorpi monoclonali nei test di tipizzazione del sangue e di valutazione della risposta al trattamento

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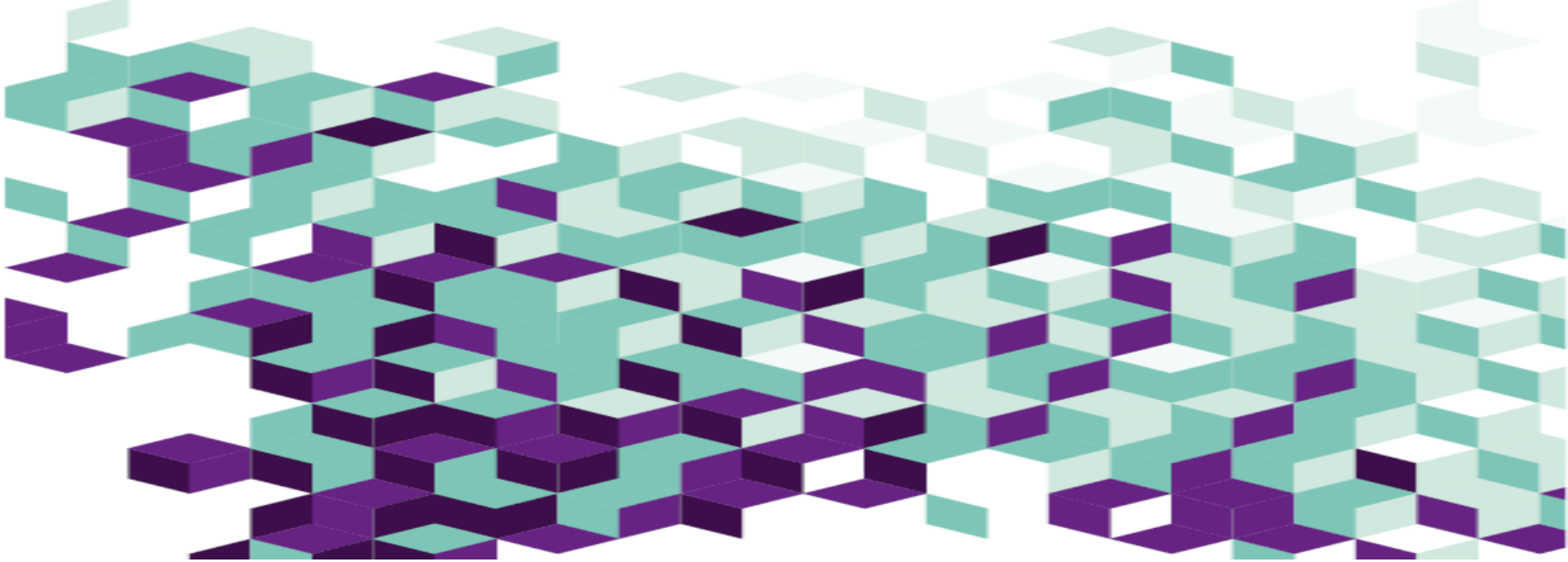
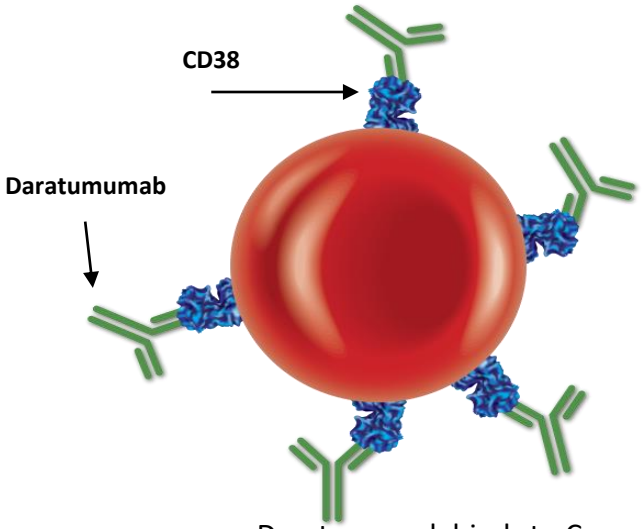


Disclosure

- I have no actual or potential conflict of interest in relation to this program/presentation.

Daratumumab Binds to CD38

- Daratumumab is a human monoclonal antibody for the treatment of multiple myeloma[1]
- Daratumumab binds to CD38[2], a protein that is ubiquitously expressed on myeloma and lymphoma cells[3-5] but at low levels on normal lymphoid and myeloid cells[6]
- CD38 is also expressed at low levels on red blood cells (RBCs)[7-9]
- CD38 monoclonal antibodies interfere with indirect antibody testing[10]



Daratumumab binds to C
on RBCs[2]

1. de Weers M, et al. *J Immunol*. 2011;186:1840-8.
2. Chapuy CI, et al. *Transfusion.* 2015;55(6Pt2):1545-1554.
3. Lin P, et al. *Am J Clin Pathol.* 2004;121:482-8.
4. Santonocito AM, et al. *Leuk Res.* 2004;28:469-77.
5. Doshi P, et al. *Haematologica.* 2014;99 (s1):138. Abstract P434.
6. Deaglio S, et al. *Leuk Res.* 2001;25:1-12.
7. Albeniz I, et al. *Hematology.* 2007;12:409-14.
8. Mehta K, et al. *FASEB J.* 1996;10:1408-17.
9. Zocchi E, et al. *Biochem Biophys Res Commun.* 1993;196:1459-65.
10. Oostendorp M, et al. Transfusion. 2015 Jun;55(6 Pt 2):1555-62.

Major and Minor Antigens

- ABO & Rh (D) are well-known major RBC antigens
- If a patient has a particular antigen, they will not produce the corresponding antibody
- However, if a patient does not express an antigen, their body automatically produces the antibody
  - For example, if the A antigen is expressed (but not the B antigen), the patient will produce anti-B antibodies (but not anti-A), and their blood type will be “A”
- Antibodies against minor antigens (irregular antibodies) are only developed by the body after exposure (ie, after a prior transfusion)
  - If the patient already has the antigen, they will not develop the antibody to it

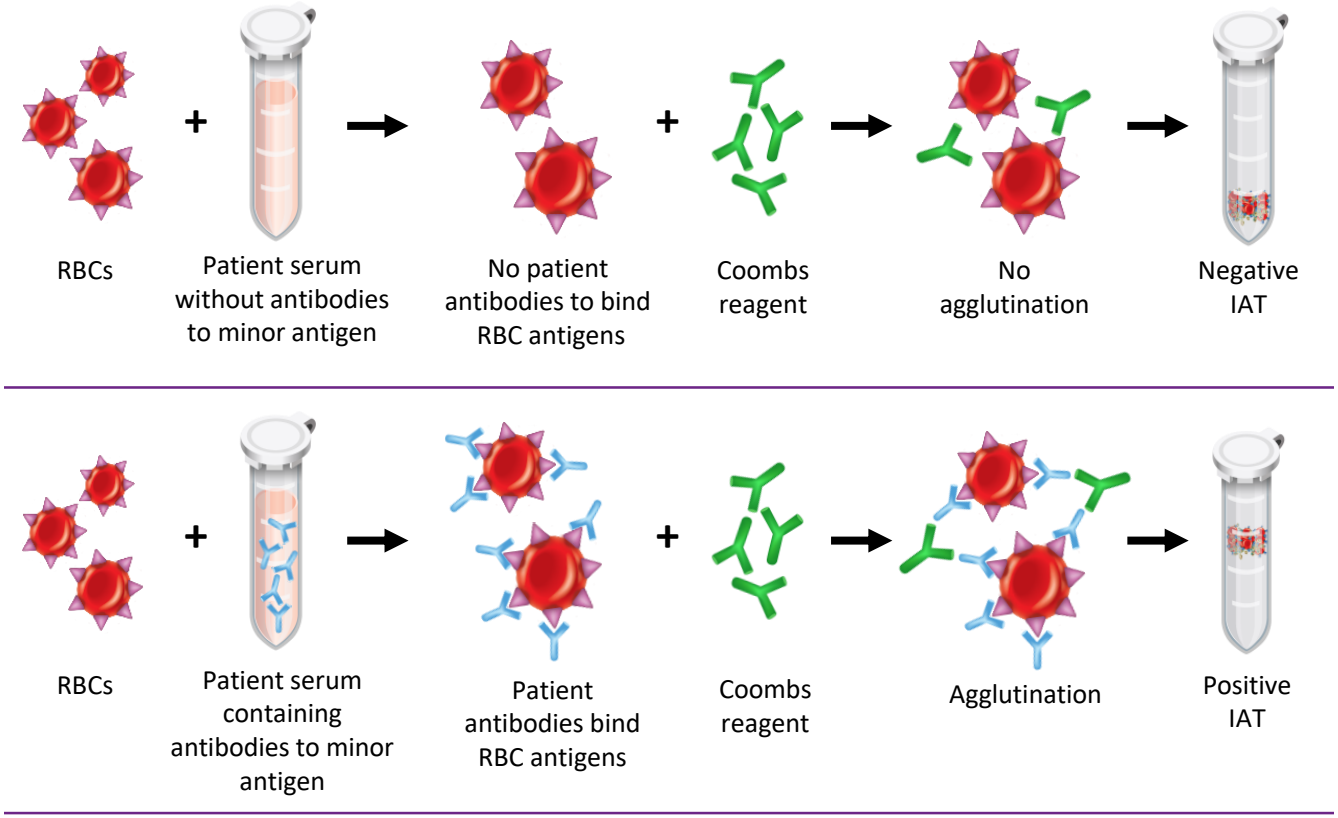
Blood transfusion compatibility testing for patients receiving CD38 mAbs

- CD38 is expressed on human red blood cells (RBCs)
- **Daratumumab binds to CD38 on RBCs → false positive** results in the Indirect Antiglobulin Test (**indirect Coombs test**)
- Daratumumab does **not interfere** with the major antigens of **ABO/RhD typing**, but with the minor ones
- **Effect is class specific for CD38 monoclonal antibodies**
- This may complicate timely release of blood products

Chapuy et al. Transfusion. 2015;55(6 Pt 2):1545-54
Oostendorp et al. Transfusion. 2015;55(6 Pt 2):1555-62

Mechanism of a Typical IAT

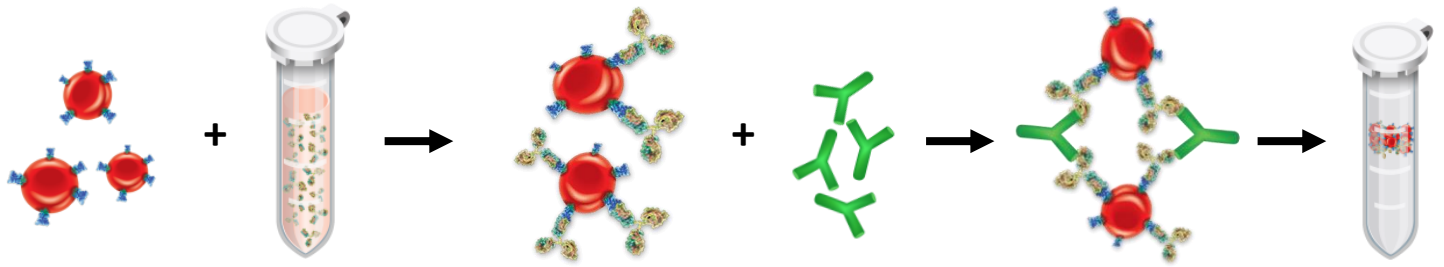
- In an IAT, antibodies to minor antigens (irregular antibodies) on reagent RBCs are detected by agglutination



Chari A, et al. Poster presented at: 2015 American Society of Hematology (ASH); December 5-8, 2015; Orlando, FL, USA (Abstract 3571).

Sera Containing Daratumumab Mimic a Positive IAT

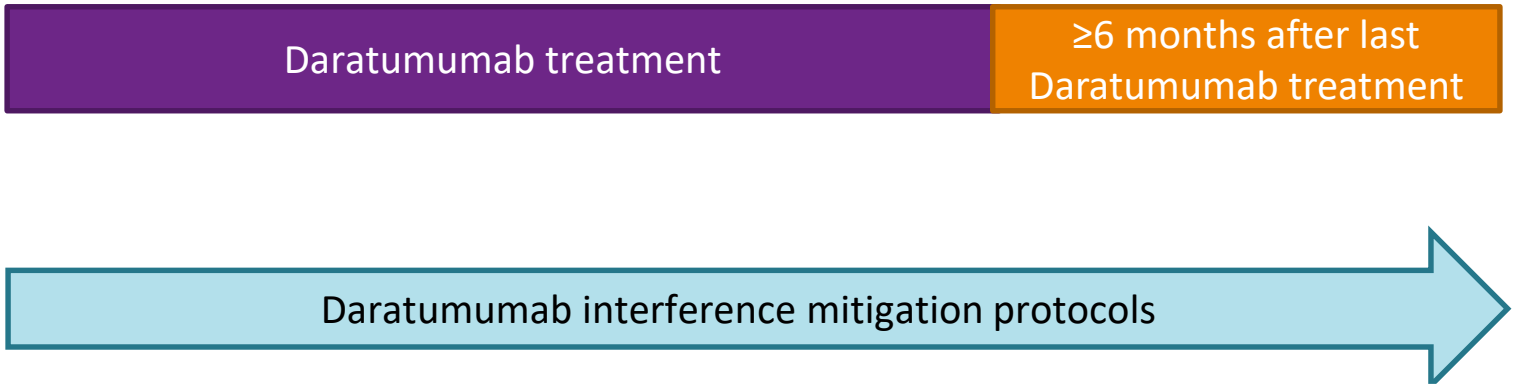
- Daratumumab in the patient's serum binds to reagent or donor RBCs in an IAT, resulting in pan-agglutination, and masking the presence of antibodies to minor antigens (irregular antibodies)[1,2,3]
- Daratumumab interference was identified when pan-agglutination was observed during RBC panel testing in 100% of patient samples from a clinical trial[1,2,3]
  - Agglutination was detected using solid phase and tube testing with PEG, LISS, or no enhancement and using LISS gel column techniques[1,2]
  - Adsorption with untreated or ZZAP-treated RBCs does not negate Daratumumab-mediated pan-agglutination, even after multiple rounds of adsorption[1]



1. Chapuy CI, et al. *Transfusion.* 2015;55(6Pt2):1545-1554.
2. Oostendorp M, et al. *Transfusion.* 2015;55(6Pt2):1555-62.
3. Chari A, et al. Poster presented at: 2015 American Society of Hamatology (ASH); December 5-8, 2015; Orlando, FL, USA (Abstract 3571)

Compatibility Testing Can Be Performed on Patients Treated with Daratumumab

- **If steps are not taken to mitigate Daratumumab interference, delays in the release of blood products for transfusion may occur**
- To avoid unnecessary delays, it is essential that mitigation protocols be applied to Daratumumab-treated patient samples
  - Once treatment with Daratumumab is discontinued, pan-agglutination may persist; the duration of this effect varies from patient to patient, but may persist for up to 6 months[2]
  - Mitigation methods should be used until pan-agglutination is no longer observed



1. Chapuy CI, et al. *Transfusion.* 2015;55(6Pt2):1545-1554.
2. Oostendorp M, et al. *Transfusion.* 2015;55(6Pt2):1555-62.
3. Chapuy CI, et al. *Transfusion.* 2016; 56(12):2964-2972.

Can Compatibility Testing Still Be Performed on Daratumumab-treated Patients?

Interference in the blood bank Conclusion: Methods to negate DARA

1. **Serotyping / genotyping before first DARA infusion**

2. **Treating reagent RBCs with DTT→ panreactivity with the samples is eliminated**

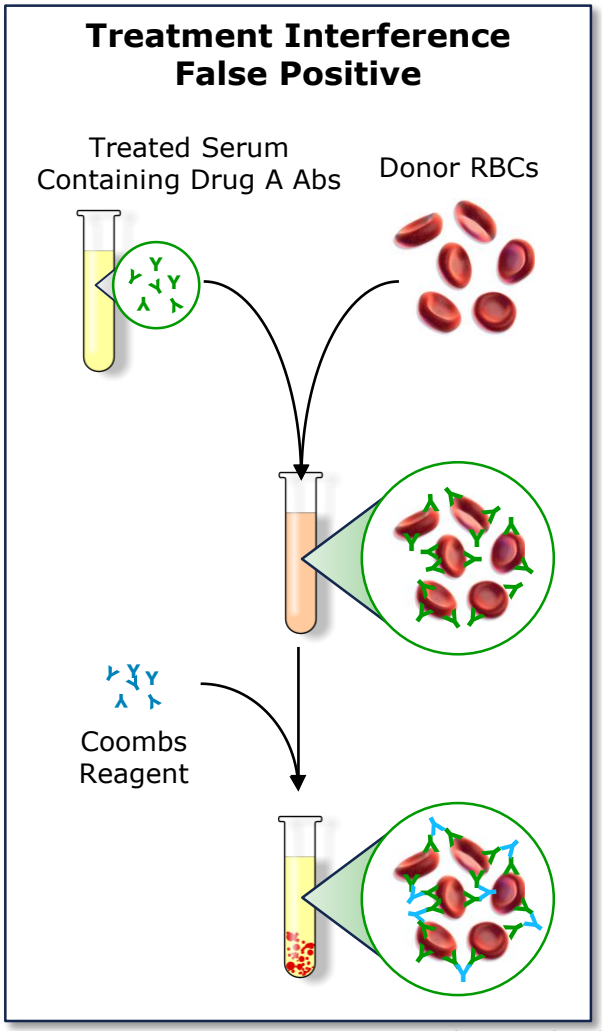
   Disruption of limited number of blood group antigens including Kell

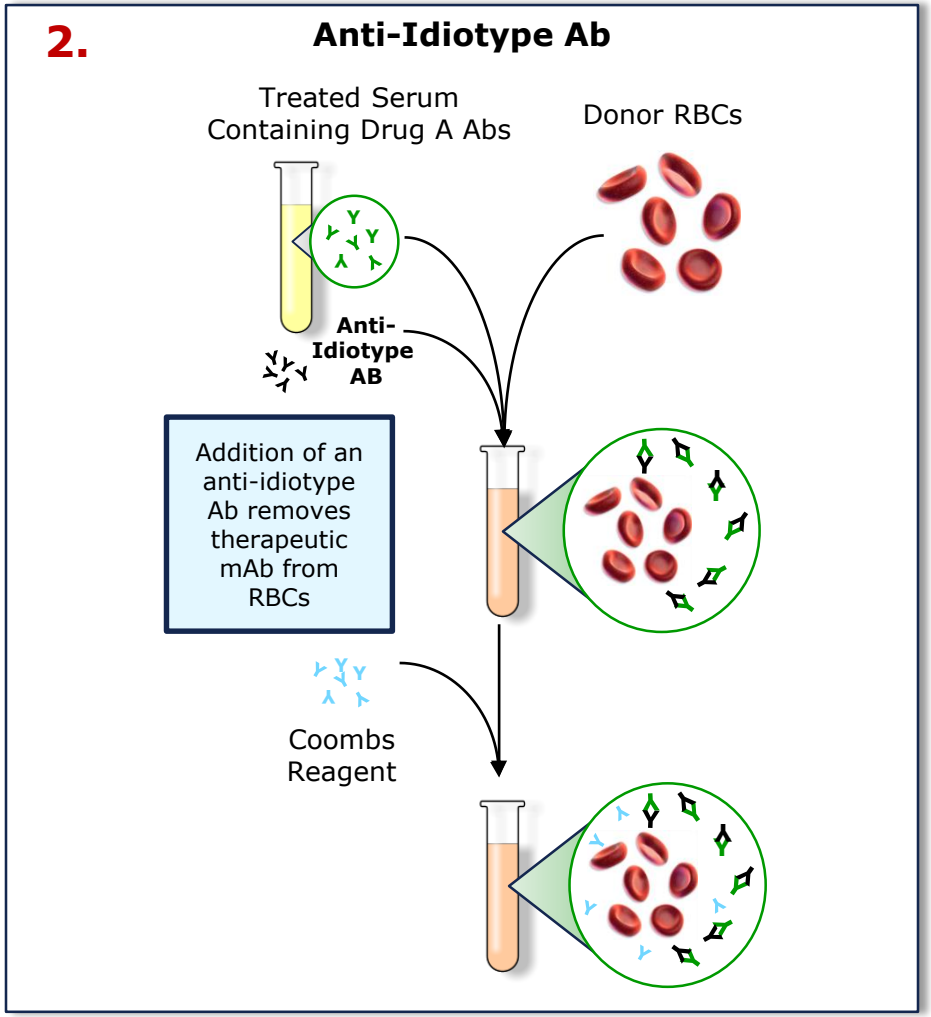
3. **Adding anti-DARA idiotype (DARA neutralizing antibody) to the plasma of DARA-treated patients eliminates positive antibody screen reactions**

   Simple but not available

Potential Solution To Assay Interference

Methods for Mitigating Monoclonal Antibody Therapy Assay Interference

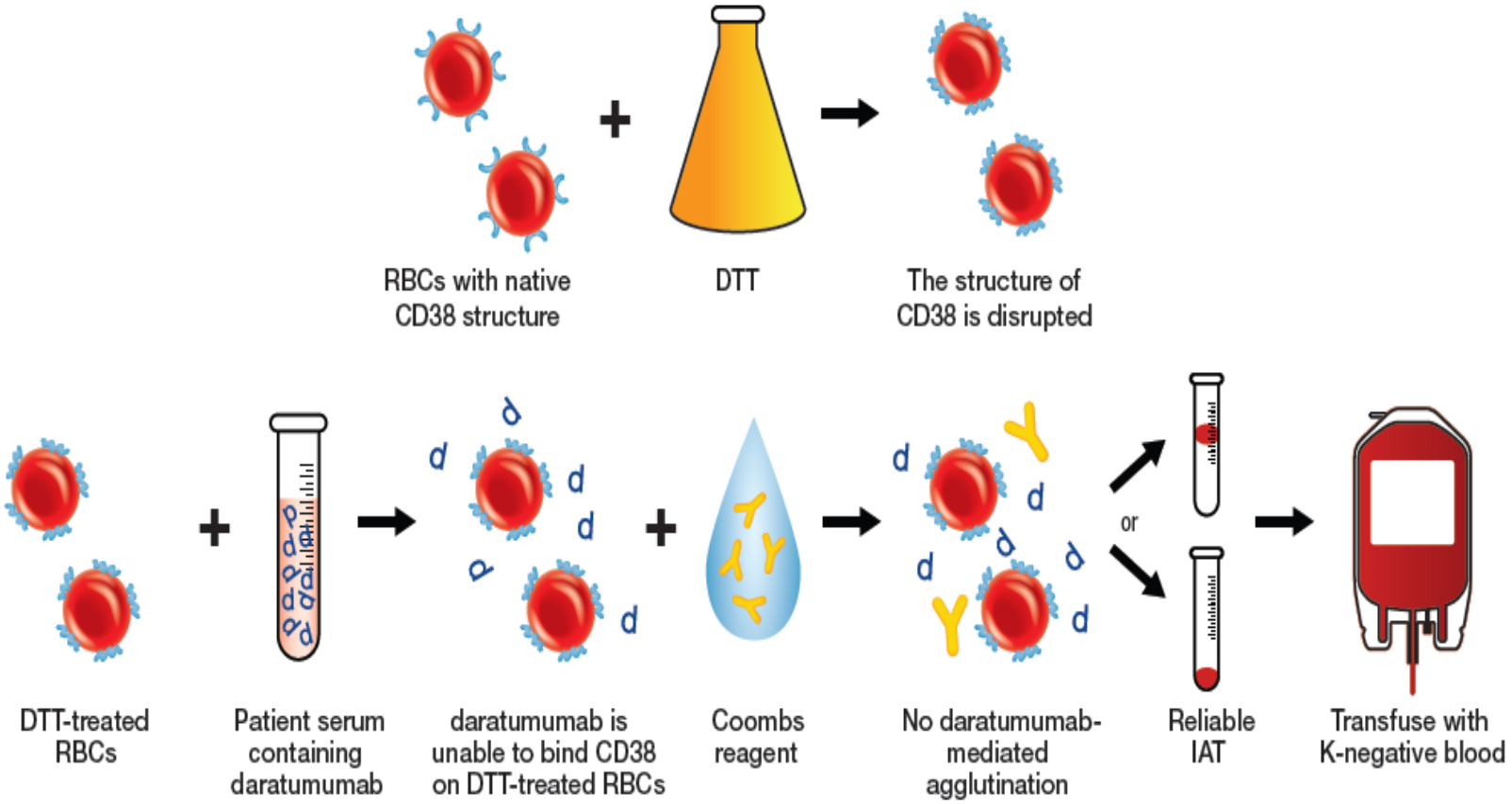




1. van de Donk Blood 2016;27(6):681–695; 2. van de Donk Immunol Rev 2016;270: 95–112

Mitigating Daratumumab Interference:
Treat Reagent RBCs with DTT or Locally Validated Methods

- Since the Kell blood group system is also sensitive to DTT treatment[2], K-negative units should be supplied after ruling out or identifying alloantibodies using DTT-treated RBCs



1. Chapuy et al. Transfusion. 2015;55(6 Pt 2):1545-54
2. Westhoff CM, Reid ME. Immunohematology. 2004;20(1):37-49

Using DTT-treated RBCs for Assays With Patient Samples

- Treating reagent RBCs with DTT eliminated pan-reactivity in 100% of Daratumumab-treated patient samples[1]

Patient	DAR dose, mg/kg	Results of antibody screen using non-DTT-treated RBCs	Results of antibody screen using DTT-treated RBCs
1	8	Pan reactive	Negative
2	8	Pan reactive	Negative
3	8	Pan reactive	Negative
4	16	Pan reactive	Negative
5	16	Pan reactive	Negative

DAR, Daratumumab; DTT, dithiothreitol; RBC, red blood cell.
Adapted from Chapuy et al. *Transfusion.* 2015;55(6Pt2):1545-1554.

- Since the Kell blood group system is also sensitive to DTT treatment,[2] K-negative units should be supplied after ruling out or identifying alloantibodies using DTT-treated RBCs
- Approximately 9% of the population is reactive to the Kell blood group system[2]; therefore, >90% of blood units will be Kell-negative and suitable for transfusion

1. Chapuy CI, et al. *Transfusion.* 2015;55(6Pt2):1545-1554.
2. Westhoff CM, Reid ME. *Immunohematology.* 2004;20:37-49.

Masked Alloantibodies Are Identifiable Using DTT-treated Reagent RBCs

- In plasma samples spiked with Daratumumab, alloantibodies masked by Daratumumab mediated pan-agglutination were identifiable after DTT treatment[1]

Screening cell	Plasma	Alloantibody	Ab screen result	Panel cells	Panel result
Cell 1 Cell 2	No DARA	-	0 0	Untreated	No reactivity
Cell 1 Cell 2	No DARA	Anti-E	0 1+	Untreated	Anti-E
Cell 1 Cell 2	+ DARA	-	1+ 1+	Untreated	Panreactivity
Cell 1 Cell 2	+ DARA	Anti-E	1+ 1+	Untreated	Panreactivity
Cell 1 + DTT Cell 2 + DTT	+ DARA	-	0 0	DTT-treated	No reactivity
Cell 1 + DTT Cell 2 + DTT	+ DARA	Anti-E	0 1+	DTT-treated	Anti-E

DAR, Daratumumab; DTT, dithiothreitol; RBC, red blood cell.
Adapted from Chapuy et al. *Transfusion.* 2015;55(6pt2):1545-1554.

1. Chapuy CI, et al. *Transfusion.* 2015;55(6Pt2):1545-1554.

What Is the Clinical Impact of Daratumumab Interference?

Daratumumab Interference Is Clinically Manageable

- To date, **no clinically significant hemolysis** has been observed in patients receiving daratumumab, and **no transfusion reactions** have occurred in patients requiring RBC transfusions
- Chari et al (2015) conducted an analysis of RBC transfusions and transfusion-related adverse events in the SIRIUS study[1]
  - Forty-seven (38%) patients received a total of 147 transfusions of packed RBCs (PRBCs) and these transfusions were not associated with complications
- To avoid unnecessary delays, it is essential that the blood bank is informed that a patient will start a CD38 monoclonal antibody or that they will receive a sample from a CD38mAb-treated patient, so th’at appropriate protocols can be applied

Chari A et al. ASH 2015 (Abstract 3571) Poster presentation

RBC, red blood cell

What Information Does the Blood Bank Need?

If a Patient Who Received Daratumumab Requires a Transfusion

It may be prudent to have clinicians notify the blood bank concerning patients receiving daratumumab. Other options are to use clinical decision support tools to automatically notify blood bank laboratory information systems (LISs) or to use regional/national databases with automatic alerting via the local LIS.

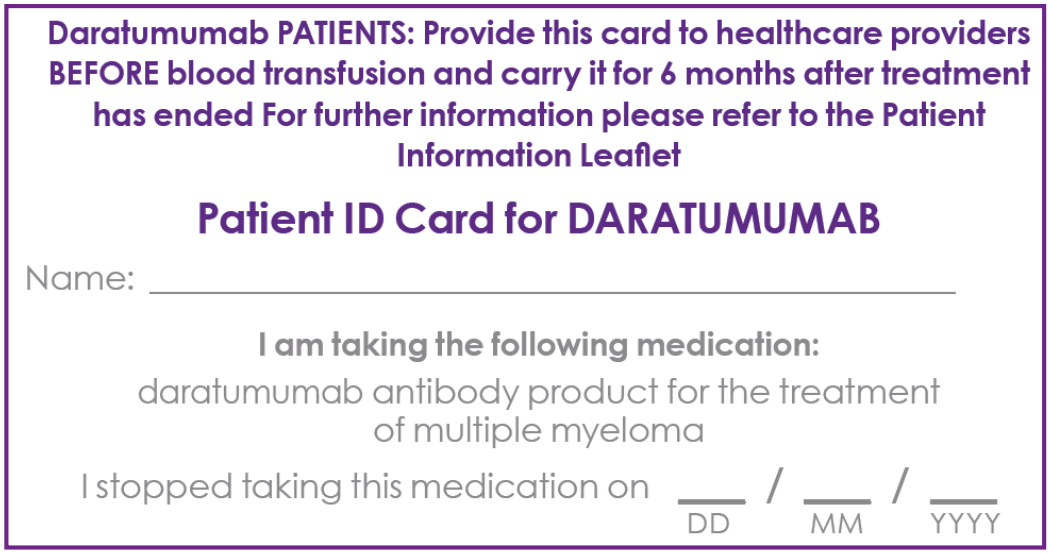
REMEMBER

If a patient who received daratumumab requires a transfusion:

- Type and screen patients prior to starting daratumumab. Inform the blood bank that your patient has been treated with daratumumab which interferes with indirect antiglobulin tests
- Ensure that your patient's blood sample is identified as containing daratumumab
- Double-check standing orders for transfusions to determine if your patient received daratumumab within the last year
- Ensure patients are given a Patient ID Card for daratumumab and provide your patient's pre-daratumumab compatibility profile, if available, to the blood bank
- Ask your patient to tell their other HCPs that they have received daratumumab, particularly before a transfusion

Ensure That Your Patients Take an Active Role in Their Treatment

- Patients should be provided with a blood group card alerting physicians on their anti-CD38 use
- Reassure your patients that compatible blood products for transfusion can still be identified
  - In the event of an emergency, protocols are in place to ensure timely transfusions per local blood bank practices
- For at least 6 months after their last Daratumumab treatment, patients should
  - Inform their HCPs that they have received Daratumumab treatment, particularly before receiving a transfusion



Daratumumab PATIENTS: Provide this card to healthcare providers BEFORE blood transfusion and carry it for 6 months after treatment has ended For further information please refer to the Patient Information Leaflet

Patient ID Card for DARATUMUMAB

Name: _____

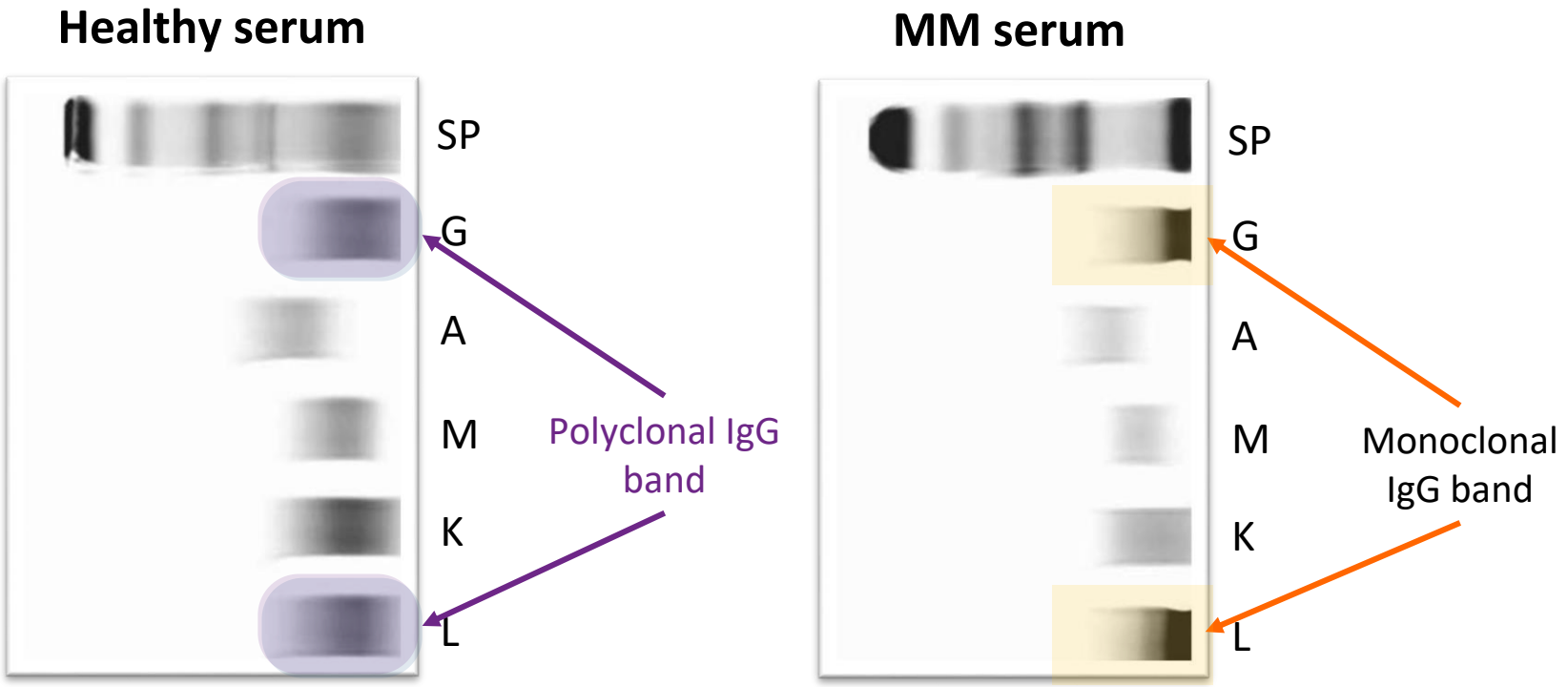
I am taking the following medication:

daratumumab antibody product for the treatment of multiple myeloma

I stopped taking this medication on ___ / ___ / ___
DD MM YYYY

Mechanism of Daratumumab Interference With Detection of M-protein

Detection of M-protein



Adapted from Katzmann J, in Gertz MA and Rajkumar SV, eds. *Multiple myeloma*. Springer, London; 2014.

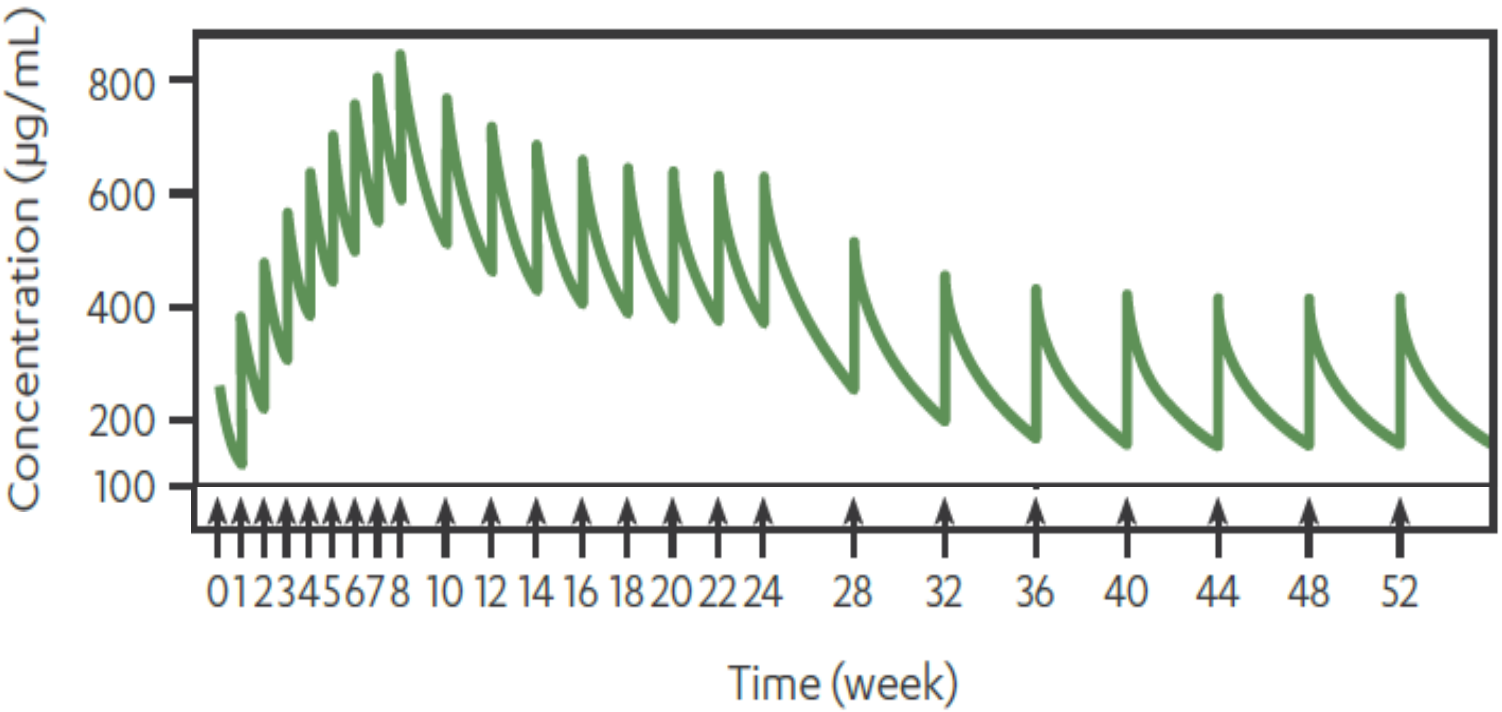
Clinical assessment of M-protein response in MM and interference through mAbs

- All therapeutic mAbs may interfere with serum electrophoresis and immunofixation
  - Difficult to discern between therapeutic antibody and the patient's clonal immunoglobulin
- Interference depends on isotype of the patient
- Daratumumab, Elotuzumab, Isatuximab and MOR202 are IgG mAbs
- Daratumumab can be detected by serum IFE and SPEP and may interfere with endogenous M-protein detection in MM samples
  - At the recommended dosing schedule (16 mg/kg weekly for 8 weeks, then every 2 weeks for 16 weeks, and every 4 weeks thereafter), daratumumab reaches peak serum concentrations of approximately 915 µg/mL (0.915 g/L) at the end of the weekly dosing period, making it readily detectable on most SPE/IFE assays

Durie et al. Leukemia. 2006;20(9):1467-1473;
McCudden et al. Clin Chem. 2010;56(12):1897-1899;
van de Donk et al. Blood 2016 ;127(6):681-695;
McCudden C, et al. Clin Chem Lab Med 2016; aop; DOI 10.1515/cclm-2015-1031

Daratumumab level

Representative PK profile of DARA for the recommended dose and schedule

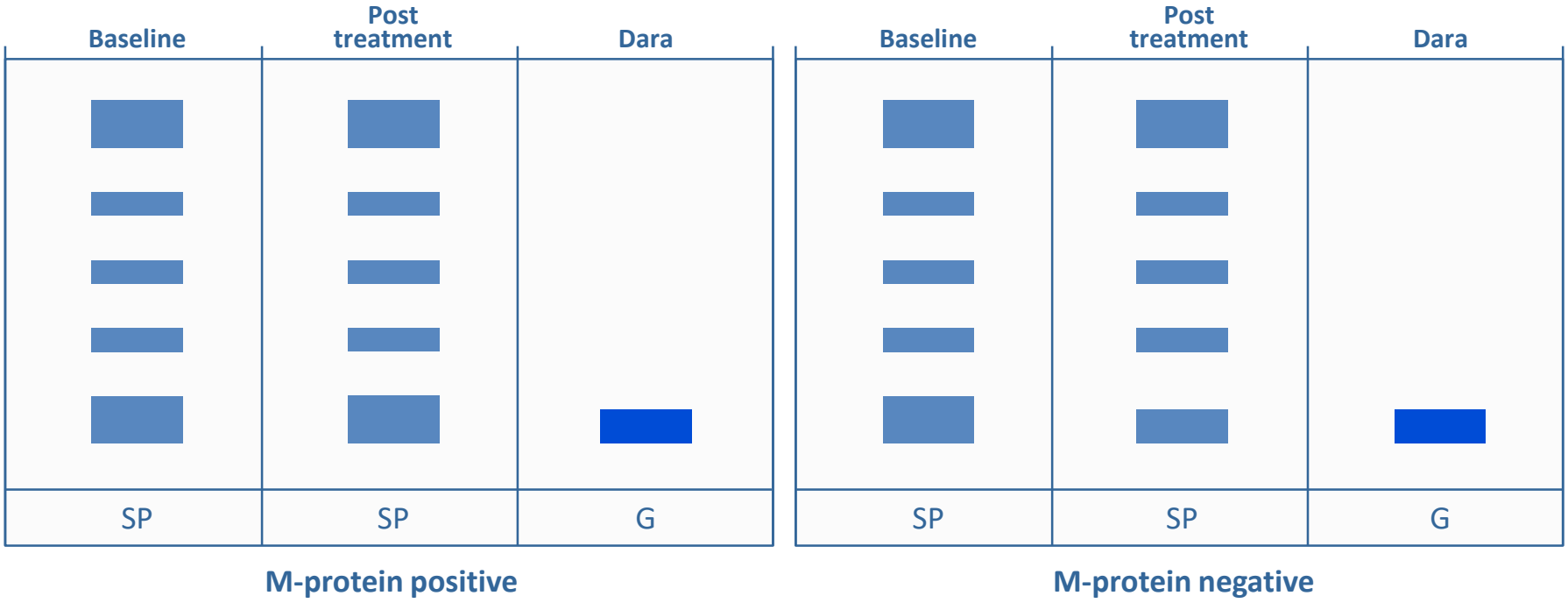


Arrows indicate that a dose was administered.

1. Xu XS, et al. Poster presented at: 2015 American Society of Hematology (ASH); December 5-8, 2015; Orlando, FL, USA (Abstract 4254).

Daratumumab Interference With SPEP/IF

- Daratumumab and residual M-protein are difficult to distinguish by standard SPEP/IF

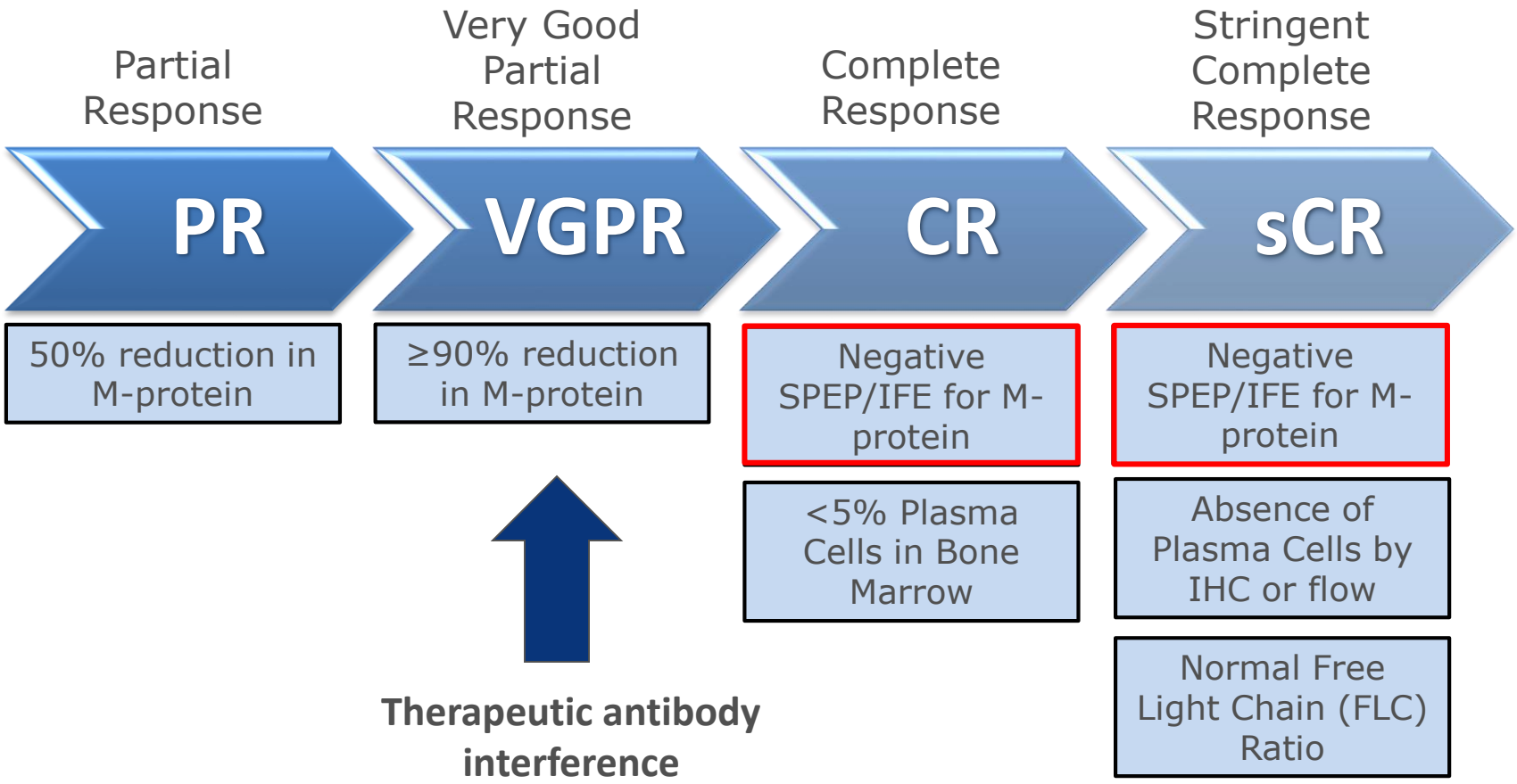


Adapted from McCudden C, et al. Clin Chem Lab Med 2016

SP, total serum protein fix; G, IgG antisera; κ, kappa antisera.

1. McCudden C, et al. *Clin Chem Lab Med.* 2016;54:1095-104

IMWG response criteria requires a negative IFE to declare patients CR

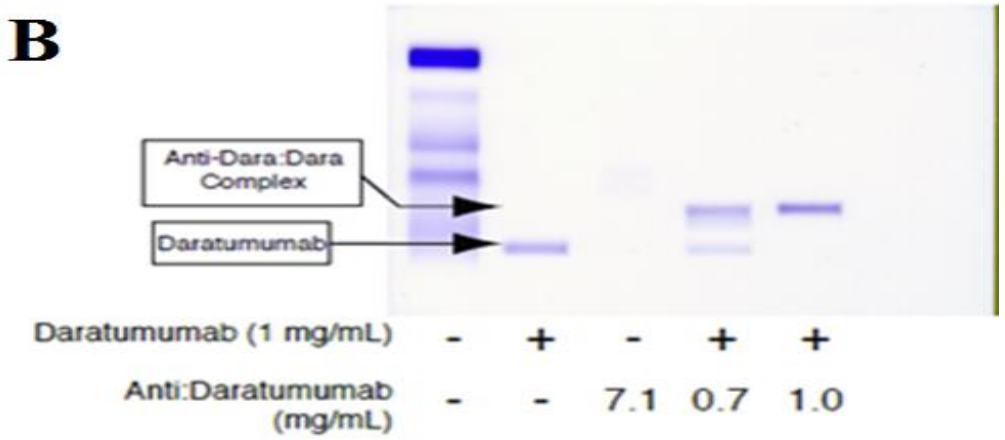


McCudden C, et al. Poster presented at: 2015 American Society of Clinical Oncology (ASCO); May 29-June 2, 2015; Chicago, IL, USA.

Daratumumab-specific IF Assay:
Distinguishing Complete Response in Multiple Myeloma From Antibody-mediated Assay Interference

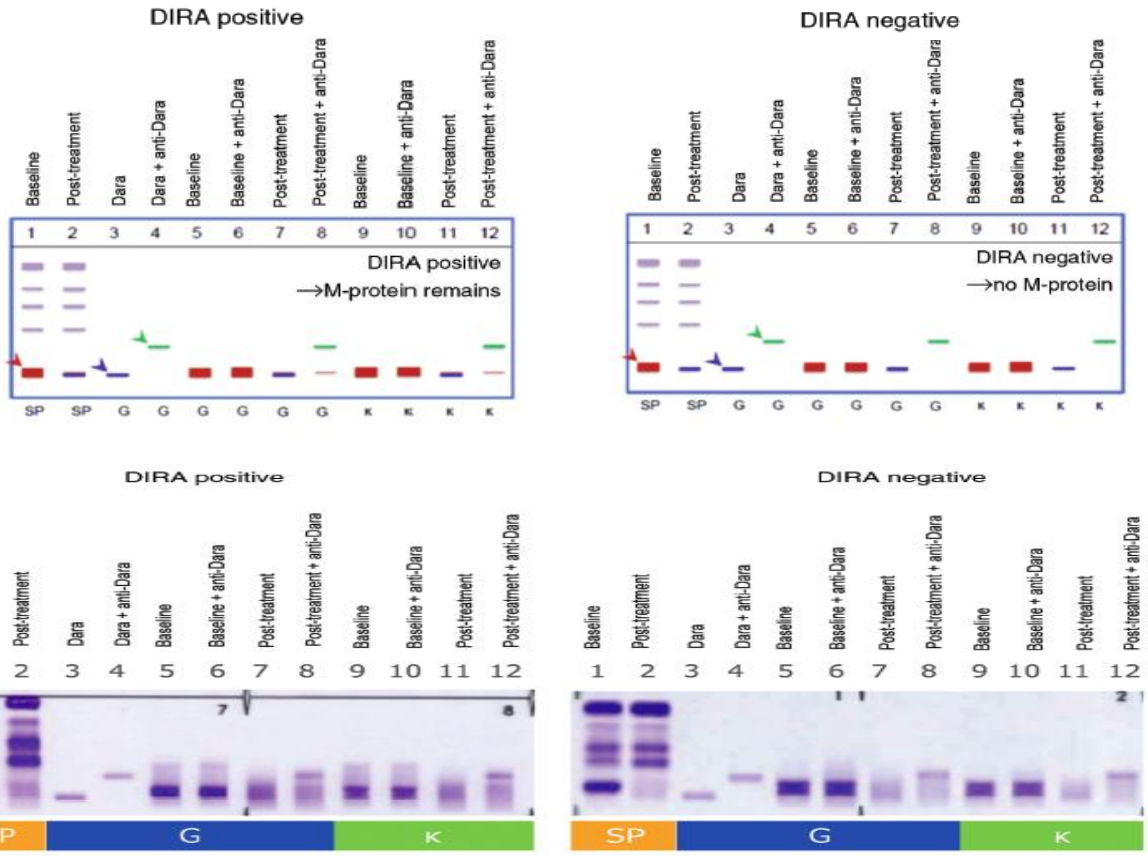
Development of an assay to distinguish M-protein from therapeutic antibody

- **Daratumumab-specific immunofixation electrophoresis reflex assay (DIRA)**
  - Incubation of serum samples of baseline and daratumumab-treated patients with or without an **anti-idiotype mAb**
  - Separation of daratumumab bands from residual endogenous M-protein
  - IFE: Daratumumab migration is shifted from the gamma region by the anti-idiotype mAb



McCudden C, et al. Clin Chem Lab Med 2016; aop; DOI 10.1515/cclm-2015-1031

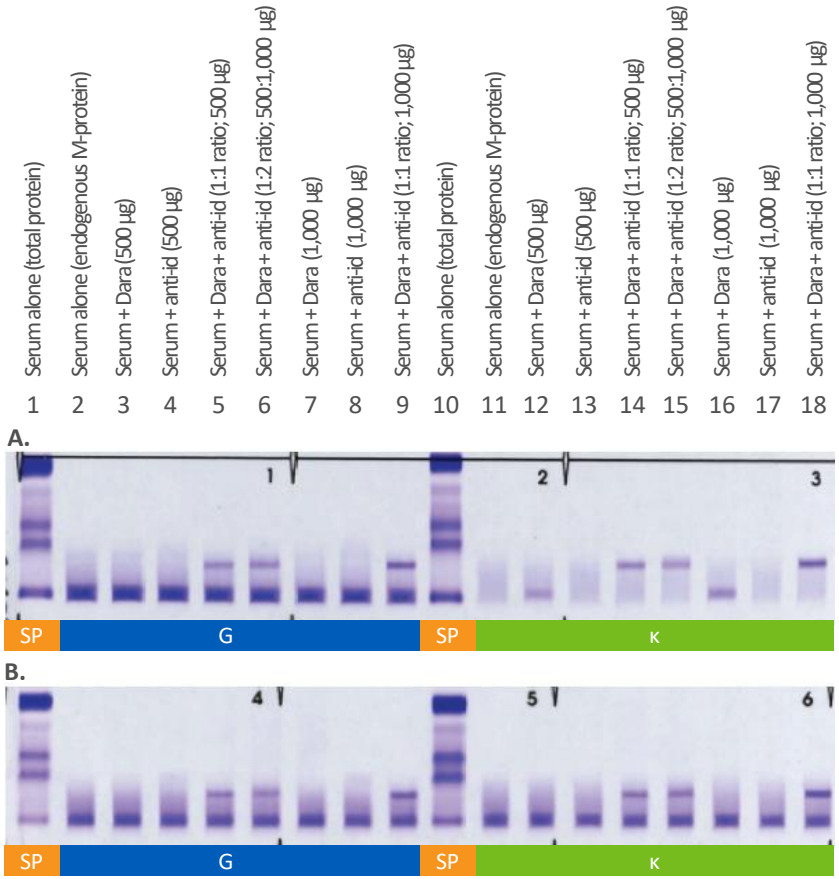
DIRA



- The DIRA template used daratumumab ±anti-idiotype as controls for migration of the therapeutic antibody and the daratumumab–anti-idiotype shifted complexes.
- Baseline and post-treatment serum ±anti-idiotype were compared to determine whether M-protein remained after shifting daratumumab.
- DIRA-positive results showed M-protein, whereas DIRA-negative results showed only a shift in daratumumab but no remaining M-protein (lanes 8 and 12)

McCudden C, et al. Clin Chem Lab Med 2016; aop; DOI 10.1515/cclm-2015-1031

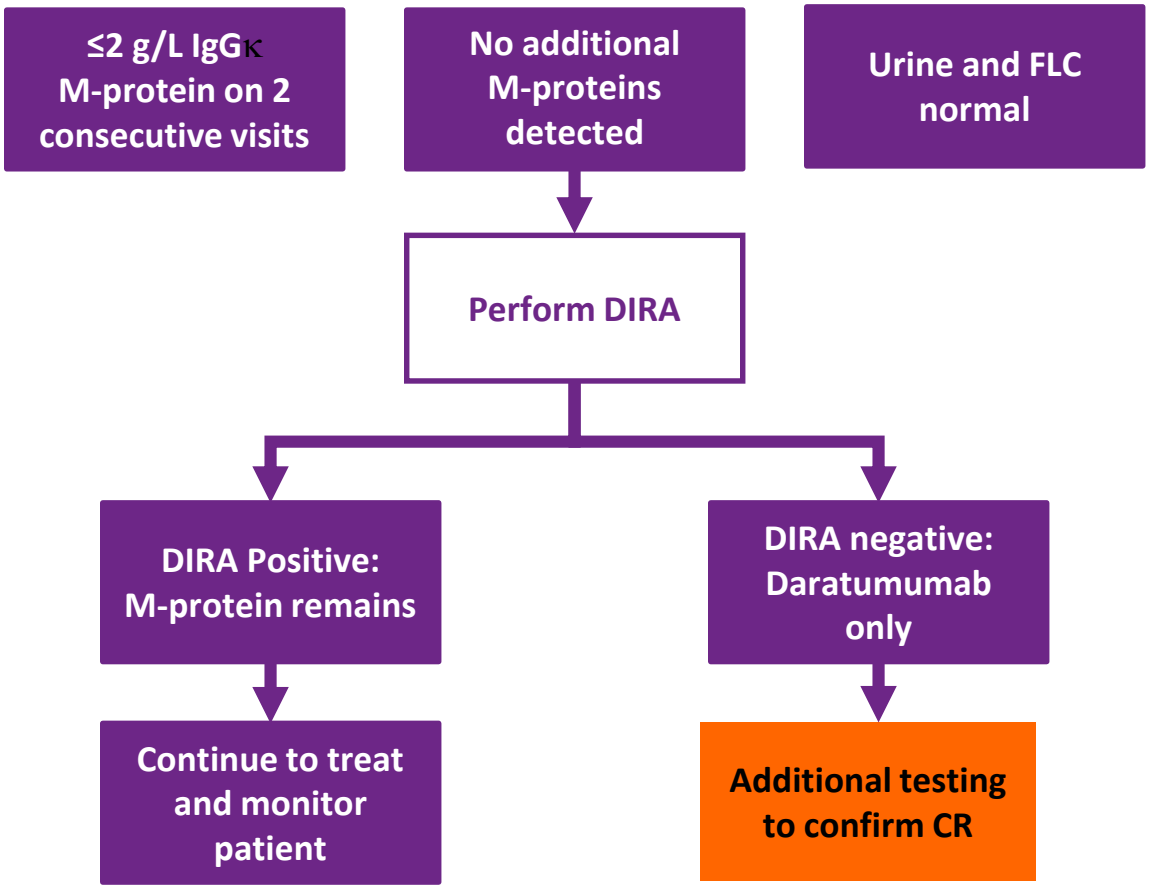
DIRA: Validation



- The DIRA limit of sensitivity was 0.2 g/L daratumumab, using spiking experiments.
- Results from DIRA were reproducible over multiple days, operators, and assays.
- The anti-daratumumab antibody was highly specific for daratumumab and did not shift endogenous M-protein.
- In conclusion, DIRA was highly specific, sensitive, and reproducible both in commercial samples spiked with daratumumab and in clinical samples from daratumumab-treated patients.

McCudden C, et al. Clin Chem Lab Med 2016; aop; DOI 10.1515/cclm-2015-1031

When to Use the Daratumumab-specific IF Assay[1]



DIRA, daratumumab-specific immunofixation electrophoresis reflex assay

1. McCudden C, et al. *Clin Chem Lab Med*. 2016;54:1095-1104.

GRAZIE PER L'ATTENZIONE