Management of Daratumumab (DARA) Patients by the Transfusion Medicine Laboratory

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Objectives:

1. What is Daratumumab and what is it used for?
   a. What is multiple myeloma?
   b. What is CD38?
2. DARA in the blood bank.
3. Case studies
4. Questions
What is Daratumumab (DARA)\(^3\)?

From the DARZALEX website:

DARZALEX\(^\circ\) (trade name of Daratumumab) Is a **CD38-Targeted Monoclonal Antibody**

DARZALEX\(^\circ\) is not chemotherapy. DARZALEX\(^\circ\) is a *monoclonal antibody* that works in several ways. One way this *monoclonal antibody* works is by attaching itself to *multiple myeloma* cells in your body and directly killing them, and/or allowing your *immune system* to destroy them.
What is Daratumumab (DARA)-cont’d 3?

DARZALEX® targets and attaches to a protein called **CD38**, which is present on the surface of certain types of cells (e.g., red blood cells) and is also present in high numbers on *multiple myeloma* cells.

Since DARZALEX® targets the **CD38 protein**, it may also affect other cells with this protein on their surface.

Single dose vials (100 mg/5 mL)
What is Multiple Myeloma?
Multiple Myeloma\(^2\):

- Greek *myelo* – meaning “marrow’ and *oma* meaning “tumour”
- A cancer of plasma cells, a type of white blood cell normally responsible for producing antibodies, causing **multiple** masses in the bone marrow or soft tissue
- Abnormal antibodies cause kidney problems and hyperviscosity syndrome (overly thick blood)
Multiple Myeloma:\n• While initially asymptomatic, at advanced stages symptoms may include: bone pain, bleeding, frequent infections and anemia
• Cause is unknown
• Considered treatable but generally incurable, with a five-year survival rate of around 49%. Untreated, typical survival is 7 months
Treatment of multiple myeloma may include:

- Chemotherapy and other drugs
- Bisphosphonates
- Radiation
- Surgery
- Stem cell transplant
- Plasmapheresis

DARA is indicated for refractory patients who have received at least 3 prior lines of therapy including proteasome inhibitors or immunomodulator y drugs (IMiDs).

IMiDs: Analogues of thalidomide used for treatment of a variety of inflammatory, autoimmune, and neoplastic diseases.
What is Daratumumab (DARA)?

- Monoclonal antibody which targets the CD38 antigen on various cell types, which is overexpressed on multiple myeloma cells.

**Chemical and physical data**

<table>
<thead>
<tr>
<th><strong>Formula</strong></th>
<th>(C_{6466}H_{9996}N_{1724}O_{2010}S_{42})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molar mass</strong></td>
<td>145,391.67 g·mol(^{-1})</td>
</tr>
</tbody>
</table>
What is Daratumumab-cont’d?

- Originally developed by Genmab but now being jointly developed with Janssen Biotech for worldwide commercialization rights

- Given **breakthrough therapy** drug status in 2013 for multiple myeloma

- Targets the CD38 antigen which is overly expressed in multiple myeloma cells, causing cells to undergo **apoptosis**

**Breakthrough therapy**: FDA designation that expedites drug development.

**Apoptosis**: programmed cell death
Daratumumab Nomenclature\textsuperscript{13}:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stem:</strong></td>
<td><strong>mab</strong></td>
</tr>
<tr>
<td></td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td><strong>u</strong></td>
<td>human</td>
</tr>
<tr>
<td><strong>tu(m)</strong></td>
<td>miscellaneous tumour</td>
</tr>
<tr>
<td><strong>Prefix:</strong> (\textit{dara})</td>
<td>carries no special meaning, just something unique for each medicine and contributes to a well-sounding name.</td>
</tr>
</tbody>
</table>
### Daratumumab Course of Treatment\(^\text{14}\):

#### BC Cancer Agency IV dosing schedule

<table>
<thead>
<tr>
<th>Cycle Description</th>
<th>Dosing Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles 1 and 2</td>
<td><strong>16 mg/kg body weight</strong> for one dose on <strong>days 1, 8, 15, and 22</strong></td>
</tr>
<tr>
<td>Cycles 3 through 6</td>
<td><strong>16 mg/kg</strong> for one dose on <strong>days 1 and 15</strong></td>
</tr>
<tr>
<td>Subsequent cycles</td>
<td><strong>16 mg/kg IV</strong> for one dose on <strong>day 1</strong></td>
</tr>
</tbody>
</table>

**Cycle length:** 4 weeks
What is CD38?

Molecule of CD38

Canadian Blood Services
it's in you to give
CD38 antigen\(^4\):

- glycoprotein found on the surface of many white blood cells

- Highly expressed on myeloma cells

- Multifunctional enzyme that catalyzes the synthesis and hydrolysis of certain amino acids essential in intracellular calcium regulation

CD (cluster of differentiation)\(^5\):

- naming protocol for the identification and investigation of cell surface molecules providing targets for immunophenotyping of cells.

- CD for humans is numbered up to 371 as of April 2016.
How DARA works\textsuperscript{6} (simplified):

- DARA (monoclonal antibody) attaches to CD38 antigen on myeloma cell surface
- Ab-Ag complex targeted by macrophages and natural killer cells, causing cell death
Side effects of DARA\textsuperscript{19} may include (but are not limited to):

- Infusion reactions
- Fatigue
- Nausea
- Back pain
- Fever
- Cough
- Upper respiratory infection
- The most common serious side effects include low lymphocyte, red blood cell, neutrophil, and platelet counts.

Patients receive premedication with corticosteroids, antipyretics and antihistamines one hour prior to every infusion to reduce side effects.
Daratumumab in the Transfusion Medicine Laboratory
Typical serological picture with DARA:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO/Rh</td>
<td>Unaffected</td>
</tr>
<tr>
<td>DAT</td>
<td>Negative to weak reactivity</td>
</tr>
<tr>
<td>Screen / panel</td>
<td>Weak panreactivity</td>
</tr>
<tr>
<td>Alloadsorption</td>
<td>Unsuccessful</td>
</tr>
</tbody>
</table>

Tried because it looks like a warm autoantibody.
Question 1:

Why do the panel cells show weak panreactivity?

Answer:

• CD38 expressed not only on myeloma cells, but also expressed, at low levels on red cells

• DARA in MM patients plasma binds to reagent red cells
Typical serological picture with DARA – cont’d:

**Question 2:**

Why is ABO/Rh unaffected?

**Answer:**

- ABO/Rh grouping reagents are IgM so DARA doesn’t interfere with this direct agglutination

- DARA is an IgG monoclonal antibody which binds to CD38 on red cells and shows agglutination when anti-human globulin is added
Typical serological picture with DARA – cont’d8:

**Question 3:** Why is alloadsorption unsuccessful?

**Answer:** Insufficient CD38 on adsorbing cells, therefore will not be able to adsorb out DARA (anti-CD38)
Managing DARA interference in TM lab:

1. Send sample to lab **before** patient goes on DARA. Lab can do a baseline antibody screen and full phenotype if not yet transfused.

2. If patient is already on DARA, when samples are sent to the lab, tell them patient is on DARA!

**Question:**

What method can be used when performing the antibody investigation to prove/exclude underlying antibodies by removing DARA interference?

- **DTT Treatment of reagent red cells**
Managing DARA interference in TM lab – cont’d:

DTT (dithiothreitol) removes CD38 from the RBC surface and eliminates the panagglutination effect.\(^\text{17}\)

Dithiothreitol is a “thiol reagent” that dissolves disulfide bonds between cysteine amino acids, disrupting the tertiary structure of proteins. Without tertiary structure, antigens cannot bind antibodies targeted towards them.\(^\text{23}\)

Red cells treated with DTT are not reactive with:
- Kell blood group antibodies
- Most antibodies in the Knops systems
- And anti-LW, -Yt, Dombrock (-Do\(^a\), -Do\(^b\), -Gy\(^a\), -Hy, and -Jo\(^a\))
**DTT**<sup>20</sup>: 

**Thiol** = *thion* (Greek for sulfur) + *alcohol*

Thiol – an organosulfur compound that contains a carbon-bonded sulhydryl group

\[ R = \text{alkyl bonded to sulfhydryl} \]
18th Ed. Method 3-18:

1. Combine 4:1 volume 0.2 M DTT with packed RBCs
2. Incubate at 37°C for 30-45 min, mixing every 5 minutes.
3. Wash cells 4x with saline then make up to 3-5 % cell suspension.
4. Test treated cells vs. plasma containing antibody.
5. Perform exclusions or look for possible pattern of reactivity of underlying antibody.

Control: Test K+ cells with anti-K

Should test negative to show DTT treatment worked
What is the most noticeable feature when using DTT?
Many thiols have strong odors and are found in strong smelling substances such as:
• onions
• garlic

and...

skunk spray

Mythbusters proven skunk smell remedy: hydrogen peroxide + baking soda + dish soap
Managing DARA interference in TM lab-cont’d⁸:

DTT treatment destroys antigens in the following blood group systems:

Knops
Scianna
Indian
Dombrock

Antibodies to these antigens are rarely encountered

Kell

Must give K neg units because can’t test for presence of anti-K
Case Studies
Case Study #1

Patient: 43 yo female
Dx: not given
DAT: positive with anti-IgG
Transfusion hx: 2 units two weeks ago
Case Study #1 – cont’d

CBS antibody investigation results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO/Rh</td>
<td>A negative</td>
</tr>
<tr>
<td>DAT</td>
<td>Positive with IgG coating red cells</td>
</tr>
<tr>
<td>Antibody screen</td>
<td>Positive</td>
</tr>
<tr>
<td>Antibody investigation</td>
<td>Very confusing!</td>
</tr>
</tbody>
</table>

CBS consults with hospital and finds out that patient has been put on daratumumab!
Case Study #2

Patient: 28 yo male
Dx: Multiple Myeloma
Transfusion hx: None within last 90 days
Hospital notes: patient will be starting daratumumab therapy in two weeks

This is an ideal sample!
Case Study #2 – cont’d

CBS antibody investigation results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO/Rh</td>
<td>O Positive</td>
</tr>
<tr>
<td>DAT</td>
<td>Negative</td>
</tr>
<tr>
<td>Antibody screen</td>
<td>Negative</td>
</tr>
<tr>
<td>Phenotype</td>
<td>C+E-c-e+; K-, M+N+S-s+; Fy(a-b+); Jk(a+b+)</td>
</tr>
</tbody>
</table>

Baseline established which will be very helpful once DARA therapy starts.
Case Study #3

Patient: 59 yo male

Dx: None given, previous history of warm autoantibodies

Transfusion hx: 2 RBC units in last two weeks

Hospital notes: patient started on daratumumab therapy 4 days ago

Send to CBS for investigation
CBS antibody investigation results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO/Rh</td>
<td>O Negative</td>
</tr>
<tr>
<td>DAT</td>
<td>Negative</td>
</tr>
<tr>
<td>Antibody screen</td>
<td>Negative after DTT treatment</td>
</tr>
<tr>
<td>Phenotype</td>
<td>N/A recently transfused</td>
</tr>
</tbody>
</table>

Sent to CBS National Immunohematology Reference Laboratory for RBC genotyping
Case Study #3-cont’d

Method for DTT treatment:\(^{23}\):

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Combine 4 parts 0.2 M DTT with 1 part packed reagent RBCs</td>
</tr>
<tr>
<td>2</td>
<td>Incubate at 37°C for 30-45 min</td>
</tr>
<tr>
<td>3</td>
<td>Wash 4x with PBS.</td>
</tr>
<tr>
<td>4</td>
<td>Resuspend RBCs to 2 – 5% in PBS.</td>
</tr>
<tr>
<td>5</td>
<td>Test DTT-treated reagent cells vs. patient plasma.</td>
</tr>
</tbody>
</table>

**Controls:**
Test DTT-treated E+ and K+ red cells vs. commercial anti-E and anti-K:
- E+ cells with anti-E, should be positive
- K+ cells with anti-K, should be **negative**

DTT destroys Kell system, not Rh
Case Study #3-cont’d

Standard CBS report comments:

Patient is receiving Daratumumab. Antibodies to all the major blood group antigens excluded by LISS IAT method using DTT treated cells. For transfusion purposes, K negative red cells should be provided because DTT treatment destroys Kell antigens.

Or PEG IAT or SIAT depending what method technologist used.
Future of DARA\textsuperscript{8}:

1. Use not going to be just restricted to a single disease. It will be tried for other conditions, lymphoma in particular, but also other uses, probably things even thought of yet.
2. Blood banks will be seeing more of this.
3. New drug Hu5F9-G4 now in clinical trials, an anti-CD47 antibody, which also binds to red cells and will cause serologic interference.\textsuperscript{27}

- Important for transfusion medicine laboratories to be given drug history.
- Clinicians should become more aware of serological implications.

Communication is key!
Wish List before sending cases to CBS:

**Pre-DARA:**
1. Sufficient sample
2. Collected pre-DARA
3. Diagnosis given: Multiple Myeloma
4. Pre transfusion so can perform full phenotype

**Post-DARA:**
1. Tell us patient is on DARA!
2. If transfused, additional sample so can send for RBC genotyping
References
References:

2. https://en.wikipedia.org/wiki/Multiple_myeloma
3. https://www.darzalex.com/
References – cont’d:


11. CD38-Targeted Immunochemotherapy in Refractory Multiple Myeloma: A New Horizon. Jacob P. Laubach and Paul G. Richardson. DOI: 10.1158/1078-0432.CCR-14-3190 Published June 2015


17. http://www.bbguy.org/education/glossary/gld17/
References – cont’d:

23. AABB Technical Manual, 18th Ed. Method 3-18, Treating Red Cells Using DTT or AET.
Questions

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604-707-3449
Canadian Blood Services

it's in you to give