



# ■ Interference in the blood bank and ways to resolve them

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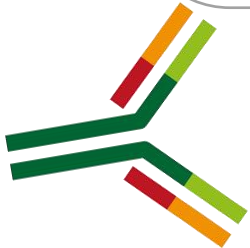
# Conflicts of Interest

Educational lectures for Immucor and Abbott

# Double trouble

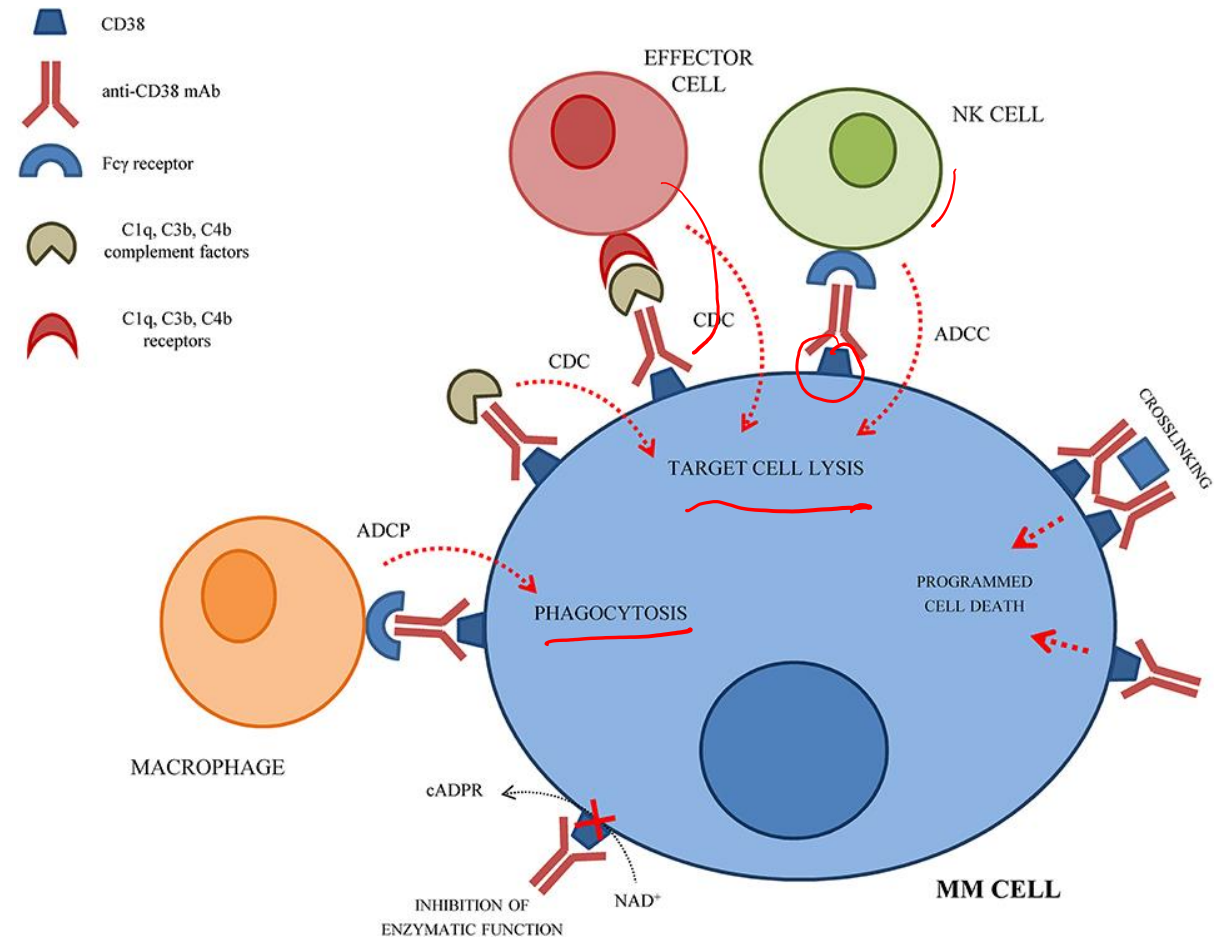
Anti-CD38

Anti-CD47



# CD38

- Cell surface enzyme that metabolizes NAD<sup>+</sup> release from damaged cells
- In cancer, suppresses T cells and promotes tumor growth
- CD38 is overexpressed in multiple myeloma and other hematological malignancies
- Low level of expression on RBCs

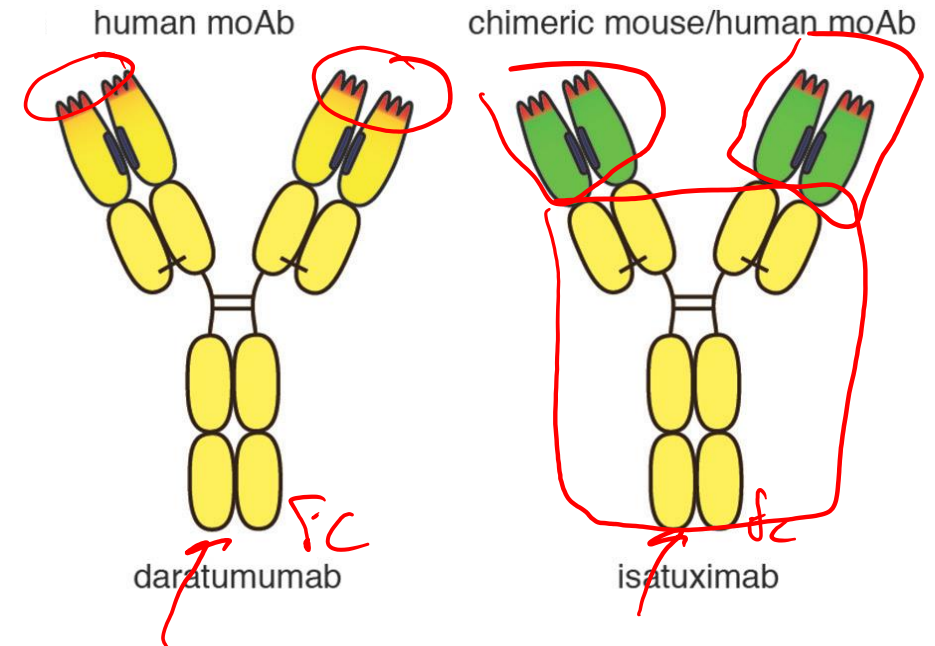


Bannas P, Koch-Nolte F. Perspectives for the Development of CD38-Specific Heavy Chain Antibodies as Therapeutics for Multiple Myeloma. *Front Immunol.* 2018 Nov 6;9:2559.

Morandi F, Horenstein AL, Costa F, Giuliani N, Pistoia V, Malavasi F. CD38: A Target for Immunotherapeutic Approaches in Multiple Myeloma. *Front Immunol.* 2018 Nov 28;9:2722.

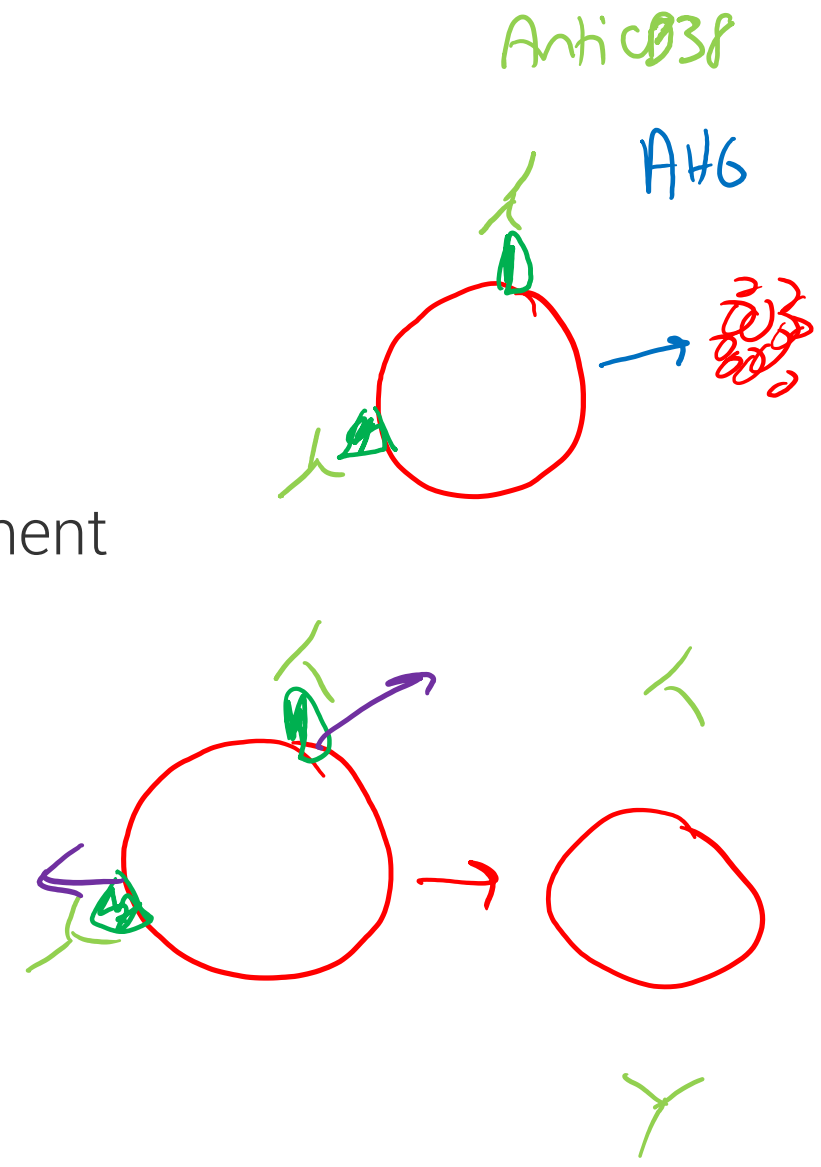
# Anti-CD38

- Daratumumab
  - » Transgenic mice immunized with human CD38
  - » Mice express IgH and IgL
- Isatuximab
  - » Mice immunized with human CD38
  - » VH, VL genetically fused to human IgG1 and kappa light chain



# Serology of CD38

- ABO/Rh typing mostly not affected
- DAT PS and IgG w-2+, if given recently 3+
  - » 75% of DATs are negative after long term treatment
- DAT complement rarely w-1+
- Antibody ID w-2+
- Eluate – rarely positive w-1+



# CD38 shedding on red blood cells

# Modifying or destroying the RBC CD38 antigen

- DTT or 2-ME

- » Destroys disulfide bridges

- » Kell, Cartwright, Dombrock, Indian, John Milton Hagen, Knops, Landsteiner-Weiner, Lutheran, and Raph destroyed

- » 0.2 M DTT standardly used<sup>1</sup>

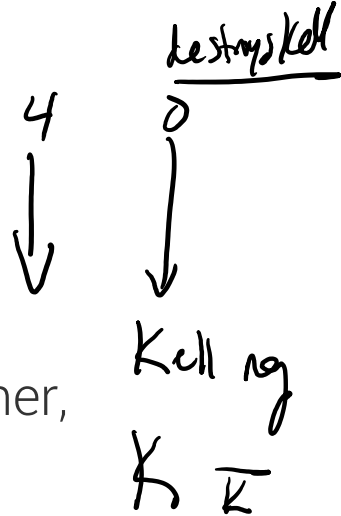
- » 0.01 M DTT pH 7.3 maintains Kell express except anti-K titre < 4<sup>2</sup>

- Used anti-A or anti-B as controls

- Trypsin/Ficin<sup>3</sup>

- » Destroys CD38, but not Kell

- » M, N, EnaTS, Lutheran, Ge2, Ge3, G34, Ch/Rg are destroyed



1. Chapuy CI, Aguad MD, Nicholson RT, et al. International validation of a dithiothreitol (DTT)-based method to resolve the daratumumab interference with blood compatibility testing. Transfusion. 2016;56(12):2964-2972.

2. Hosokawa M, Kashiwagi H, Nakayama K, et al. Additional validation of Osaka method (0.01 mol/L dithiothreitol) for negating the daratumumab interference. Transfusion. 2019;59(7):2479-2480.

3. Ibeh N, Baine I, Rudon LF, Lomas-Francis C, Jhang JS, Galdon P, Westhoff CM, Velliquette RW, Arinsburg SA. Use of an in-house trypsin-based method to resolve the interference of daratumumab. Transfusion. 2021 Oct;61(10):3000-3007.



# Modified or destroyed RBC CD38 antigen

- Polybrene<sup>1</sup>
  - » 2 drops patient sera, 1 drop RBCs, 600  $\mu$ L LIS  $\rightarrow$  2 drops Polybrene
- Cord RBCs<sup>2</sup>
  - » Express very little to no CD38
  - » A small typed panel can do simple rule outs without alloantibodies

O77  
↓  
kell

cord  
O k+

O k+

O k+

1. Yeh TJ, Yeh CJ, Liu YC, Hsiao HH. Manual polybrene method for pretransfusion test could overcome the interference of daratumumab therapy in myeloma. *Transfusion*. 2019 Aug;59(8):2751-2752.  
2. De Vooght KM, Oostendorp M, van Solinge WW. Dealing with anti-CD38 (daratumumab) interference in blood compatibility testing. *Transfusion*. 2016;56(3):778-779.

# Modified or destroyed RBC CD38 antigen

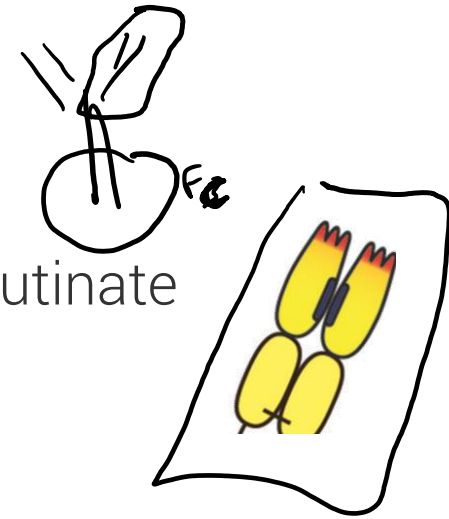
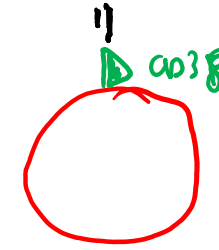
- In(Lu)<sup>1</sup>
  - » Weakened expression of CD38
  - » Usually hard for blood banks to have a panel large enough
- Fy(a-,b-) cells reported to be negative/weaker
- DARA treated patients with non-reactive RBCs

1. Celliquette R, Shakarian G, Jhang J, Rodriguez M, Morrison M, Adamy J, et al. Daratumumab-derived anti-CD38 can be easily mistaken for clinically significant antibodies to Lutheran antigens or to Knops antigens [abstract]. *Transfusion* (2015) 55:26A.

# Blocking CD38 on RBCs

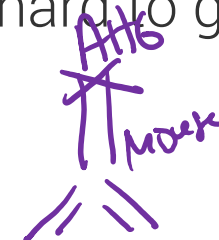
- Anti-CD38 F(ab) fragments<sup>1</sup>

- » CD38 binding fragment binds the antigen without being able to agglutinate
- » Limitation: expensive, hard to get



- Anti-CD38 + monospecific anti-human IgG<sup>2</sup>

- » RBC + anti-CD38 + antihuman IgG (Fc specific) → plasma incubation + AHG
- » Limitation: must titrate anti-CD38 agglutination to 1+, expensive, hard to get



- Non-human anti-CD38<sup>3</sup>

- » Mouse derived anti-CD38
- » AHG reagent does not recognize mouse antibody

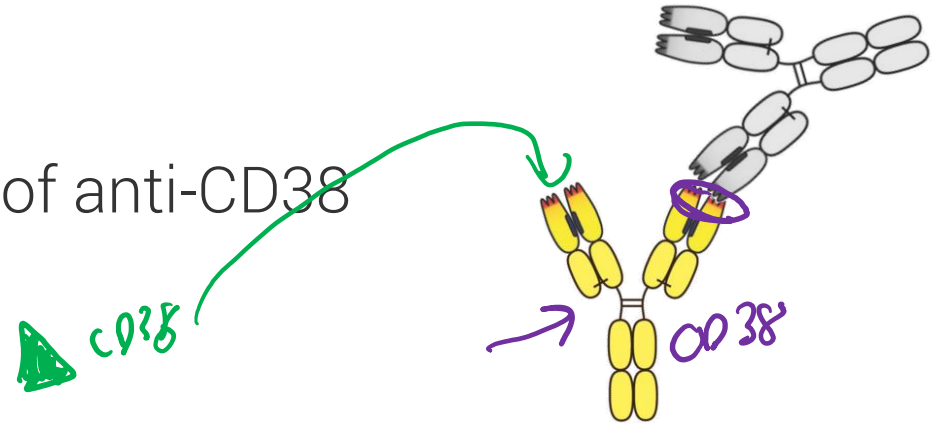
1. Selleng K, Gebicka PD, Thiele T. F(ab')<sub>2</sub> Fragments to Overcome Daratumumab Interference in Transfusion Tests. *N Engl J Med*. 2018 Jul 5;379(1):90-91. doi: 10.1056/NEJMc1804751.

2. Chinoca Ziza KN, Paiva TA, Mota SR, et al. A blockage monoclonal antibody protocol as an alternative strategy to avoid anti-CD38 interference in immunohematological testing. *Transfusion*. 2019;59 (5):1827-1835.

3. Baig NA, Dukek BA, Falbo DK, et al. Daratumumab interference in flow cytometric anti-granulocyte antibody testing can be overcome using non-human blocking antibodies. *Vox Sang*. 2021;116 (1):116-122.

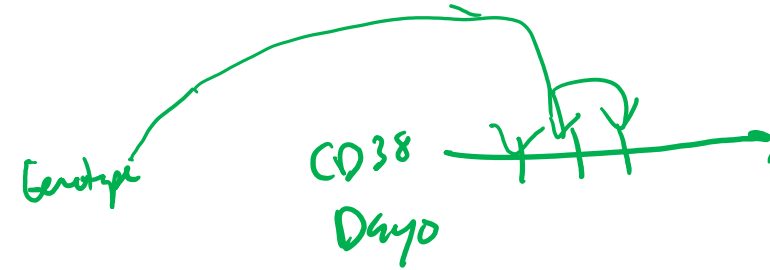
# Neutralization of CD38

- Anti-idiotypic antibody<sup>1</sup>
  - » Antibody that binds the variable region of anti-CD38
  - » Limitation: expensive, hard to get
- Soluble CD38 antigen<sup>2</sup>
  - » Limitation: expensive, hard to get
- CD38 antigen from myeloma cell lysate or stroma<sup>3</sup>
  - » Daudi B-cell disrupted by sonication and ultracentrifuged
  - » Limitation: specialized equipment and standardization
- Anti-CD38 aptamers
  - » RNA or ssDNA that binds anti-CD38
  - » Limitation: expensive, hard to get



1. Oostendorp M, Lammerts van Bueren JJ, Doshi P, et al. When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. *Transfusion*. 2015;55(6 Pt 2):1555–1562.  
2. Chapuy CI, Nicholson RT, Aguad MD, et al. Resolving the daratumumab interference with blood compatibility testing. *Transfusion*. 2015;55(6 Pt 2):1545–1554.  
3. Tapia-Alveal C, Olsen TR, Worgall TS. Personalized immunoglobulin aptamers for detection of multiple myeloma minimal residual disease in serum. *Commun Biol*. 2020;3(1):781.

# Genotyping

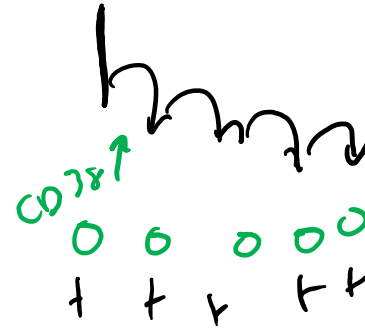


- Genotype before starting anti-CD38
- Can be faster to issue units
- Do ab screen and serological crossmatch - if reactivity goes up by 1+ then ignore, 2+ goes to medical director for review
- Extended match RhD, RhCE, K/k, Jk<sup>a</sup>, Jk<sup>b</sup>, Fy<sup>a</sup>, Fy<sup>b</sup>, S/s
- Limitation: large genotyped/phenotyped inventory, costly in some countries, allo-antibody formation is not higher than baseline population

Anani W.Q., Duffer K., Kaufman R.M., Denomme G.A. How do I work up pretransfusion samples containing anti-CD38? *Transfusion*. 2017;57(6):1337–1342.

Anani W.Q., Marchan M.G., Bensing K.M., Schanen M., Piefer C., Gottschall J.L. Practical approaches and costs for provisioning safe transfusions during anti-CD38 therapy. *Transfusion*. 2017;57(6):1470–1479.

# Other considerations



- Solid phase carry over
  - » High concentrations of anti-CD38 can contaminate solid phase assays
  - » Subsequent samples will be pan-reactive
  - » Must run 'blanks' until pan-reactivity abates

# When anti-CD38 does not read the book

|   | Rh-Hr |   |   |   |   | Kell           |   |   |   |   | Duff/           | Kidd            | Lewis           | P               | MN              |                 |                 |                 | Luth            | X               | Additional Antigen | Test Results |   |   |   |   |                 |                 |                 |                  |     |     |                  |                  |
|---|-------|---|---|---|---|----------------|---|---|---|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|--------------------|--------------|---|---|---|---|-----------------|-----------------|-----------------|------------------|-----|-----|------------------|------------------|
|   | D     | C | E | c | e | C <sup>w</sup> | f | V | K | k | Kp <sup>a</sup> | Kp <sup>b</sup> | Js <sup>a</sup> | Js <sup>b</sup> | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> |                    | P1           | M | N | S | s | Lu <sup>a</sup> | Lu <sup>b</sup> | Xg <sup>a</sup> | WtG <sup>a</sup> | PEG | DTT | Fic <sup>a</sup> | Fic <sup>b</sup> |
| 1 | +     | + | 0 | 0 | + | 0              |   | 0 | + | 0 | +               | 0               | +               | +               | 0               | +               | 0               | +               | 0               | +               | +                  | +            | 0 | + | 0 | + | +               | +               | 2               | 1                | 0   | 0   |                  |                  |
| 2 | +     | 0 | + | + | 0 | 0              |   | 0 | + | 0 | +               | 0               | +               | 0               | +               | +               | +               | 0               | +               | +               | 0                  | +            | 0 | + | 0 | + | 0               | +               | 2               | 1                | 0   | 0   |                  |                  |
| 3 | 0     | 0 | 0 | + | + | 0              |   | + | + | 0 | +               | 0               | +               | 0               | +               | 0               | +               | 0               | +               | 0               | +                  | +            | 0 | + | 0 | 0 | +               | +               | 2               | 1                | 0   | 0   |                  |                  |
| 4 | 0     | 0 | 0 | + | + | 0              |   | + | 0 | 0 | +               | 0               | +               | +               | +               | +               | +               | +               | 0               | +               | +                  | +            | 0 | + | 0 | + | 0               | +               | 2               | 1                | 0   | 0   |                  |                  |
| 5 | +     | + | 0 | 0 | + | 0              |   | 0 | + | 0 | +               | 0               | +               | +               | 0               | +               | 0               | 0               | +               | +               | +                  | +            | 0 | 0 | + | 0 | +               | 0               | 2               | 2                | 0   | 0   |                  |                  |

Handwritten notes: "Cell" circled above the table; "ed" written to the left of the Test Results column; "DTT" circled above the Test Results column; "Fic" written above the Test Results column; "GP.Mur, Mi(a+)" written in the Additional Antigen column for row 5; "NOTE: Mia is resistant to DTT. Cell # 5 (Mia+)" written at the bottom; "AUTO" written above the Test Results for row 5; "0, 0" circled in the Test Results for row 5.

# Autoadsorbed plasma x3

OOO + plasma →

|   | Rh-Hr |   |   |   |   |                |   |   | Kell |   |                 |                 |                 | Duffy           |                 | Kidd            |                 | Lewis           |                 | P               | MN |   |   |   | Luth |                 | X               | Additional Antigens | Test Results    |  |  |  |
|---|-------|---|---|---|---|----------------|---|---|------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----|---|---|---|------|-----------------|-----------------|---------------------|-----------------|--|--|--|
|   | D     | C | E | c | e | C <sup>w</sup> | f | V | K    | k | Kp <sup>a</sup> | Kp <sup>b</sup> | Js <sup>a</sup> | Js <sup>b</sup> | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P1 | M | N | S | s    | Lu <sup>a</sup> | Lu <sup>b</sup> |                     | Xg <sup>a</sup> |  |  |  |
| 1 | +     | + | 0 | 0 | + | 0              |   | 0 | +    | 0 | +               | 0               | +               | +               | 0               | +               | 0               | +               | 0               | +               | +  | + | 0 | + | 0    | +               | +               |                     |                 |  |  |  |
| 2 | +     | 0 | + | + | 0 | 0              |   | 0 | +    | 0 | +               | 0               | +               | 0               | +               | +               | +               | 0               | +               | +               | 0  | + | 0 | + | 0    | +               | +               |                     |                 |  |  |  |
| 3 | 0     | 0 | 0 | + | + | 0              |   | + | +    | 0 | +               | 0               | +               | 0               | +               | 0               | +               | 0               | +               | 0               | +  | 0 | + | 0 | 0    | +               | +               |                     |                 |  |  |  |
| 4 | 0     | 0 | 0 | + | + | 0              |   | + | 0    | 0 | +               | 0               | +               | +               | w               | +               | +               | +               | 0               | +               | +  | + | 0 | + | 0    | +               | 0               |                     |                 |  |  |  |

Handwritten circled notes in the 'Additional Antigens' column, possibly indicating 'MNSs'.

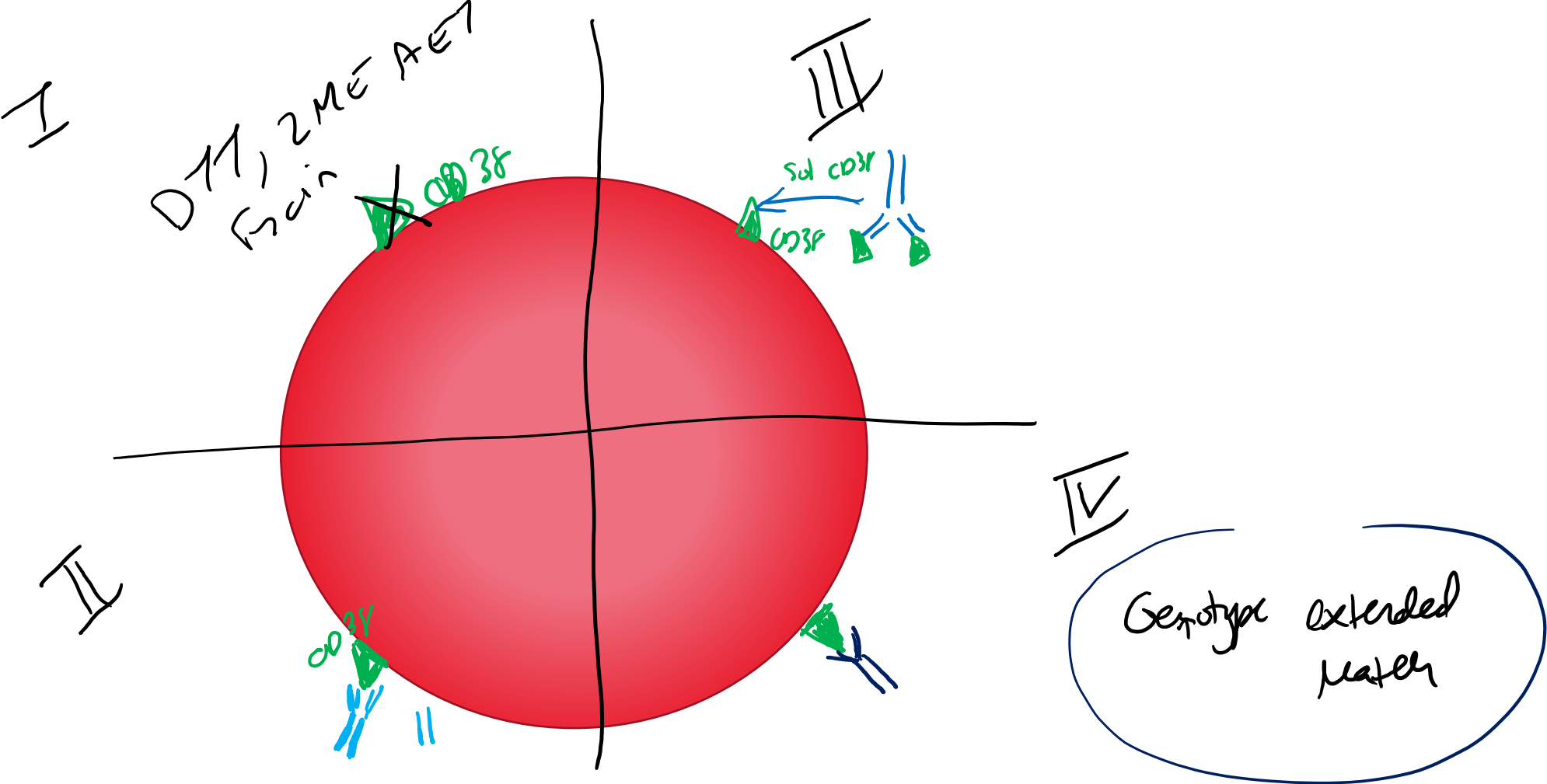


# Eluate

|   | Rh-Hr |   |   |   |   |                |   |   | Kell |   |                 |                 |                 | Duffy           |                 | Kidd            |                 | Lewis           |                 | P               | MN |   |   |   | Luth |                 | X               | Additional Antigens |
|---|-------|---|---|---|---|----------------|---|---|------|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----|---|---|---|------|-----------------|-----------------|---------------------|
|   | D     | C | E | c | e | C <sup>w</sup> | f | V | K    | k | Kp <sup>a</sup> | Kp <sup>b</sup> | Js <sup>a</sup> | Js <sup>b</sup> | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P1 | M | N | S | s    | Lu <sup>a</sup> | Lu <sup>b</sup> |                     |
| 1 | +     | + | 0 | 0 | + | 0              |   | 0 | +    | 0 | +               | 0               | +               | +               | 0               | +               | 0               | +               | 0               | +               | +  | + | 0 | + | 0    | +               | +               |                     |
| 2 | +     | 0 | + | + | 0 | 0              |   | 0 | +    | 0 | +               | 0               | +               | 0               | +               | +               | +               | 0               | +               | +               | 0  | + | 0 | + | 0    | +               | +               |                     |
| 3 | 0     | 0 | 0 | + | + | 0              |   | + | +    | 0 | +               | 0               | +               | 0               | +               | 0               | +               | 0               | +               | 0               | +  | 0 | + | 0 | 0    | +               | +               |                     |
| 4 | 0     | 0 | 0 | + | + | 0              |   | + | 0    | 0 | +               | 0               | +               | +               | w               | +               | +               | +               | 0               | +               | +  | + | 0 | + | 0    | +               | 0               |                     |

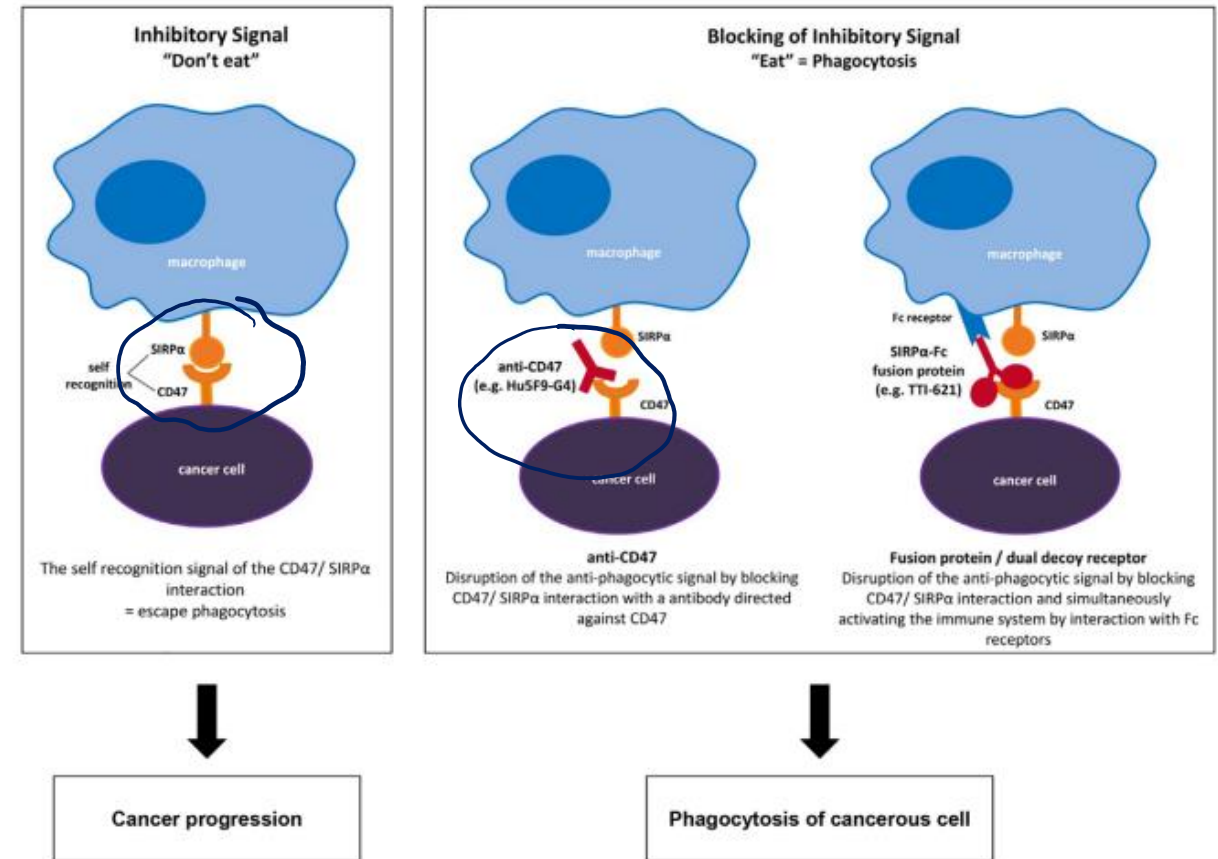
<sup>W</sup>  
<sup>EL</sup>  
 Test Results  
 Lu<sup>m</sup>Lu<sup>s</sup>  
 EL m<sup>s</sup>Lu<sup>s</sup>  
 0  
 0  
 0  
 0  
 0  
 2  
 1 (wk)  
 1  
 1 (wk)

# Overall methods to approach anti-CD38 patients



# CD47

- Transmembrane protein that mediates cell proliferation, migration, phagocytosis, apoptosis
- Macrophages use CD47 to tell self from non-self
- Expressed on non-malignant cells and some cancer cells



Zhang W, Huang Q, Xiao W, et al. Advances in Anti-Tumor Treatments Targeting the CD47/SIRPα Axis. *Front Immunol.* 2020;11:18.

Velliquette RW, Aeschlimann J, Kirkegaard J, Shakarian G, Lomas-Francis C, Westhoff CM. Monoclonal anti-CD47 interference in red cell and platelet testing. *Transfusion.* 2019 Feb;59(2):730-737.

# Anti-CD47

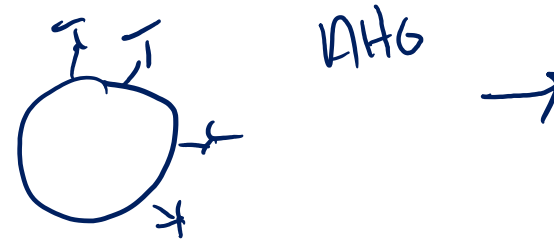
- Mouse Fc region grafted to human IgG4 and used to immunize mice
- Agglutination of platelets and red blood cells
- Expressed with RhCE complex (ce > DcE/DcE > D-- > Rh<sub>null</sub>)
- Older RBCs may be more susceptible to phagocytosis with anti-CD47
- Hgb and platlet count drop for approximately 21 days after starting

Liu J, Wang L, Zhao F, Tseng S, Narayanan C, Shura L, Willingham S, Howard M, Prohaska S, Volkmer J, Chao M, Weissman IL, Majeti R. Pre-Clinical Development of a Humanized Anti-CD47 Antibody with Anti-Cancer Therapeutic Potential.

Mouro-Chanteloup I, Delaunay J, Gane P, et al. Evidence that the red cell skeleton protein 4.2 interacts with the Rh membrane complex member CD47. Blood 2003;101:338-44. 17. Cartron JP. RH blood group system and molecular basis of Rhdeficiency. Baillieres Best Pract Res Clin Haematol 1999;12:655-89

# Serological characteristics of anti-CD47

- ABO/Rh typing
  - » Forward typing weak panreactivity
  - » Reverse typing 3-4+ panreactive
  - » Rh antigen typing weak panreactivity
- DAT – negative or microscopic +
  - » Steric hindrance or AHG blockade
- Eluate
  - » 3-4+ panreactive Ortho anti-IgG
  - » No reactivity with Immucor Gamma-clone anti-IgG (does not detect IgG4)
- Warm washes and EGA did not remove antibody interference



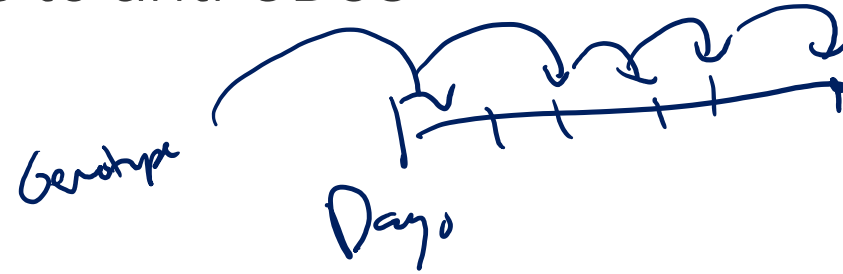
# Serological characteristics of anti-CD47

- Antibody identification – 3+-4+ reactivity
  - » Resistant to ficin, papain, trypsin,  $\alpha$ -chymotrypsin, 0.2M DT, and W.A.R.M. reagent
  - » Removed by
    - Papain alloadsorptionx4 with papain treated R<sub>1</sub>R<sub>1</sub>, R<sub>2</sub>R<sub>2</sub>, and rr RBCs (both AHG reagents negative)
      - › Some samples had m+ agglutination
    - Adsorptionx4 with single donor platelets
      - › Neg with Immucor Gamma-clone
      - › Positive with Ortho anti-IgG
    - Adsorption with HPCx3 decreased reactivity to 2+
  - » PEG adsorptions per manufacturer are negative, but controls are negative as well

Velliquette RW, Aeschlimann J, Kirkegaard J, Shakarian G, Lomas-Francis C, Westhoff CM. Monoclonal anti-CD47 interference in red cell and platelet testing. Transfusion. 2019 Feb;59(2):730-737.

# Genotyping

- Similar practice to anti-CD38





*ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.*