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
- 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% in 2006 due to improved chemotherapy)
DARZALEX

- Human CD38-directed (IgG1κ) monoclonal antibody
- First monoclonal antibody to treat multiple myeloma
- Initially approved as a monotherapy to 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
- Recently in combination with lenalidomide and dexamethasone, or bortezomib and dexamethasone, for treatment of patients who have received at least one prior therapy
CD38

- Glycoprotein found on the surface of many types of cells including B lymphocytes, T lymphocytes, natural killer, plasma cells and red blood cells
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)
DARZALEX

• Given breakthrough designation on 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
DARZALEX

• Accelerated approval allows approval of a drug to treat a serious or life-threatening disease
• Provides earlier patient access to promising new drugs while the company conducts confirmatory clinical trials
• Brand name for Daratumumab (DARA)
• Marketed by Janssen Biotech, Inc - a pharmaceutical company of Johnson & Johnson
DARZALEX® Combination Therapies

- Data from Phase 3 clinical trials demonstrated DARA in combination with standard of care therapy had a 61% reduction in the risk of disease progression or death compared to standard of care therapy alone (bortezomib and dexamethasone)
- Improved complete response or better (19% vs 9%)
- Improved partial response (59% vs 29%)
- Overall response rate improved (83% vs 63%)
## Dosage and Administration

<table>
<thead>
<tr>
<th>Monotherapy and in combination with Lenalidomide and dexamethasone</th>
<th></th>
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<tbody>
<tr>
<td>Weeks 1 to 8</td>
<td>once weekly</td>
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<tr>
<td>Weeks 9 to 24</td>
<td>once every 2 weeks</td>
</tr>
<tr>
<td>Weeks 25 onwards until disease progression</td>
<td>once every 4 weeks</td>
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</table>

<table>
<thead>
<tr>
<th>In combination with Bortezomib and dexamethasone</th>
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<tbody>
<tr>
<td>Weeks 1 to 9</td>
<td>once weekly</td>
</tr>
<tr>
<td>Weeks 10 to 24</td>
<td>once every 3 weeks</td>
</tr>
<tr>
<td>Weeks 25 onwards until disease progression</td>
<td>once every 4 weeks</td>
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</table>
Dosage and Administration

- Dosage of 16 mg/kg; 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)
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

• Panreactivity or variable reactivity in antibody screen or antibody identification testing
  • Can create delays if unaware of DARA treatment
  • To the BB, can look like a autoantibody, multiple antibodies or high frequency antibody
• Adsorptions using ZZAP-treated or untreated RBCs fail 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
Treating RBCs with DTT

- DTT is a reducing agent that disrupts double bonds formed between cysteine residues
- 5 disulfide bonds in CD38
- Treatment of RBC with DTT destroys structure of CD38
- Antigens sensitive to DTT include Kell, Knops, Dombrock, Lutheran, Cartwright, LWa and JMH. Cromer antigens may be weakened and some examples of anti-Vel do not react with DTT-treated RBCs
DTT Treatment

• 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 37°C 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

  ➢ Some abstracts have suggested stability of DTT treated RBC up to 9 days at 2-8°C or up to 14 days when stored in Alsever’s solution at 2-8°C
## DTT sensitivity and reactions

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Clinical significance for transfusion reaction</th>
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<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>
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</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
Ruling out underlying antibody

- 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
  - Molecular testing (PreciseType® HEA) helpful tool
- “DARA RBCs” - use of serological CD38 negative genotyped RBCs from patients receiving DARA therapy
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.
DTT and Neutralization

- Recombinant soluble human CD38 or anti-daratumumab idiotype antibody can eliminate interference however neither reagent is widely available at this time.
- Implementing DTT by the blood bank requires validation, procedures, and staff training.
TJUH mitigation Strategies

- Preapproval meeting with Janssen
- 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
- Decided to treat DARA reactivity as if an autoantibody following the same algorithm
TJUH Mitigation Strategies

- 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
TJUH Mitigation Strategies

• 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 PreciseType™ HEA molecular testing

• 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
Blood Bank Algorithm

- Emergent Tx - unXM ABORh compatible
- T/S & RBC Ag profile by PreciseType™HEA
- Pt name, MR, DOB and date of first dose
- Hard stop-predrug spec in BB? Drug therapy initiated
- Add DARA special message on pt record
- If pos ABSC, Ref Lab for DTT and ABID Document on Ref request DARA pt
- Give Kell neg RC if unable to rule out and pt Kell neg (undetermined)
- ABID every 31 days unless change in serology
Crossmatching

- Neg antibody screen, an electronic or ISXM
- Current Pos antibody screen, LIS will require extended crossmatch. These units may be incompatible. If reference lab has documented that all testing is complete, least incompatible units may be given with physician signature
- Known alloantibodies, AHG XM with Ag negative red cells will be provided. These units may be incompatible. If reference lab has documented that all testing is complete, least incompatible units may be given with physician signature
Follow up

• Oncology gives patient completed ID card for Darzalex treatment
• We have since identified the need to add to our physician acknowledgement form a category for
  • Drug related antibody not clinically relevant causing incompatibility
    • in the absence of clinically significant allo-antibodies
    • In the presence of clinically significant allo-ab; Ag negative units provided
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® will be reimbursable
Use of PreciseType® HEA

- Pre-DARA molecular testing makes for timely transfusion decisions rather than waiting for a problem and delay in obtaining results (our reference lab returns results in one week).
- TJUH gives prophylactic Rh and Kell matched units to prevent antibody development in sickle cell patients (this can be extended to Fy, Jk and Ss) use same principle in DARA pts.
Use of PreciseType® HEA

• If unable to rule out certain clinically significant antibodies or the patient demonstrates hemolysis, can rule out using the red cell antigen profile. For example, patient is Lub+, don’t need to give expensive rare Lutheran null units.

• Incidentally, 1 DARA patient that had pre-DARA PreciseType HEA® performed discovered hybrid altered C antigen on molecular/serology testing and is able to make anti-C.
TJUH Experience

- 25 patients to date (11/28/16)
- 24 pre-DARA antibody screen negative; 1 hx of Anti-E
- 3/24 Kell positive
- 1 patient DARA stopped after significant infusion reaction
TJUH Experience

• 13 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

- 20 positive post DARA screens after receiving 1-11 doses
  - Specimens collected 1-14 days after last dose
- 8/20 DAT Poly and IgG Positive (microscopic to weak); C3 negative
- 8 eluates performed; 5 negative, 3 panreactive/no specificity
TJUH Experience

- Variable reactivity 0-4+ ECHO SP (0-3+ NEO)
- 1-2+ Gel (had more uniform panreactivity)
- 0-3+ PEG (3+ in one patient, all others 0-2+)
- 0-2+ LISS
- 1 patient non-reactive with ficin
- *Incidentally, 2 patients discovered hybrid altered C antigen on molecular/serology testing*
- 8 patients with GATA mutation
TJUH Experience

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

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• 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
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