

# CD 38 & CD 47 – Blood Bank's Kryptonite

Shay Jones MLS (ASCP)<sup>CM</sup>, BB<sup>CM</sup>

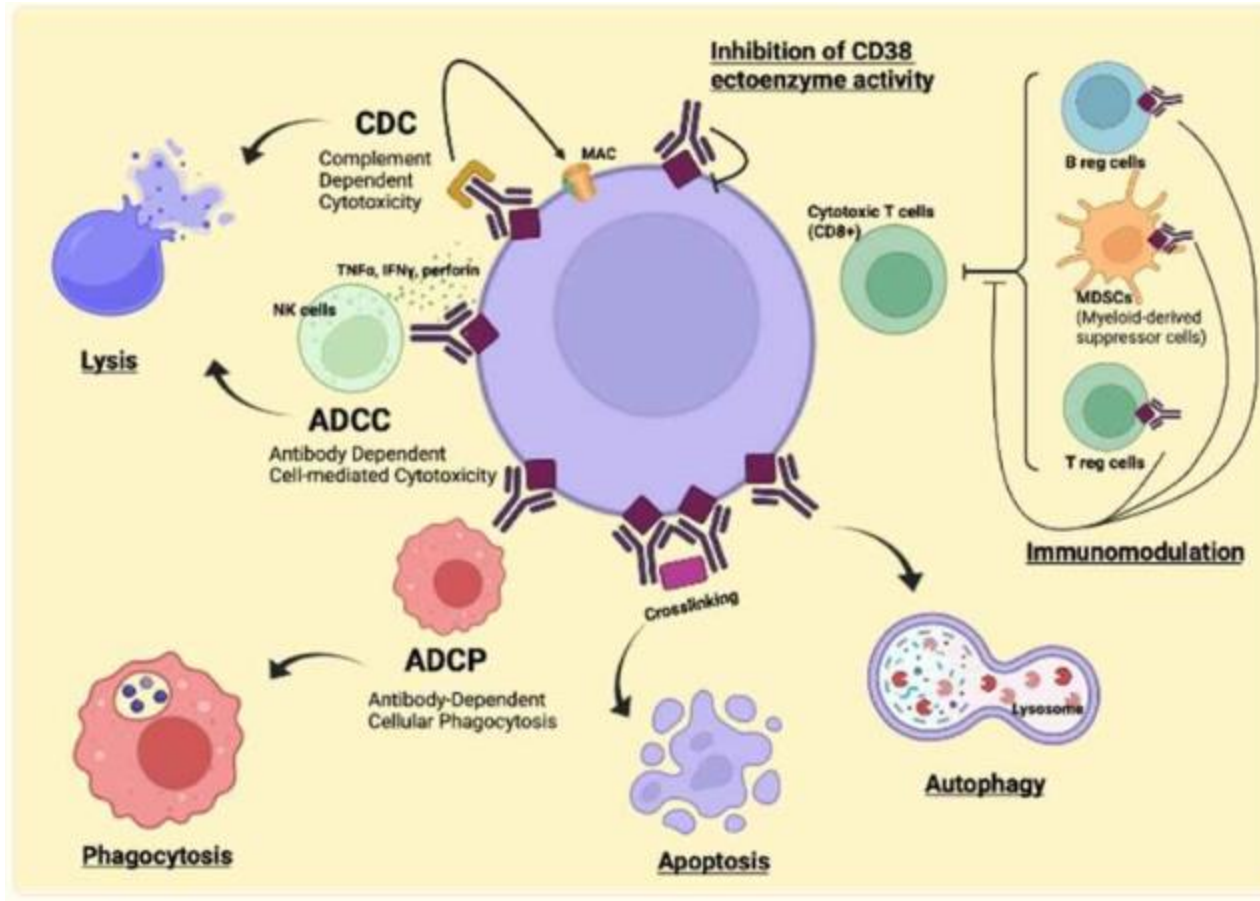
# Objectives

- At the end of this presentation, participants should be able to distinguish the differences between reactivity due to anti-CD38 and anti-CD47 therapies
- At the end of this presentation, participants should be able to design a workflow to allow for the resolution of reactivity due to anti-CD38 and anti-CD47 therapies

# Drug Interference - CD 38

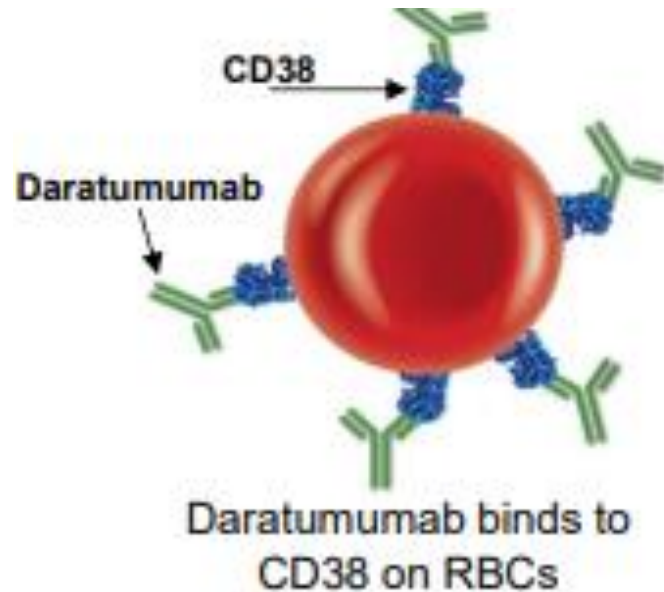
- Daratumumab works through different mechanisms of action
  - Antibody-dependent cellular cytotoxicity
  - Antibody-dependent cellular phagocytosis
  - Complement-dependent cytotoxicity
  - Direct cytotoxicity through apoptosis by Fc gamma receptor-mediated

# Drug Interference - CD 38

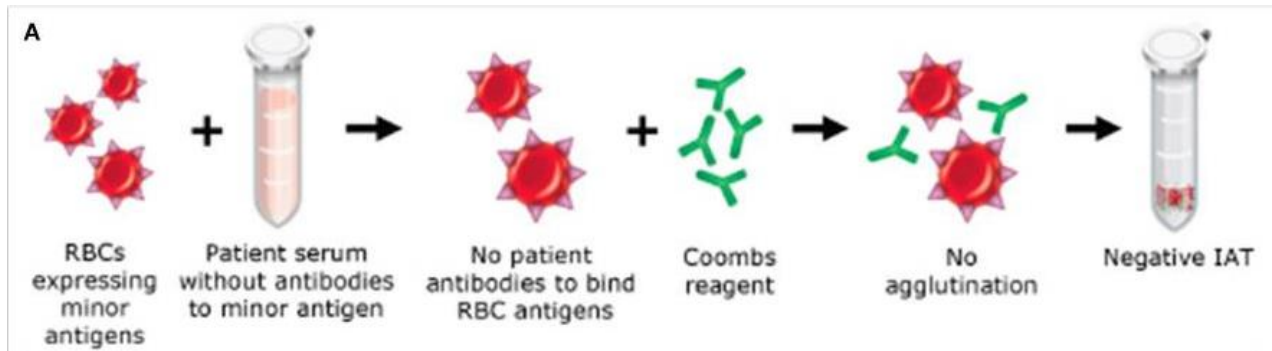


# Drug Interference - CD 38

- What does this mean for blood banks?
  - CD 38 proteins are found in minimal amounts on red blood cells – including reagent red blood cells
  - Patients receiving anti-CD38 therapy may show weak reactivity during serologic testing (DAT, antibody screen, antibody ID, XM)



# Drug Interference - CD 38



# Drug Interference - CD 38

- The immediate concern is the masking of potential, clinically significant alloantibodies
- There are a recommendations for patients before starting anti-CD38 therapy
  - An initial type and screen to detect any new or historical antibodies present
  - Phenotype or genotype may be helpful in providing pRBCs for future transfusions (more on that later)
- Like everything in blood bank, communication is key! The more prepared for the observed reactivity, the quicker the patient can receive transfusion

# Drug Interference - CD 38 Case Study #1

- Initial reactions observed during antibody screen testing
  - Patient history indicates recent anti-CD38 treatment

	Rh					MNSs				Kell		Duffy		Kidd		Results	
	D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	5' RT	PEG IAT
1	+	+	0	0	+	+	+	0	+	+	+	0	+	+	0	0	1+
2	+	0	+	+	0	+	0	0	+	0	+	+	0	0	+	0	1+
3	0	0	0	+	+	0	+	+	+	+	+	0	+	+	+	0	1+



# Drug Interference - CD 38

- Like most panreactivity, alloadsorptions may be the blood banks/ IRLs first thought...
  - Adsorptions using untreated or treated cells fails to adsorb out the CD 38 antibody
- Dithiothreitol (DTT) treated cells are successful in removing the reactivity
  - Keep in mind that DTT treatment does not just remove the CD 38 proteins (Kell, Lutheran, YT, JMH, LW, Cromer, Indian, Dombrock and Knops)
  - As some of these antigens are low prevalence or clinically insignificant, the most common concern is Kell system antibodies

# Drug Interference - CD 38 Case Study #1

- Successful DTT treatment

	Rh					MNSs				Kell		Duffy		Kidd		Results
	D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	DTT treated reagent RBCs
1	+	+	0	0	+	+	+	0	+			0	+	+	0	0√
2	+	0	+	+	0	+	0	0	+			+	0	0	+	0√
3	0	0	0	+	+	0	+	+	+			0	+	+	+	0√

		Rh					MNSs				Kell		Duffy		Kidd		Results
		D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	DTT treated reagent RBCs
1	R1R1w	+	+	0	0	+	+	+	0	+			+	0	+	0	0√
2	R1R1	+	+	0	0	+	+	0	0	+			+	+	0	+	0√
3	R2R2	+	0	+	+	0	+	0	0	+			+	+	0	+	0√
4	Ror	+	0	0	+	+	+	+	+	0			0	0	+	0	0√
5	r'r	0	+	0	+	+	0	+	0	+			+	0	+	0	0√
6	r''r	0	0	+	+	+	0	+	0	+			0	+	+	+	0√
7	rr	0	0	0	+	+	0	+	+	+			0	+	+	0	0√
8	rr	0	0	0	+	+	+	0	+	0			+	+	+	+	0√
9	rr	0	0	0	+	+	0	+	0	+			+	+	0	+	0√
10	rr	0	0	0	+	+	0	+	+	0			0	+	0	+	0√
11	R1R1	+	+	0	0	+	+	0	+	0			0	+	+	0	0√

# Drug Interference - CD 38


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# TRANSFUSION

IMMUNOHEMATOLOGY

## **Use of an in-house trypsin-based method to resolve the interference of daratumumab**

Nnaemeka Ibeh, Ian Baine, Louella Fuentes Rudon, Christine Lomas-Francis, Jeffrey S. Jhang, Patricia Galdon, Connie M. Westhoff, Randall W. Velliquette, Suzanne A. Arinsburg 

First published: 01 September 2021 | <https://doi.org/10.1111/trf.16635> | Citations: 1

# Drug Interference - CD 38 Case Study #1

- Trypsin treatment can also be helpful in mitigating the anti-CD38 reactivity while being able to rule out antibodies to Kell system antigens

	Rh					MNSs				Kell		Duffy		Kidd		Results
	D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	Trypsin treated reagent RBCs
<b>1</b>	+	+	0	0	+	+	+	0	+	+	+	0	+	+	0	0√
<b>2</b>	+	0	+	+	0	+	0	0	+	0	+	+	0	0	+	0√
<b>3</b>	0	0	0	+	+	0	+	+	+	+	+	0	+	+	+	0√

# Drug Interference - CD 38 Case Study #2

- Initial reactions observed during antibody screen testing
  - This patient is from out of town
  - Up to date medical history is limited
  - Patient mentions the diagnosis of multiple myeloma

	Rh					MNSs				Kell		Duffy		Kidd		Results	
	D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	5' RT	PEG IAT
<b>1</b>	+	+	0	0	+	+	+	0	+	+	+	0	+	+	0	0	1+
<b>2</b>	+	0	+	+	0	+	0	0	+	0	+	+	0	0	+	0	1+
<b>3</b>	0	0	0	+	+	0	+	+	+	+	+	0	+	+	+	0	1+

# Drug Interference - CD 38 Case Study #2

- Due to the history of MM, the tech takes a wild guess

	Rh					MNSs				Kell		Duffy		Kidd		Results
	D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	DTT treated reagent RBCs
1	+	+	0	0	+	+	+	0	+			0	+	+	0	0√
2	+	0	+	+	0	+	0	0	+			+	0	0	+	0√
3	0	0	0	+	+	0	+	+	+			0	+	+	+	0√

## Drug Interference - CD 38 Case Study #2

- At this point, the provider is consulted to try and get a clear medical history...
  - After some time, it is determined that the patient IS NOT receiving any anti-CD38 therapy...





		Rh					MNSs				Kell		Duffy		Kidd		Results
		D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	DTT treated reagent RBCs
1	R1R1w	+	+	0	0	+	+	+	0	+			+	0	+	0	0√
2	R1R1	+	+	0	0	+	+	0	0	+			+	+	0	+	0√
3	R2R2	+	0	+	+	0	+	0	0	+			+	+	0	+	0√
4	Ror	+	0	0	+	+	+	+	+	0			0	0	+	0	0√
5	r'r	0	+	0	+	+	0	+	0	+			+	0	+	0	0√
6	r''r	0	0	+	+	+	0	+	0	+			0	+	+	+	0√
7	rr	0	0	0	+	+	0	+	+	+			0	+	+	0	0√
8	rr	0	0	0	+	+	+	0	+	0			+	+	+	+	0√
9	rr	0	0	0	+	+	0	+	0	+			+	+	0	+	0√
10	rr	0	0	0	+	+	0	+	+	0			0	+	0	+	0√
11	R1R1	+	+	0	0	+	+	0	+	0			0	+	+	0	0√

# Drug Interference - CD 38 Case Study #2

- This sure smells like anti-CD38...
- Patient information is key!
  - This patient is being seen by another facility!
  - The blood bank reaches out to the outside facility and learns the patient IS receiving anti-CD-38 therapy!
  - With that information, and the observed reactivity, we are able to determine that the reactivity seen is due to the anti-CD38

# Drug Interference - CD 38

<b>Test</b>	<b>Negative (no interference)</b>	<b>Positive (reactive with all cells)</b>	<b>Negative or positive</b>
ABO/RhD typing	X	N/A	N/A
Antibody detection ("screen")	N/A	X	N/A
Antibody identification	N/A	X	N/A
DAT	N/A	N/A	X
IS crossmatch	X	N/A	N/A
AHG crossmatch*	N/A	X	N/A

# Drug Interference - CD 38

- The University of Kansas Health System policy
  - Initial antibody screen is performed in solid phase method – confirmation that patient is receiving anti-CD38 treatment
  - Patient's plasma is tested with 3 cell screening cells in LISS IAT
  - Patient's plasma is tested with previously DTT treated or newly DTT treated reagent red blood cells are tested
  - If the LISS cells are positive and DTT cells are negative, K negative units are provided

# Drug Interference - CD 38

Review: Effects of anti-CD38 monoclonal antibodies on red blood cell transfusion and interventions

[Jia Song](#)<sup>1</sup> and [Rong Fu](#)<sup>1</sup>

- 47 patients received 147 units of RBCs for transfusion
  - No patients had any subsequent transfusion reaction or hemolysis
  - Cited efficacy of transfusing phenotyped matched RBCs

# Drug Interference - CD 38


**Vox Sanguinis**

The International Journal of Transfusion Medicine



Original Paper

## **Risk of RBC alloimmunization in multiple myeloma patients treated by Daratumumab**

Zhan Ye  Laurie A. Wolf, Daniel Mettman, Fred V. Plapp

- A retrospective study of 45 MM patients
  - June 2015 – December 2018
  - All cases of positive aby screens were DTT treated
  - Transfusion history was monitored from first dose of DARA to the last
  - Control group: 46 MM patients receiving transfusion but NOT DARA

# Drug Interference - CD 38


**Vox Sanguinis**

The International Journal of Transfusion Medicine



Original Paper

## **Risk of RBC alloimmunization in multiple myeloma patients treated by Daratumumab**

- Zhan Ye  Laurie A. Wolf, Daniel Mettman, Fred V. Plapp
  - 184 aby screens were performed on 45 patients
    - Patients transfused with ABO-Rh compatible RBCs, pheno-matched units or both
  - No detectable antibody after DTT treatment
    - 2 patients with historical antibodies – no new alloantibodies detected
  - Risk of alloantibody formation is very low, no significant difference between ABO-Rh compatible and pheno-matched RBCs

# Drug Interference - CD 38

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## TRANSFUSION

BRIEF REPORT

### **Efficient neutralization of daratumumab in pretransfusion samples using a novel recombinant monoclonal anti-idiotypic antibody**

Fleur Aung, Jeff Spencer, David Potter, Thuy-Dung Pham, Naheed Farooqui, Kathryn R. Platt, Raja Zayat, Melanie Oliveira, Robin Smeland-Wagman, Eric Petersen, Richard M. Kaufman 



# Drug Interference - CD 38

- Recombinant monoclonal rabbit anti-DARA antibody was created
- IAT's in gel were performed to determine the ratio of anti-DARA
- Anti-DARA was used in tube tests to determine, and confirm, the ability to detect underlying alloantibodies

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**TRANSFUSION**

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# Drug Interference - CD 38

- At a ratio of 1:1 (or greater), anti-DARA was able to neutralize the DARA reactivity
- Anti-E and anti-K were identified in patient's neutralized plasma

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**TRANSFUSION**

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## Drug Interference - CD 38

- The use of anti-CD38 therapy will continue to effect blood bank testing results
- Communication is key!
  - There are many examples of patient medication charts not being updated
  - Often a patient will remember that they may have received treatment at another facility
  - Having updated patient information is key (to not only all of blood bank 😊) to properly identifying reactivity due to anti-CD38 treatment

# Drug Interference - CD 47

- CD 47 is a transmembrane protein and an important tumor antigen, overexpressed on multiple types of tumor cells
- CD 47 provides tumor cells with a “don’t eat me” signal that allows tumor cells to evade the immune system
- Multiple clinical trials in the past were halted due to severe hemolytic reaction of anti-CD47

# Drug Interference - CD 47

## CD47-SIRP $\alpha$



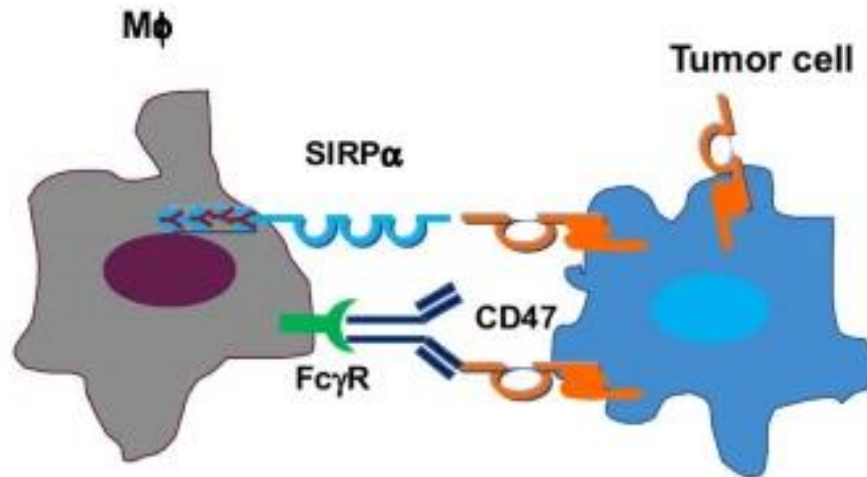
CD47

CD47 — Integrin associated protein (IAP)

- Ig-like protein
- 5 membrane spanning segments
- Short cytoplasmic tail
- Ubiquitous expression including T, B, RBC, platelet, HSC

# Drug interference – CD47

- CD47 working with SIRP $\alpha$  will create the “don’t eat me” signal – “The CD47-SIRP $\alpha$  axis”



- CD47 on cancer cells can inhibit myeloid cell-mediated clearance

# Drug Interference – CD47

- Studies focusing on blocking the CD47-SIRP $\alpha$  axis are of interest.....
- The ultimate goal is to block the CD47 action to allow and even enhance phagocytosis

# Drug Interference – CD47

- What does it mean for blood banks?
  - Like anti-CD38, anti-CD47 is found in large numbers on RBCs and PLTs
  - Unlike CD38, CD47 causes interference in all phase of testing (IS, RT, AHG)

## Monoclonal anti-CD47 interference in red cell and platelet testing

Randall W. Velliquette, Judith Aeschlimann, Julie Kirkegaard, Gayane Shakarian, Christine Lomas-Francis, Connie M. Westhoff 

- This study found that plasma from recipients of anti-CD47 therapies still had reactivity with DTT, trypsin, papain,  $\alpha$ -chymotrypsin treated RBCs



# Drug Interference – CD47

Test RBCs	IS	IAT* Ortho anti-IgG	IAT Carryover from IS Gamma-clone anti-IgG
D- rr (ce/ce)	4+	4+	mi-1+
D+	3+	4+	mi-1+
R <sub>2</sub> R <sub>2</sub> (DcE/DcE)			
D- -	0	4+	0/0
Rh <sub>mod</sub>	0	3+	0/0
Rh <sub>null</sub>	0	2+	0/0
D+ cord	3+	2+	mi-1+
Reverse (A or B)	3+		
Auto/DAT	0	0/mi	0/mi
Eluate	NT	4+	mi-1+

# Drug Interference – CD47

## Monoclonal anti-CD47 interference in red cell and platelet testing

Randall W. Velliquette, Judith Aeschlimann, Julie Kirkegaard, Gayane Shakarian, Christine Lomas-Francis, Connie M. Westhoff 

- Platelet testing
  - This study found that patient's that had not had prior plt transfusion, who received anti-CD47 had positive reactions observed with both Capture-P tests but negative reactions by ELISA testing

# Drug Interference – CD47

Sample	Sex	Monoclonal antibody therapy	Capture-P (% positive wells of 8)	Capture-P ready-screen (% positive wells of 13)	PakPlus
1	Male	Hu5F9-G4	Pos (100%)	Pos (100%)	Neg
2	Female	Hu5F9-G4	Pos (100%)	Pos (100%)	Neg
3	Male	DARA	Neg	Neg	Neg
4	Male	DARA	Pos (75%)	Pos (8%)	GP Ib/IX, IV HLA
5	Male	DARA	Pos (100%)	Pos (30%)	Neg
6	Male	DARA	Pos (100%)	Pos (30%)	Neg
7	Male	DARA	Pos (100%)	Neg	GP IIb/IIIa
8	Male	DARA	Pos (100%)	Pos (23%)	Neg

# Drug Interference – CD47

- There is still hope!
  - Reactivity was found to be removed with multiple alloadsorptions with papain-treated RBCs OR pooled platelets....
- There is even better hope!
  - Anti-CD47 is an IgG4 antibody
  - The use of Gamma-clone anti-IgG does not detect IgG4
  - Anti-CD47 reactivity is not usually observed in SPRCA testing

# Drug Interference – CD47 Case Study

- Initial reactions observed during ABO/Rh testing

Front Type					Back Type				
Anti-A	Anti-B	A1 Lectin	Anti-D	Rh Control	A <sub>1</sub>	A <sub>2</sub>	B	O	Auto control
4+	0	0	3+	0	4+	4+	4+	4+	4+

# Drug Interference – CD47 Case Study

- Initial reactions observed during antibody screen
  - Patient has a history of anti-E

	Rh					MNSs				Kell		Duffy		Kidd		Results	
	D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	5' RT	PEG IAT
1	+	+	0	0	+	+	+	0	+	+	+	0	+	+	0	3+	4+
2	+	0	+	+	0	+	0	0	+	0	+	+	0	0	+	3+	4+
3	0	0	0	+	+	0	+	+	+	+	+	0	+	+	+	4+	4+

# Drug Interference – CD47 Case Study

- At KU, we do not use the Gama-clone IgG
- We do have SPRCA....

	Rh					MNSs				Kell		Duffy		Kidd		Results
	D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	SPRCA
1	+	+	0	0	+	+	+	0	+	+	+	0	+	+	0	0
2	+	0	+	+	0	+	0	0	+	0	+	+	0	0	+	2+
3	0	0	0	+	+	0	+	+	+	+	+	0	+	+	+	0

		Rh					MNSs				Kell		Duffy		Kidd		Results
		D	C	E	c	e	M	N	S	s	K	k	Fy a	Fy b	Jk a	Jk b	SPRCA
1	R1R1w	+	+	0	0	+	+	+	0	+	+	+	+	0	+	0	0√
2	R1R1	+	+	0	0	+	+	0	0	+	0	+	+	+	0	+	0√
3	R2R2	+	0	+	+	0	+	0	0	+	0	+	+	+	0	+	2+
4	Ror	+	0	0	+	+	+	+	+	0	0	+	0	0	+	0	0√
5	r'r	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0√
6	r''r	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+	2+
7	rr	0	0	0	+	+	0	+	+	+	+	+	0	+	+	0	0√
8	rr	0	0	0	+	+	+	0	+	0	+	0	+	+	+	+	0√
9	rr	0	0	0	+	+	0	+	0	+	0	+	+	+	0	+	0√
10	rr	0	0	0	+	+	0	+	+	0	0	+	0	+	0	+	0√
11	R1R1	+	+	0	0	+	+	0	+	0	0	+	0	+	+	0	0√



# Drug Interference – CD47 Case Study

- We were unable to resolve the ABO discrepancy at KU

Front Type					Back Type				
Anti-A	Anti-B	A1 Lectin	Anti-D	Rh Control	A <sub>1</sub>	A <sub>2</sub>	B	O	Auto control
4+	0	0	3+	0	4+	4+	4+	4+	4+

- We called the ABO type “NTD” and gave O positive units whenever transfusion was needed

# Drug Interference – CD47 Case Study

- Interestingly, KU's CD47 patient's reactions changed.....

Front Type					Back Type				
Anti-A	Anti-B	A1 Lectin	Anti-D	Rh Control	A <sub>1</sub>	A <sub>2</sub>	B	O	Auto control
4+	0	0	3+	0	0	0	4+	0	0

- The patient received their last dose of anti-CD47 in December 2022 – ABO/Rh = NTD
- This ABO/Rh type was done exactly one month later, January 2023

# Drug Interference – CD47

- Transfusion recommendations can vary
  - Some facilities, if using alloabsorptions, opt to provide phenotypically matched
  - Other facilities, if using Gamma-clone IgG4, will provide XM compatible units

# Conclusion – Things to remember: CD38

- Anti-CD38 reactivity is typically weak
- Anti-CD38 may affect multiple tests (DAT, Aby screen, XM, etc)
- Anti-CD38 is susceptible to DTT and trypsin treatment – keeping in mind what antigens are destroyed in these processes
- Most policies provide K antigen negative units – remember the XM will be incompatible
- There are studies to suggest that patients receiving CD38 therapy are not prone to alloantibody formation

# Conclusion – Things to remember: CD47

- Anti-CD47 reactivity is typically very strong
- Anti-CD47 may affect multiple tests (ABO, DAT, Aby screen, XM, etc)
- Anti-CD47 is NOT susceptible to DTT and trypsin treatment – the use of alloadsorptions or different testing methods/reagents is useful
- Transfusion protocols differ

# Conclusion

- Anti-CD38 and anti-CD47 therapies are becoming more and more prevalent
- Knowing patient medical history is key to formulating a workflow for these patients
- Having a workflow for each treatment will allow for decreased turn-around-times for workups and will provide units to the patient more quickly

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