Handouts

2019 Advanced Track Webinars

11 September 2019  ImmuLINK for Laboratories of All Sizes
6 November 2019   Sickle Cell: In the Transfusion Service and In Real Life

Link to register:
https://immucor.webinato.com/register
Continuing Education

- PACE, Florida and California DHS
- 1.0 Contact Hours
- Each attendee must register to receive CE at: https://www.surveymonkey.com/r/MonoclonalTherapyandtheBloodBank
- Registration deadline is August 23, 2019
- Certificates will be sent via email only to those who have registered September 6, 2019

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THE IMPACTS OF AN UNDISCLOSED MONOCLONAL ANTIBODY THERAPY ON SEROLOGICAL TESTING

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TECHNICAL SUPERVISOR, TRANSFUSION SERVICE
UTMB
OBJECTIVES

• Recognize serological testing results associated with monoclonal antibody therapy

• Identify when a patient is being treated with monoclonal antibody therapy based on what other drugs the patient is on

• Recognize monoclonal antibody therapy drugs that can interfere with serological testing

CASE STUDY

• O pos 72 year old female

• Came through the ER with an accidental overdose
  • Patient overdosed on Sotalol (used to treat arrhythmias).
  • No other medications listed on order form

• Received 2 units of RBC in July of 2015

REFERENCE LAB TEST RESULTS
PATIENT'S PHENOTYPE

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</table>

Patient is a R1r.

RUN PHENOMATCHED CELLS TO DETERMINE HIGH OR MULTIPLES

Since all 3 phenomatched cells are positive, we are looking at an antibody to a high frequency antigen.

TITER

Antibody titers like an antibody that is directed toward what used to be called “HTLA” antigens. “HTLA” antibodies have High Titer but Low Avidity for the antigens they are directed toward, thus the weak reactions in the titer.
Cell treatments and titer indicate the antibody is toward an antigen in the Knops-McCoy system.

Clinically significant antibodies to common red blood cell antigens are ruled out.

Since the antibody is reacting toward Helgeson’s cells (which are negative for the Knops-McCoy system antigens), the antibody is not an antibody toward the Knops-McCoy system.
WHAT ELSE CAN IT BE?

Drug problem?
- No medication list was provided with the patient request form
- Called the hospital and requested a medication list
- Patient was on antibiotics, antiviral, anti-fungal, lenalidomide, and dexamethasone. Last doses were about 5 months prior to workup being sent to the reference lab.

WHAT ELSE CAN IT BE?

We suspected that the patient was treated with Daratumumab due to the combination of drugs that the patient was on.
- DARA is approved by the FDA to be given with lenalidomide or bortezomib and dexamethasone.
- Most of the patients who are on DARA are also on a combination of antibiotics, antiviral, and anti-fungal.

WHAT ELSE CAN IT BE?

We asked the blood bank tech to dig deeper into the patient’s chart for a history of multiple myeloma and treatment with Daratumumab.
- Patient had multiple myeloma and is currently in remission.
- Her last dose of DARA was 5 months ago. Tech did not think DARA could be contributing to serological testing problem due to last dose being 5 months ago.
- The DARA treatment information was found in an oncology note and not in the patient’s medication history.
DARATUMUMAB

- Anti-CD38 binds to plasma cells and red blood cells
- Interferes with serological testing
- Interferences with serological testing can persist for up to 6 months after the last dose of DARA infusion

IMPKACTS ON PATIENT SEROLOGICAL TESTING

<table>
<thead>
<tr>
<th></th>
<th>Undisclosed Monoclonal Antibody Therapy</th>
<th>If we knew Patient was on Daratumumab Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>2 days before workup can be completed (had to wait for hospital to respond with medication list and oncology treatment)</td>
<td>2 hours for workup to be completed</td>
</tr>
<tr>
<td>Cost</td>
<td>$1200</td>
<td>$200</td>
</tr>
</tbody>
</table>

PLEASE INCLUDE MEDICATION LIST AND PATIENT DIAGNOSIS!

- Reference lab techs are trained to recognize drugs that are associated with monoclonal antibody therapy treatments.
  - Oncology treatments are not always listed under the medication list in a patient’s chart.
  - Oncology treatments are usually given as part of a drug cocktail.
- Patient diagnosis is also very important to help the reference lab figure out what kind of serological testing problems the patient might be experiencing.
### MONOCLONAL ANTIBODY THERAPY DRUGS THAT INTERFERE WITH SEROLOGICAL TESTING

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type of Monoclonal Antibody</th>
<th>Use to treat</th>
<th>FDA approved to be given with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daratumumab</td>
<td>Anti-CD38</td>
<td>Multiple myeloma, diffuse intrinsic brain tumors, mantle cell lymphoma</td>
<td>Lenalidomide, bortezomib or pomalidomine and dexamethasone along with antibiotics, anti-fungal</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Anti-CD20</td>
<td>Hematological cancers, autoimmune diseases such as RA, SLE, MG, and GPA, Off-label use: organ transplant recipients</td>
<td>Methotrexate along with antibiotics</td>
</tr>
<tr>
<td>Hu5F9-G4</td>
<td>IgG4 Anti-CD47</td>
<td>Hematological cancers and solid tumors</td>
<td>Currently in clinical trial</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>NA</td>
<td>Ovarian cancer, lung cancer, head and neck cancer, breast cancer, haematological and solid tumors</td>
<td>Filgrastim</td>
</tr>
</tbody>
</table>

### REFERENCES


**THANK YOU!**
HU5F9-G4 monoclonal Anti-CD47 therapy

A First Experience with Interference in Antibody Identification

Christine Howard-Menk, MS, MT(ASCP)SBB

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Case Report

- L.W., 58-year-old Caucasian female
- Hx of B-cell lymphoma
- s/p peripheral stem cell transplant (sibling)
- 7.0 g/dl hemoglobin
- Hx of warm and cold autoagglutinin
- Referring hospital reports a panagglutinin using gel
- Sent to outside laboratory for additional testing
- Additional transfusion and medication history not readily available

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Initial testing

Gel as primary method

<table>
<thead>
<tr>
<th>ABO reverse discrepancy</th>
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<th></th>
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</tbody>
</table>

Panagglutinin at AGT, all cells tested 4+

Polyspecific DAT: Negative
Tube testing

ABO reverse discrepancy

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-AB</th>
<th>Anti-D</th>
<th>PeG/LISS</th>
<th>Ficin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Panel results: PeG/LISS, Ficin all cells reactive 3-4+ at IS and AGT

Negative DAT (auto control neg)

Pretransfusion specimen

- Still available for testing
- DAT negative
- Antigen typing—including rare specificities
- Providing antigen negative units prior to workup completion

Additional testing

- Phenotypically matched cells (R2R2, K-) vs:
  - Ficin
  - DTT
  - Glycine acid-EDTA
- Aged cells (no greater than 5 months)
- Cord cells (not matched)
- All reactions at AGT 3-4+
High Frequency?

- Results indicated a potent antibody to high frequency antigen.
- Rare specificities tested based on reactivity and race of patient:
  - Lan, Vel, pP1Pk (Tja), En+, I, H, Cs+, Co+

Other testing

Eluate:

4+ with all cells tested.
Last Wash negative.

Autoantibody with specificity (loss of antigen status in patient)

Titrations

Plasma vs. phenotype matched cell (R2R2, K-)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tbody>
<tr>
<td>Resp</td>
<td></td>
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<td>Resp</td>
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</tbody>
</table>

Can show titering as characteristic
Amount of absorptions required
**Allo Absorption**

Allo absorption with human stroma (enzyme treated)

<table>
<thead>
<tr>
<th>Serum</th>
<th>Group B</th>
<th>Rh Positive</th>
<th>Anti-A</th>
<th>Anti-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No underlying alloantibodies

Group B, Rh positive

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**Patient History**

Complete history not immediately available

Results suggested high frequency antibody

Additional history confirmed patient in clinical trial for anti-CD47 therapy

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**HU5F9-G4**

Human monoclonal IgG4 antibody.

Targets CD47 for treatment of B-Cell lymphomas; solid tumors

CD47 is a cell surface protein which regulates phagocytosis and broadly expressed, including red cells.
Literature Search

-- Reactivity not detected when anti-IgG4 not present
-- Specific IgG coombs sera (Immucor Gamma clone, monoclonal)
  required to resolve reactivity at AGT
-- Gamma clone does NOT contain anti-IgG4

Resolution

• ABO discrepancy resolved using alloabsorbed plasma at IS
• Patient is group B, Rh positive
• Plasma reactivity resolved using Immucor Gamma clone anti-IgG,
  omitting IS and 37°C phases.
Conclusion

Monoclonal therapies will continue to emerge

Extensive communication between clinical service and blood bank necessary to provide timely results to patient

Antibody screening

patients on monoclonal antibodies

Julie Staves
Oxford University Hospitals NHS Foundation Trust

Monoclonal Anti-CD38

- Over the last 20 years – monoclonal antibody based treatment have been developed
- Over the last 5 years – more of these treatments have received licences and the use has increased
- Anti-CD38 granted UK Licence in 2017
  - Most commonly used drug is Daratumamab
  - Main use in the treatment of Multiple Myeloma
  - CD38 is overexpressed on myeloma cells
  - Anti-CD38 binds to the antigens on the myeloma cells and causes them to apoptosis
Monoclonal Anti-CD47

- Hu5F9-G4 is a human monoclonal IgG4 antibody recognising CD47
- Currently in clinical trials to treat haematologic and solid malignancies
  - Therefore the potential impact is much greater than CD38 in terms of patient numbers
- CD47 is also highly expressed on RBCs so interferes with routine pre-transfusion testing
- Not only antibody screening and crossmatch, but also ABO typing
  - Unexpected positive reactions seen in the reverse group.

The Problem

- Monoclonal antibodies such as anti-CD38 and –CD47 bind to the antigens expressed on red cells, including reagent red cells
  - Can result in a pan-reactive positive screen
  - You’ve got to then decide on next steps
  - Without prior knowledge of the patient treatment – you could assume its an antibody to a high frequency antigen or AIHA!
  - All this causes delays to the transfusion

What to do?

- The key to provision of blood for this type of patient is communication
  - If you know what drug the patient is receiving you can have a pre-determined strategy
  - The strategy will vary depending on the drug
Anti-CD38

- The most commonly used strategy for anti-CD38 is antibody screening with DTT treated reagent red cells
- This breaks down the bonds in the antigens and prevents binding of the antibody.
- but it also destroys some clinically significant antigen system

Potential other strategies

- Addition of an antibody neutralising reagent to the patient plasma
  - Some in development, and some availability are reference services

Capture-R

- Experience with Capture-R shows that not all patients on anti-CD38 will present with a pan reactive screen (compared to CAT)
- At OUH we've found that approximately 40% of patients have a negative screen
- This allows us to use our standard crossmatching protocols and allows rapid provision of red cells
CD38 Results – Capture vs CAT

<table>
<thead>
<tr>
<th>Patient</th>
<th>CD38 Dose</th>
<th>CAT Screen</th>
<th>Solid Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>low</td>
<td>per reactive</td>
<td>neg</td>
</tr>
<tr>
<td>2</td>
<td>low</td>
<td>per reactive</td>
<td>neg</td>
</tr>
<tr>
<td>3</td>
<td>low</td>
<td>per reactive</td>
<td>1 cell positive</td>
</tr>
<tr>
<td>4</td>
<td>low</td>
<td>per reactive</td>
<td>neg</td>
</tr>
<tr>
<td>5</td>
<td>low</td>
<td>per reactive</td>
<td>neg</td>
</tr>
<tr>
<td>6</td>
<td>low</td>
<td>per reactive</td>
<td>neg</td>
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<tr>
<td>7</td>
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<tr>
<td>11</td>
<td>low</td>
<td>per reactive</td>
<td>neg</td>
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</tbody>
</table>

Low dose: 15mg/Kg  Medium Dose: 20mg/Kg

Anti-CD47

Potential Strategies:
- Auto/Allo absorptions to remove the monoclonal antibody
- There is no similar reagent to DTT
- Development of a neutralising reagent is a potential (not yet available)

Capture

- The currently used anti-CD47 is an anti-IgG4 molecule
- Capture indicator cells will not detect pure anti-IgG4 antibodies
- Therefore the anti-CD47 is not detected and the antibody screen result is clinically relevant
We have published a paper – looking at transfusion issues associated with the first anti-CD47 trial in the UK.

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

- This was a multicentre trial and a number of different strategies were used to provide red cells.
Genotype/phenotype matching

- Knowing what clinically significant antibodies a patient could potentially make is helpful in these cases
- It provides knowledge of what you need to exclude (e.g., if a patient is K pos – means you don’t need to be concerned they have an anti-K)
- We are getting genotypes on our monoclonal patients prior to treatment

Matching

- Provision of antigen matched red cells for patients who whom you effectively can’t do an antibody screen (e.g., anti-CD38)
- Although this is a strategy a number of us use – we need to recognize that it’s not without problems

Potential problems

- Delay in the provision if not readily available
- You are only matching for the common clinically significant antibodies so you may not account for something
- Remember to ensure clinical area is aware of this and monitors accordingly.
What do we do?

- **CD47**
  - Not an issue for us
  - We do genotype prior to treatment
  - But just use our standard protocols for negative screen
  - We antigen match if we get a allo antibody
  - When the IgG3 anti-CD47s start appearing – you’ll confirm Capture R performance

CD38

- We genotype prior to treatment
  - If screen negative – use standard protocols
  - For positive screens – we antigen match

Conclusions

- Monoclonal antibodies seem to be here to stay
- Pre-planning and communication is essential
- There are some different solutions to the problems
- Current ongoing work is looking for neutralising agents to add to the patient plasma to block the interference
- At present Capture-R technology does not appear to be as affected by monoAb interference to the same extent as some other technologies
Thank you!