


MAYO CLINIC


Monoclonal Antibody Therapeutics
 Potential Interferents on Protein Electrophoresis and Related Tests

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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?

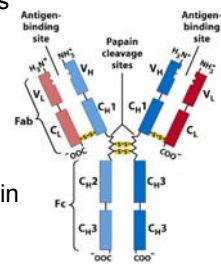
- Definition
 - Use of monoclonal antibody to block and/or clear molecules or cells involved in disease process
 - Occurs through activation of endogenous immune system
- Antigen targets

Soluble molecules	Membrane-bound molecules
<ul style="list-style-type: none"> • TNF • VEGF • IgE 	<ul style="list-style-type: none"> • CD20 • CD38 • PD-1/PDL1



The Immunoglobulin Structure


- Composed of 4 peptide chains
 - 2 identical heavy chains
 - Gamma > Delta
 - Mu > Epsilon
 - Alpha
 - 2 identical light chains
 - Kappa > Lambda
- Ig class defined by heavy chain
- IgG divided into 4 subclasses



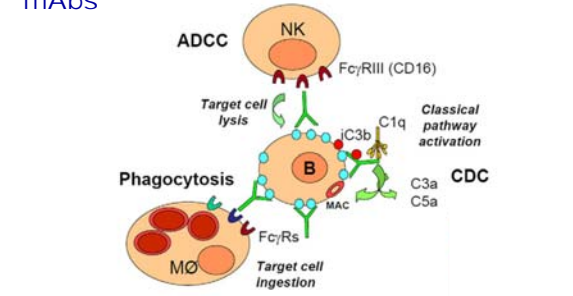
There are Four IgG Subclasses

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


	IgG1	IgG2	IgG3	IgG4
Circulating concentrations, mg/dL	341-894	171-632	18.4-106	2.4-121
% of total	40-75%	15-50%	2-5%	1-10%
Complement fixation	++	+	+++	-
FcR binding	++	-	+++	-
Half-life (days)	20-24	20-24	7-10	20-24

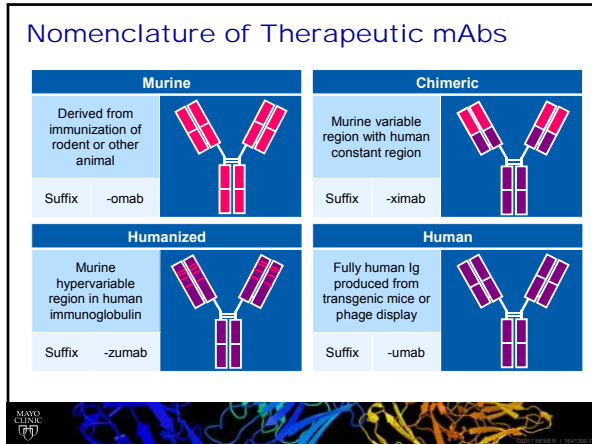


Mechanism of Action of Therapeutic mAbs



J Golay and M Inrona. Arch Biochem Biophys. 526 (2012) 146-153

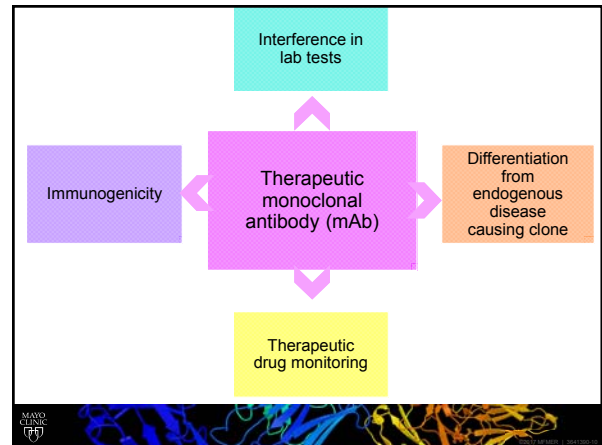
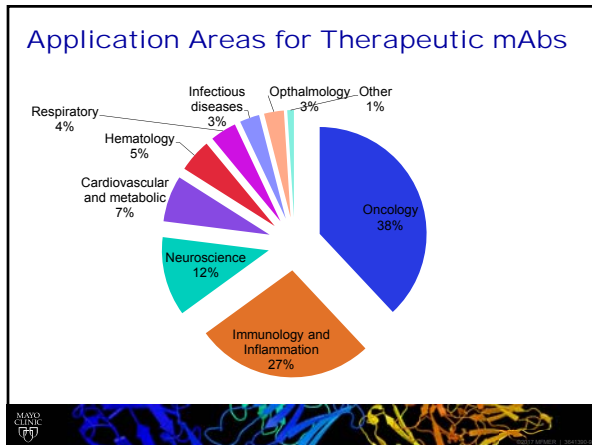




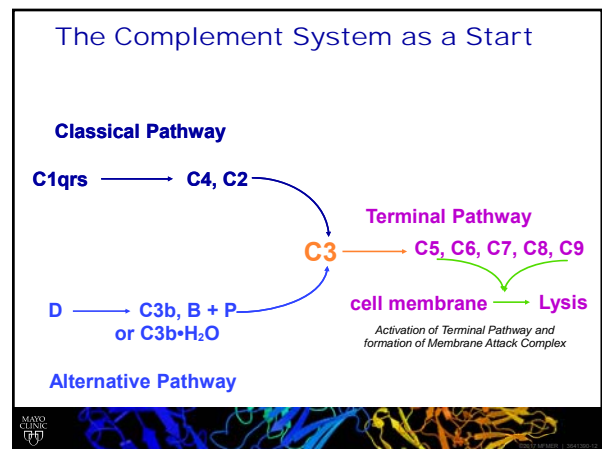
Therapeutic Monoclonal Antibodies

Specific Examples

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



Therapeutic mAbs as Interferences in Lab Tests

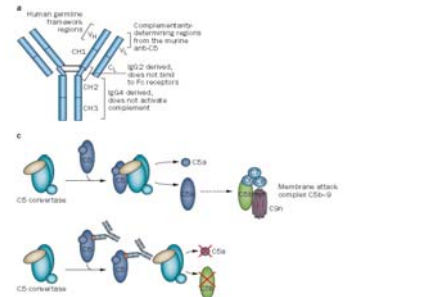


Types of Complement Serology Tests

- 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



Zuber, J et al. Nat. Rev. Nephrol. 2012. doi:10.1038/nrneph.2012.214

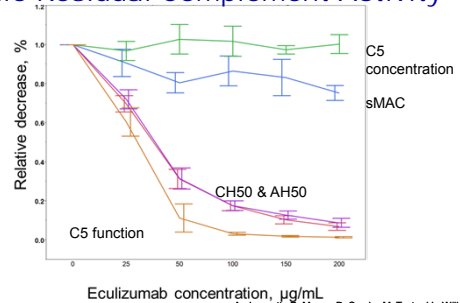


How can Eculizumab Impact Results Experiments

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



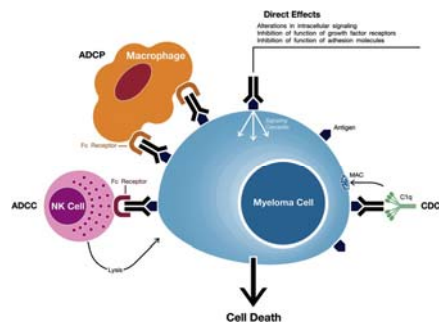
At 50 $\mu\text{g}/\text{mL}$ of Eculizumab, There is Little Residual Complement Activity



Andreguetto B, Murray D, Snyder M, Tostrud L, Willich MA. Mol Immunol. 2015;67(1):119-20.



Daratumumab: IgG1 targeting CD38



Niels W. C. J. van de Donk et al. Blood 2016;127:681-695




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



DTT Protocol to Reduce Immunocomplexes on RBC Surface

- 0.2M DTT: dithiothreitol, reducing agent

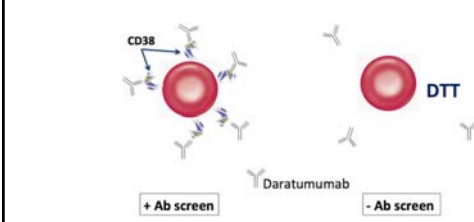




Image from www.bloodbankguy.org – Dr. Kaufmann slides, BWH

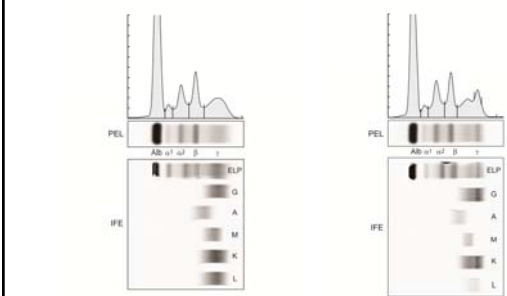



A new IgG kappa on Immunofixation

- 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?

	RI (g/dL)	Result		RI (g/dL)	Result
Total Protein	6.3-7.9	5.7	Alpha-2	0.6-1.0	1.0
Albumin	3.4-4.7	3.0	Beta	0.7-1.2	1.1
Alpha-1	0.1-0.3	0.3	Gamma	0.6-1.6	0.3

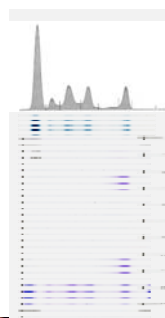

RI, reference interval

Case #2, 62yo male

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

	RI (g/dL)	Result		RI (g/dL)	Result
Total Protein	6.3-7.9	6.7	Alpha-2	0.6-1.0	1.3
Albumin	3.4-4.7	2.9	Beta	0.7-1.2	0.9
Alpha-1	0.1-0.3	0.5	Gamma	0.6-1.6	1.1
			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?

	RI (g/dL)	Result		RI (g/dL)	Result
Total Protein	6.3-7.9	5.7	Alpha-2	0.6-1.0	1.1
Albumin	3.4-4.7	3.1	Beta	0.7-1.2	0.8
Alpha-1	0.1-0.3	0.4	Gamma	0.6-1.6	0.4

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

A SPEP and serum IFE at baseline

B SPEP and serum IFE at cycle 34

Niels W. C. J. van de Donk et al. Blood 2016;127:681-695

Daratumumab Interference on IFE

DIRA – Daratumumab IFE Reflex Assay

McCudden et al., CCLM 2016 doi: 10.1515/cclm-2015-1031.
Van de Donk et al., CCLM 2016 doi: 10.1515/cclm-2015-0888.

Result: IF with shifted dara

Without HYDRASHIFT 2/4 daratumumab: daratumumab/anti-daratumumab immune complex, Patient peak: IgG Kappa.

With HYDRASHIFT 2/4 daratumumab: Dara/anti-dara immune complex.

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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 → daratumumab therapy (16mg/Kg) → C2D1 → C4D1

IF With HYDRASHIFT 2/4 daratumumab: Persistence of Paul's monoclonal IgG K. Paul reached Complete Response according to IMWG response criteria.

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Example 2

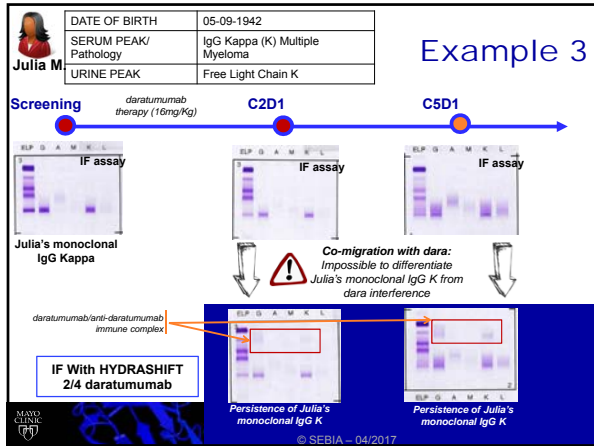
Anna B.

DATE OF BIRTH	12-23-1948
SERUM PEAK/ Pathology	IgG Kappa (K) Multiple Myeloma
URINE PEAK	Free Light Chain K

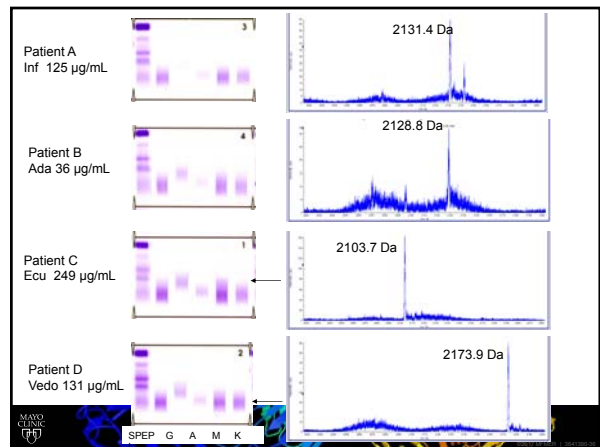
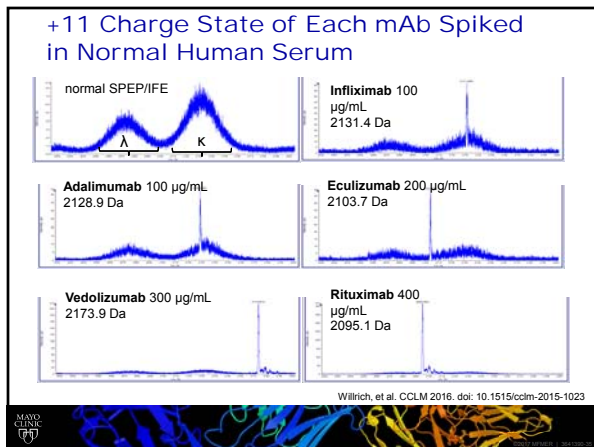
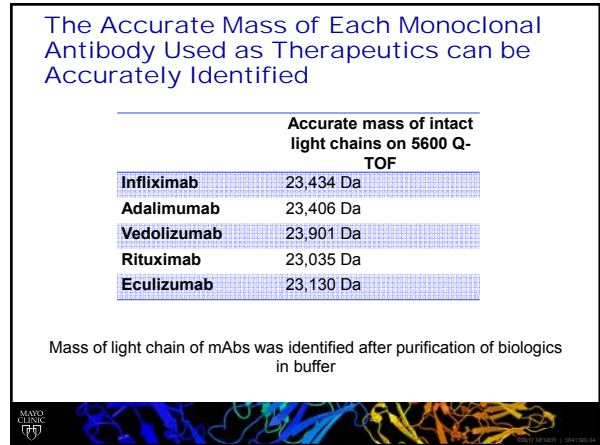
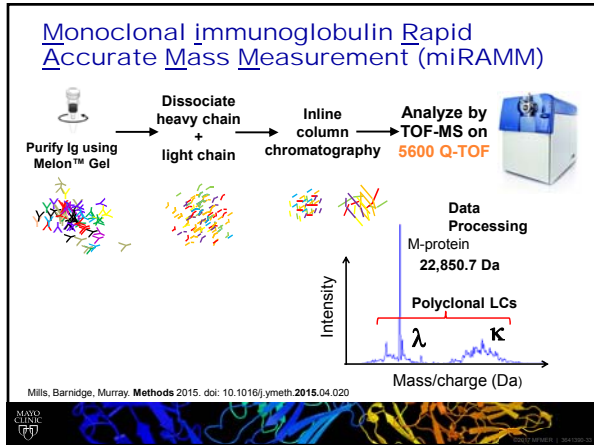
Screening → daratumumab therapy (16mg/Kg) → C3D1 → C5D1

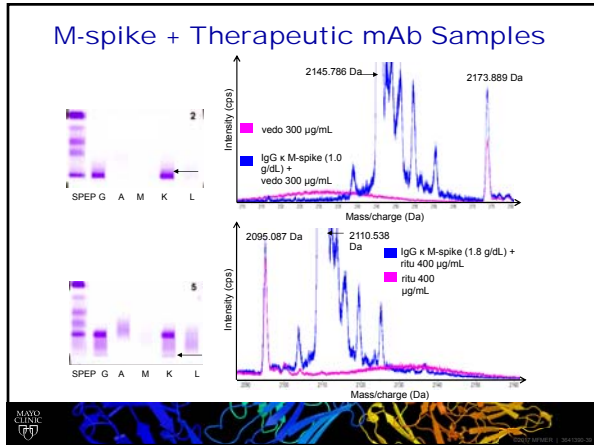
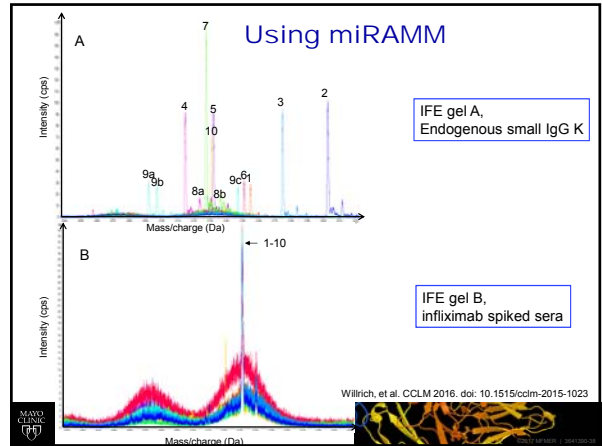
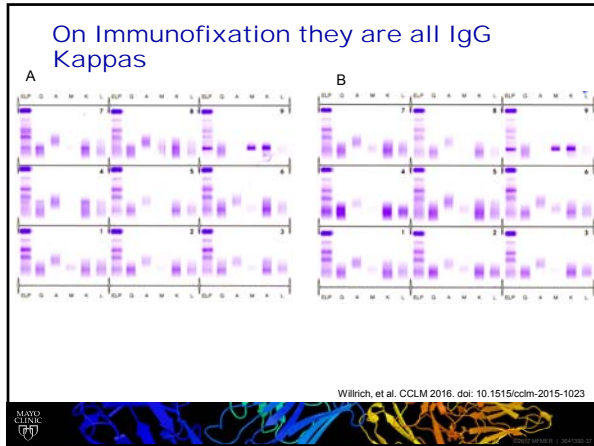
IF With HYDRASHIFT 2/4 daratumumab: Persistence of Anna's monoclonal IgG K. Anna reached Complete Response according to IMWG response criteria.

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Mass Spectrometry Assays





- ### In Summary
- Therapeutic mAbs may impact lab test results
 - Use and administration should be known by the laboratory
 - Interpretation of results should take mAbs into consideration when reporting
 - An abnormal complement result
 - A pan-reactive RBC antibody screen
 - A new IgG kappa on SPEP/IFE
 - New interferences will be discovered as their use becomes more common
 - There are ways to mitigate the interferences
 - DTT, gel shift assays, mass spectrometry

- ### 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
-
- Wilrich, et al. CCLM 2016. doi: 10.1515/cclm-2015-1023

- ### Acknowledgements
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 - David R. Barnidge, Ph.D.
 - Linda Tostrud
 - Karen Lockington
 - Rachel Young
- Wilrich, et al. CCLM 2016. doi: 10.1515/cclm-2015-1023



Questions & Discussion
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