Function, Action and Interference of Anti-CD38 (DARA) Antibody in Blood Banking

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Objectives

- Define Multiple Myeloma, its diagnosis, prognosis and treatment
- Describe how anti-CD38 (DARA or Darzalex™) is used for treatment of these patients
- Discuss how anti-CD38 may cause interference with antibody screening and identification in the transfusion service
- Demonstrate ways to mitigate this interference by anti-CD38
Multiple Myeloma

- Cancer of plasma cells which accumulate in the bone marrow
- Feature production of a paraprotein or abnormal immunoglobulin light chain (M protein)
- Common symptoms (CRAB)
  - C - calcium elevated
  - R - renal failure
  - A - anemia
  - B - bone lesions
Treatment of Multiple Myeloma

• Considered incurable but treatable
• Focused on the decrease the clonal plasma cell population
  ➢ Steroids
  ➢ Chemotherapy
  ➢ Proteasome inhibitors such as bortezomib
  ➢ Immunomodulatory drugs such as thalidomide or lenalidomide
  ➢ Autologous hematopoietic stem cell transplantation
    ✓ Transplantation of patient’s own stem cells after chemotherapy
  ➢ Radiation therapy to reduce pain from bone lesions
Relapse

• It is not uncommon to relapse following treatment
• May be due to tumor heterogeneity
  ➢ Different tumor cells can show distinct morphological and phenotypic profiles, including cellular morphology, gene expression, metabolism, motility, proliferation, and metastatic potential
• Re-treatment with the original agent, use of other agents (such as melphalan, cyclophosphamide, thalidomide or dexamethasone, alone or in combination) and a second autologous stem cell transplant
• Later can develop “treatment resistance”
Prognosis

- With high-dose therapy followed by autologous stem cell transplantation, median survival has been estimated in 2003 to be about 4.5 years compared to median 3.5 years with standard therapy.
- Overall 5 year survival rate is around 35% (45% 2006)
BREAKTHROUGHS AND ACCELERATED APPROVAL

**DARZALEX**

- Breakthrough designation was given May 2013 based on preliminary clinical evidence suggesting drug may offer a substantial improvement over available therapies.
- Granted accelerated approval by the FDA 11/16/2015.
- Accelerated approval allows approval of a drug to treat a serious or life-threatening disease based on clinical data showing the drug has an affect on a surrogate endpoint reasonably likely to predict clinical benefit to patients.
DARZALEX

- Accelerated approval also provides earlier patient access to promising new drugs while the company conducts confirmatory clinical trials
- Brand name for Daratumumab
- Marketed by Janssen Biotech, Inc - a pharmaceutical company of Johnson & Johnson
CD38

- Cluster of differentiation 38
- Also known as Cyclic ADP ribose hydrolase
- Glycoprotein found on the surface of many types of cells including B lymphocytes, T lymphocytes, natural killer, plasma cells and red blood cells
- Synthesizes and hydrolyzes an intracellular calcium ion mobilizing messenger
Daratumumab (DARA)

- Human CD38-directed (IgG1κ) monoclonal antibody
- First monoclonal antibody to treat multiple myeloma
- Treat relapsed/refractory patients who have received at least three prior lines of therapy including a proteasome inhibitor and an immunomodulatory agent or who are double-refractory
How it works

• CD38 is a transmembrane protein that is highly represented in malignant myeloma cells.
• By binding to CD38, DARA inhibits growth of these cells and induces tumor cell death through a variety of mechanisms including Fc-dependent effector mechanisms such as complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP).
Van de Donk et al, Blood, February 11, 2016: 127 (6)
Dosage and Administration

• Weeks 1 to 8: 16 mg/kg once weekly
• Weeks 9 to 24: 16 mg/kg once every 2 weeks
• Weeks 25 and beyond: 16 mg/kg once every 4 weeks until disease progression
• IV infusion only. Premedication with corticosteroids, antipyretics, and antihistamines
• Infusion should be completed within 15 hours
Warnings and Precautions

• Infusion reactions: First infusion 46-48%, second infusion 5%, subsequent 4%
  • Nausea, diarrhea, constipation
  • Respiratory: cough, upper respiratory, nasal congestion, dyspnea
  • Back pain, leg pain, musculoskeletal chest pain
  • Fatigue, headache, chills, fever, hypertension
  • Darzalex may also result in low counts of WBC (lymphocytopenia, neutropenia, and leukopenia) or RBC (anemia) and platelets (thrombocytopenia)
Warnings and Precautions

- Interference with serological testing
- Interference with determination of complete response as DARA is a human IgG kappa monoclonal antibody that can be detected on both the serum protein electrophoresis and immunofixation assays
- This can interfere with determination of complete response and disease progression of some patients with IgG kappa myeloma proteins. May need to consider other methods to evaluate response.
Interference with Serological Testing

- Increased CD38 expression on RBC membranes of cancer patients and weak expression on normal RBCs.
- DARA binds to CD38 on RBCs and may result in positive indirect antiglobulin tests. DARA-mediated positive indirect antiglobulin tests may persist for up to 6 months after the last DARA infusion.
- Determination of ABO and Rh blood type are not impacted.
Interference with Serologic Testing - Chapuy, et al

• Chapuy, et al found that five of five patients who received DARA had positive antibody screens
• Panreactivity in routine serologic tests
• Adsorptions using ZZAP-treated or untreated RBCs failed to remove the interference
• CD38 found to be sensitive to denaturation by DTT and enzymatic digestion with trypsin can cleave CD38 from the cell surface
Interference with Serologic Testing - Chapuy, et al

- Observed panreactivity (weak pos to 1+) in 18 of 18 DARA patient samples (SP - Tango, PEG)
- 3/5 patients had a positive DAT (IgG only) and positive autocontrol
- Panreactivity in eluate
- None of the five DARA-treated patients showed signs of hemolysis
- Use of soluble CD38 reduced DARA binding in a dose dependent manner
Treating RBCs with DTT

• DTT is a reducing agent that disrupts double bonds formed between cysteine residues
• Antibodies that recognize antigens in the context of the protein conformation will not react with DTT-treated RBCs
• Antigens sensitive to DTT include Kell, Knops, Dombrock, Lutheran, Cartwright, LW\textsuperscript{a} and JMH. Cromer antigens may be weakened and some examples of anti-Vel do not react with DTT-treated RBCs
DTT Treatment

- 5 disulfide bonds in CD38
- Treatment of RBC with DTT destroys structure of CD38
- Procedure
  - Dilute 1M DTT with 4 parts PBS pH 8.0
  - Mix 4 volumes of dilute DTT with 1 volume of packed RBCs washed 4x
  - Incubate at 37C for 30 minutes
  - Resuspend the treated RBCs to a 3-5% suspension with PBS and use in tests with the sera under investigation
  - Test treated and untreated RBCs with anti-k or other antibodies directed to antigens inactivated by DTT. Reactivity with untreated RBCs should be 2-4+ and untreated cells will be negative
## DTT sensitivity and reactions

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Clinical significance for transfusion reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kell</td>
<td>Mild to severe/delayed hemolytic</td>
</tr>
<tr>
<td>Knops</td>
<td>No</td>
</tr>
<tr>
<td>Dombrock</td>
<td>Delayed and acute/hemolytic</td>
</tr>
<tr>
<td>Lutheran</td>
<td>No to mild/moderate</td>
</tr>
<tr>
<td>Cartwright</td>
<td>No to moderate/rare/delayed</td>
</tr>
<tr>
<td>Lw^a</td>
<td>No to mild/delayed</td>
</tr>
<tr>
<td>JMH</td>
<td>No</td>
</tr>
<tr>
<td>Cromer</td>
<td>No to moderate to severe</td>
</tr>
<tr>
<td>Vel</td>
<td>No to severe/hemolytic</td>
</tr>
</tbody>
</table>
Ruling out underlying antibody

- DARA sometimes does not react with antigen typed cord cells (very little CD38)
- Enzyme treated cells such as trypsin rule out Kell system, Yt and LW
- DARA may have specificity to Knops - if available Knops negative cells may help
- In(LU) [Lu(a-b-)] cells appear to have low CD38 but can potentially give the false impression that the patient has an anti-Lu antibody - HEA beadchipping can help
DTT and Neutralization

• Recombinant soluble human CD38 or anti-daratumumab idiotype antibody can eliminate interference however neither reagent is widely available at this time
• If not previously utilized by the blood bank, validation, procedures and staff training are required.
TJUH Pre-mitigation Strategies - Round One

• Initiated early December
• Physician/Blood Bank discussions and education about DARA and the interference with blood bank testing
• Physician to notify blood bank of patients to receive DARA
TJUH Experience - Patient BN

- 38 F compassionate use
- Received DARA 12/18/15 and 12/24/15 (BB was not notified of DARA use)
- Specimen collected 12/25/15
- ECHO SP variable results 0 to 3+ reactivity
- PEG variable 0-W+
- PEG XM variable 0-2+ incompatibility
- DAT Poly negative
TJUH Experience - Patient BN

- After almost two days of workup, specimen sent to reference lab
- Reference lab - negative DAT Poly, IgG, C3d
- Reactive with Alb and PEG, 0.2M DTT removed reactivity. Reactive to dilution of 64
- 9 of 10 rgt red cells reactive in ficin
- Blood bank notified of DARA use
- Reference Lab - Anti-DARA
TJUH Experience - Patient BN

- Specimen collected 1/3/16
- ECHO SP panreactive 1-3+
- PEG panreactive w+
- Gel (Ortho) panreactive 1-2+
- Reference lab - negative DAT Poly, IgG, C3d
- Reactive with Alb and PEG, 0.2M DTT removed reactivity. Reactive to dilution >512
- Patient expired 1/4/16
TJUH Mitigation Strategies - Round 2

• Discussions and education with Physicians, pharmacy, Oncology nursing personnel, reference laboratory staff and blood bank staff. Identification of DARA patients and notification of the Blood Bank is KEY!

• Participated in educational sessions for oncology nursing

• Pre-DARA specimens is 2 EDTA - one for preDARA Type and Screen; one for HEA beadchip

• Post-DARA specimens is 3 EDTA - one for BB to perform Type and Screen. If positive, send two specimens to reference lab for DTT treatment and antibody identification
TJUH Mitigation Strategies - Round 2

• Initial physician driven notification efforts did not work, pharmacy hard stop implemented.
• Prior to dispense of first dose, pharmacy must verify notification to blood bank and pre-DARA specimen collections
• LIS build of Special Message “DARA - Pt on DARA - Refer to ARC for DTT”
• New antibody “Anti-DARA” built as clinically insignificant
**Guidance for Patients receiving Anti-CD38 (Daratumumab or DARA)**

1. Oncology will order a Type and Screen with Red Blood Cell Antigen profile prior to starting treatment. For inpatients, orders will be placed in the HIS. The comment section should include that the patient will be receiving DARA and expected first dose. Oncology shall provide the blood bank with 2 EDTA tubes. Notification of patient receiving DARA may be given verbally. Remind patient care team that a pre-drug T&S and RBC antigen profile needs to be ordered/collected if not previously done.

2. If emergency transfusion is required, non-crossmatched ABORh compatible red cells will be issued per Blood Bank protocol.

3. Oncology/pharmacy will have a hard stop to confirm that pre-drug specimens have been received before the patient receives drug.
Guidance for Patients receiving Anti-CD38 (Daratumumab or DARA)

4. Receive notification of patient name, Medical Record number, DOB and when the patient is to receive the first dose.

5. Add special message of DARA in the BB Information System. DARA - Pt on DARA; send to ARC for DTT

6. Receive and perform pre-drug Type and Screen and send a specimen to reference lab for PreciseType® HEA.
Guidance for Patients receiving Anti-CD38 (Daratumumab or DARA)

7. After patient receives drug: If the patient has an order for Type and Screen or blood products, Oncology will provide the blood bank with 3 EDTA tubes. Perform Type and Screen. If positive, send specimen to reference lab for DTT treatment and antibody identification. Document on reference lab request that patient is receiving DARA.

8. If the reference lab cannot rule out Kell antibody in the presence of DARA and the patient is Kell antigen negative or undetermined, provide Kell negative red cells.

9. Rework up (DTT treatment and full antibody identification) every 31 days unless there is a change in serology.
Crossmatching

- For patients with a negative antibody screen using DTT-treated RBCs, an electronic or immediate-spin crossmatch with ABO/RhD compatible units may be performed.
- For patients with known alloantibodies, antiglobulin crossmatched antigen negative red cells will be provided. These units will be incompatible. If reference lab has documented that all testing is complete, least incompatible units may be given with physician signature

➢ Note: If the current antibody screen is positive, the LIS will require extended crossmatch. If screen is currently negative, electronic or immediate-spin crossmatch with ABO/RhD compatible units may be performed
Follow up

- Oncology gives patient completed ID card for Darzalex treatment
- Blood Bank sends antibody ID cards to patient
Why PreciseType® HEA vs serologic phenotyping

• 35 antigens vs the few BB would test
• Serologic phenotyping is time consuming for the blood bank
• Most patients have been recently transfused within the previous 3 months
• Predicted phenotype for many high-prevalence antigens that are destroyed by DTT
• Can make decisions for product selection if complicated serology (similar to sickle cell process)
• PreciseType HEA® is likely reimbursable
PreciseType HEA® is likely reimbursable
TJUH Experience

- 15 patients to date (5/8/16)
- All pre-DARA antibody screen negative
- 13/14 Kell negative
- 2/6 DAT Poly and IgG Positive (Weak and microscopically respectively); C3 negative
- 2 eluates performed; 1 negative, 1 panreactive/no specificity
- 1 patient DARA stopped after significant infusion reaction
TJUH Experience

- 4 negative screens after receiving 5-8 doses
  - Specimens collected 2-8 days after last dose
- Decrease in hemoglobin from start of DARA to first transfusion
  - Range: 0.4-3.8 g/dl
  - Average 1.5 g/dl
TJUH Experience

- 6 positive post DARA screens after receiving 1-11 doses (1,2,2,5,5,11)
  - Specimens collected 1-14 days after last dose
- Variable reactivity 0-4+ ECHO SP
- 1-2+ Gel (had more uniform panreactivity)
- W-3+ PEG
- 0-2+ LISS
- 1 patient non-reactive with ficin
- Incidentally, 1 patient discovered hybrid altered C antigen on molecular/serology testing
TJUH Experience

• 1 patient no specimen or testing post DARA after 6 doses; hgb stable
• 4 patients - just starting treatment


Manufacture insert, DARZALEX, Janssen Biotech, Inc, 2015

Resolving the daratumumab interference with blood compatibility testing, Chapuy, C., et al, Transfusion, Volume 55, June 2015, pages 1545-1554

When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy, Oostendorp, M., et al, Transfusion, Volume 55, June 2015, pages 1555-1562


AABB Recommendations for Mitigating the Anti-CD38 Interference with Serological Testing, AABB, Dec 10, 2015