Monoclonal Antibody Therapeutics: Potential Interferents on Protein Electrophoresis and Related Tests

Maria Alice V. Willrich, Ph.D., DABCC

Learning Objectives

- At the end of the presentation, participants will be able to:
  1. List major classes of monoclonal antibody therapeutics and their clinical applications.
  2. Discuss how the presence of monoclonal therapeutic antibodies can impact and/or interfere in existing clinical tests, such as protein electrophoresis and immunofixation.
  3. Analyze alternatives to mitigate the issue in the clinical laboratory.

What is a Therapeutic Monoclonal Antibody?

- Definition
  - Use of monoclonal antibody to block and/or clear molecules or cells involved in disease process
  - Occurs through activation of endogenous immune system

- Antigen targets

<table>
<thead>
<tr>
<th>Soluble molecules</th>
<th>Membrane-bound molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>CD20</td>
</tr>
<tr>
<td>VEGF</td>
<td>CD38</td>
</tr>
<tr>
<td>IgE</td>
<td>PD-1/PDL1</td>
</tr>
</tbody>
</table>

The Immunoglobulin Structure

- Composed of 4 peptide chains
  - 2 identical heavy chains
    - Gamma
    - Delta
    - Mu
    - Epsilon
  - 2 identical light chains
    - Kappa
    - Lambda

- Ig class defined by heavy chain
- IgG divided into 4 subclasses

There are Four IgG Subclasses

- >95% sequence homology in constant region across IgG subclasses

<table>
<thead>
<tr>
<th></th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulating concentrations, mg/dL</td>
<td>341.884</td>
<td>171.632</td>
<td>18.4-106</td>
<td>2.4-121</td>
</tr>
<tr>
<td>% of total</td>
<td>40.75%</td>
<td>15.50%</td>
<td>2.5%</td>
<td>1-10%</td>
</tr>
<tr>
<td>Complement fixation</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>FcR binding</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Half-life (days)</td>
<td>20-24</td>
<td>20-24</td>
<td>7-10</td>
<td>20-24</td>
</tr>
</tbody>
</table>

Mechanism of Action of Therapeutic mAbs

- ADCC
- Phagocytosis
- NK cell killing
- FeR binding
- Classical pathway activation

J Golay and M Introna, Arch Biochem Biophys. 526 (2012) 146-153
Nomenclature of Therapeutic mAbs

<table>
<thead>
<tr>
<th></th>
<th>Murine</th>
<th>Chimeric</th>
<th>Humanized</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derived</td>
<td>Murine</td>
<td>Murine variable</td>
<td>Murine hypervariable</td>
<td>Fully human Ig</td>
</tr>
<tr>
<td>from</td>
<td>region with human</td>
<td>region in human</td>
<td>produced from</td>
<td>produced from</td>
</tr>
<tr>
<td>immunization</td>
<td>constant region</td>
<td>immunoglobulin</td>
<td>transgenic mice</td>
<td>transgenic mice</td>
</tr>
<tr>
<td>animal</td>
<td>Suffix -omab</td>
<td>Suffix -ximab</td>
<td>or phage display</td>
<td>or phage display</td>
</tr>
<tr>
<td>Suffix</td>
<td>-omab</td>
<td>-ximab</td>
<td>-zumab</td>
<td>-umab</td>
</tr>
</tbody>
</table>

Therapeutic Monoclonal Antibodies
Specific Examples

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Muromonab-CD3</th>
<th>Rituximab</th>
<th>Trastuzumab</th>
<th>Adalimumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand Name</td>
<td>Orthoclone OKT3</td>
<td>Rituxan</td>
<td>Herceptin</td>
<td>Humira</td>
</tr>
<tr>
<td>Approval Date</td>
<td>1986</td>
<td>1997</td>
<td>1998</td>
<td>2002</td>
</tr>
<tr>
<td>Class</td>
<td>Murine</td>
<td>Chimeric</td>
<td>Humanized</td>
<td>Human</td>
</tr>
<tr>
<td>Target</td>
<td>CD3</td>
<td>CD20</td>
<td>HER2</td>
<td>TNF-α</td>
</tr>
<tr>
<td>Disease</td>
<td>Transplant rejection</td>
<td>Non-Hodgkin lymphoma</td>
<td>Breast carcinoma</td>
<td>Various autoimmune disorders</td>
</tr>
</tbody>
</table>

Application Areas for Therapeutic mAbs

- Oncology: 38%
- Immunology and Inflammation: 27%
- Neuroscience: 12%
- Cardiovascular and metabolic: 7%
- Hematology: 5%
- Respiratory: 4%
- Infectious: 3%
- Ophthalmology: 3%
- Other: 1%
- Immunology and Inflammation: 27%
- Oncology: 38%
- Neuroimmunology: 12%
- Cardiovascular and metabolic: 7%
- Hematology: 5%
- Respiratory: 4%
- Infectious: 3%
- Ophthalmology: 3%
- Other: 1%

Therapeutic mAbs as Interferences in Lab Tests

- Interference in lab tests
- Immunogenicity
- Therapeutic monoclonal antibody (mAb)
- Differentiation from endogenous disease causing clone
- Therapeutic drug monitoring

The Complement System as a Start

- Classical Pathway
- Terminal Pathway
- Alternative Pathway

Classical Pathway
- C1qrs
- C4, C2

Terminal Pathway
- C3, C5, C6, C7, C8, C9
- Activation of Terminal Pathway and formation of Membrane Attack Complex

Alternative Pathway
- D
- C3b, B + P
- or C3b+H2O
- Lysis
- Activation of Terminal Pathway and formation of Membrane Attack Complex
Types of Complement Serology Tests

- Functional or Activity Assays
  - CH50, total complement function
  - AH50, alternative pathway function
  - E.g. Hemolytic assays
- Concentration of each complement component
  - C3, C4, C5
  - Soluble MAC
  - E.g. nephelometry, turbidimetry, ELISA

Eculizumab: IgG2/4 mAb targeting C5

How can Eculizumab Impact Results

Experiments

- 12 residual waste serum with normal total complement function
- Spiked with increasing concentrations of eculizumab
  - 0 > 25 µg/mL > 50 µg/mL > 100 µg/mL > 150 µg/mL > 200 µg/mL
- Tested
  - CH50, AH50
  - C5 antigen concentration, C5 function
  - sMAC

At 50 µg/mL of Eculizumab, There is Little Residual Complement Activity

Daratumumab: IgG1 targeting CD38

CD38 is not Present Only in Plasma Cells...

- CD38 is present in
  - Myeloid cells
  - Lymphoid cells
  - Red blood cells
  - Other tissues
**CD38 and Blood Transfusions**

- Prior to RBC blood transfusions, a test to detect significant Ab to RBC Ag is required.
- If screening test is positive, additional individual Ab tests are performed.
- Daratumumab may cause:
  - Screening test to be positive
  - All Individual Ab tests to be positive “pan-reactivity”
  - Positive crossmatches with all units

**DDT Protocol to Reduce Immunocomplexes on RBC Surface**

- 0.2M DTT: dithiothreitol, reducing agent

**A new IgG kappa on Immunofixation**

- Therapeutic mAbs are being used to treat multiple myeloma
- May show up as abnormalities on protein electrophoresis and immunofixation
  - Daratumumab, isatuximab, elotuzumab
- Other mAbs in high concentrations may be visible
  - E.g. Rituximab (Anti-CD20)

**Serum Protein Electrophoresis and Immunofixation**

**Case #1, 58yo female**

- History of Multiple Myeloma
- IgG lambda migrating in the beta-fraction
- Shows up for laboratory follow-up
- How to report findings?

<table>
<thead>
<tr>
<th>Protein</th>
<th>RI (g/dL)</th>
<th>Result</th>
<th>RI (g/dL)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein</td>
<td>6.3-7.9</td>
<td>5.7</td>
<td>Alpha-2</td>
<td>0.6-1.0</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.4-4.7</td>
<td>3.5</td>
<td>Beta</td>
<td>0.7-1.2</td>
</tr>
<tr>
<td>Alpha-1</td>
<td>0.1-0.3</td>
<td>0.3</td>
<td>Gamma</td>
<td>0.6-1.6</td>
</tr>
</tbody>
</table>

RI: reference interval

**Case #2, 62yo male**

- History of IgG kappa multiple myeloma, relapse
- M-spike in gamma fraction
- How to report findings?

<table>
<thead>
<tr>
<th>Protein</th>
<th>RI (g/dL)</th>
<th>Result</th>
<th>RI (g/dL)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein</td>
<td>6.3-7.9</td>
<td>6.7</td>
<td>Alpha-2</td>
<td>0.6-1.0</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.4-4.7</td>
<td>3.9</td>
<td>Beta</td>
<td>0.7-1.2</td>
</tr>
<tr>
<td>Alpha-1</td>
<td>0.1-0.3</td>
<td>0.5</td>
<td>Gamma</td>
<td>0.6-1.6</td>
</tr>
</tbody>
</table>

M-spike 0.8
Case #3, 75yo male

- History of a small monoclonal kappa in the gamma fraction
- Shows up for follow-up
- How to report findings?

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RI (g/dL)</th>
<th>Result</th>
<th>RI (g/dL)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein</td>
<td>5.2-7.9</td>
<td>5.7</td>
<td>5.0-10</td>
<td>1.1</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.4-4.7</td>
<td>3.1</td>
<td>3.7-1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Alpha-1</td>
<td>0.1-0.3</td>
<td>0.4</td>
<td>0.6-1.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Elotuzumab can be Detected in SPEP and IFE in Samples from Patients Treated with Elotuzumab


Daratumumab Interference on IFE

DIRA – Daratumumab IFE Reflex Assay


Example 1

Paul K.

DATE OF BIRTH 02-09-1955

Serum Peak/Pathology

IgG Kappa (K) Multiple Myeloma

Urine Peak

Free Light Chain K

Screening

IF With HYDRASHIFT 2/4 daratumumab

Persistence of Paul’s monoclonal IgG Kappa

Paul reached Complete Response according to IMWG response criteria

Example 2

Anna B.

DATE OF BIRTH 12-23-1948

Serum Peak/Pathology

IgG Kappa (K) Multiple Myeloma

Urine Peak

Free Light Chain K

Screening

IF With HYDRASHIFT 2/4 daratumumab

Anna’s monoclonal IgG Kappa

Co-migration with dar: impossible to differentiate Anna’s monoclonal IgG Kappa from daratumumab interference
Julia M.

DATE OF BIRTH: 05-09-1942
SERUM PEAK Pathology: IgG Kappa Multiple Myeloma
URINE PEAK: Free Light Chain K

Example 3

Screening C2D1 C5D1

Julia's monoclonal IgG Kappa

Co-migration with dara: Impossible to differentiate Julia's monoclonal IgG K from dara interference

Persistence of Julia's monoclonal IgG K

Daratumumab/anti-daratumumab immune complex

IF With HYDRASHIFT 24 daratumumab

Mass Spectrometry Assays

The Accurate Mass of Each Monoclonal Antibody Used as Therapeutics can be Accurately Identified

Accurate mass of intact light chains on 5600 Q-TOF

- Infliximab 23,434 Da
- Adalimumab 23,406 Da
- Vedolizumab 23,901 Da
- Rituximab 23,035 Da
- Eculizumab 23,130 Da

Mass of light chain of mAbs was identified after purification of biologics in buffer

Patient A Inf 125 µg/mL
2131.4 Da

Patient B Ada 36 µg/mL
2128.8 Da

Patient C Ecu 249 µg/mL
2103.7 Da

Patient D Ved 131 µg/mL
2173.9 Da


Purify Ig using Melon™ Gel
Dissociate heavy chain + light chain
Inline column chromatography
Analyze by TOF-MS on 5600 Q-TOF

Monoclonal immunoglobulin Rapid Accurate Mass Measurement (miRAMM)

+11 Charge State of Each mAb Spiked in Normal Human Serum

- Adalimumab 100 µg/mL 2128.9 Da
- Eculizumab 200 µg/mL 2103.7 Da
- Vedolizumab 300 µg/mL 2173.9 Da
- Rituximab 400 µg/mL 2095.1 Da

On Immunofixation they are all IgG Kappas

Using miRAMM

Different Challenges in the Future

• Therapeutic mAbs may come in different forms
  • Monoclonal Antibody Fragments
  • Bispecific mAbs
  • Antibody-Drug Conjugates (ADCs)
  • New challenges for the laboratory

Acknowledgements

• David L. Murray, M.D. Ph.D.
• Melissa R. Snyder, Ph.D.
• Paula M. Ladwig
• David R. Barnidge, Ph.D.
• Linda Tostrud
• Karen Lockington
• Rachel Young