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PEARLS OF LABORATORY MEDICINE

Daratumumab Interference in Pre-Transfusion Testing

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Daratumumab as treatment for Multiple Myeloma

Multiple myeloma (MM) is a hematologic malignancy affecting antibody producing plasma cells.

One of the targeted antibody based therapies is Daratumumab (DARA), a human $IgG1_K$ monoclonal antibody that targets the CD38 glycoprotein located on the cellular surface of the neoplastic plasma cells.

It is currently approved by FDA for MM patients who have received only one prior therapy. This approval by the FDA came as the result of two simultaneous clinical trials demonstrating similar and “substantial improvements” in progression free survival despite different accompanying drug regimens, proving that DARA was the primary agent responsible for the improved clinical endpoints

CD 38 expression and Daratumumab interference

- CD38 besides being expressed on plasma cells is also expressed on multiple other hematologic cell types including red blood cells (RBCs), medullary thymocytes, activated B and T cells, natural killer cells, monocytes, granulocytes, and others.
- This expression led to interference of Daratumumab in the assays that use secondary anti-human IgG antibody for amplification of signal, such as AHG phase of red cell antibody screen and others.

Case presentation and initial antibody screen

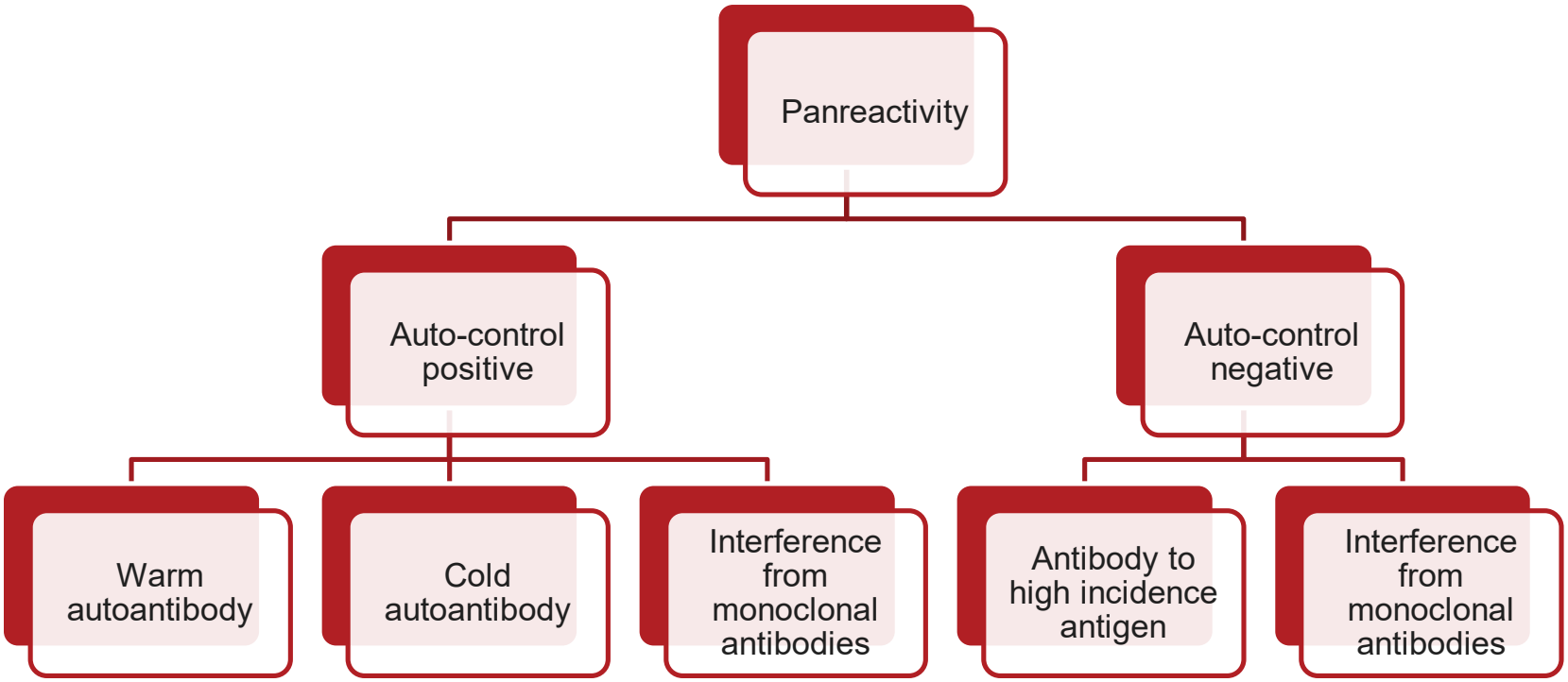
60 year old female patient, undergoing treatment for multiple myeloma was found to have low hemoglobin and needed transfusion. A routine red cell antibody screen test was submitted to hospital's transfusion laboratory and results are shown below.

Cell	D	C	c	E	e	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	IS	AHG	CC
I	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	+	+	+	+	+	0	+	+	-	w+	na
II	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	0	0	+	0	+	0	-	w+	na
III	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	0	+	+	0	0	+	+	-	w+	na
Patient's cells																												-	-	+

Antibody screen, extended panel

Cell	D	C	c	E	e	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	IS	AHG	CC
I	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	+	-	W+	
II	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	+	0	+	+	-	W+	
1	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	-	W+	
2	+	0	+	0	+	0	0	0	+	0	+	+	0	0	0	+	+	0	0	+	0	+	0	+	+	+	+	-	W+	
3	0	+	+	0	+	0	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	+	+	0	+	+	-	W+	
4	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	-	W+	
5	0	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	+	-	W+	
6	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+	-	W+	
7	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	+	-	W+	
8	+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	-	W+	
9	+	0	+	+	0	0	0	+	0	0	+	0	+	+	+	+	+	0	+	W	+	0	+	+	+	+	+	-	W+	
10	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	0	+	0	+	0	-	W+	
Patient's cells																												-	-	2+

Patterns of antibody screen associated with pan reactivity



Immunomodulation as possible mechanism of negative autocontrol results

- Immunomodulation is selective removal of antigen from RBC membrane by spleen monocytes, when they are passing through the spleen
- It was shown in the models explaining RhIG and anti Kell immunoglobulin prophylaxis

Interpretation of antibody screen results

Negative autocontrol effectively rules out warm and cold autoantibodies.

The differentiation between antibody toward high incidence antigen and Daratumumab or other monoclonal therapeutic antibody interference requires review of medication administration records and/or special technics in transfusion laboratory.

Special techniques that help in distinguishing Daratumumab interference

DTT (Dithiothreitol) treatment.

- Destroys CD38 – target for Daratumumab
- Destroys Kell blood group system antigens

Other techniques, like using enzymes, (papain) can be useful in distinguishing high incidence antigens

Antibody screen, after DTT treatment

Cell	D	C	c	E	e	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	IS	AHG	CC
I	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	+	-	-	2+
II	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	+	0	+	+	-	-	2+
1	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	-	-	2+
2	+	0	+	0	+	0	0	0	+	0	+	+	0	0	0	+	+	0	0	+	0	+	0	+	+	+	+	-	-	2+
3	0	+	+	0	+	0	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	+	+	0	+	+	-	-	2+
4	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	-	-	2+
5	0	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	+	-	-	2+
6	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+	-	-	2+
7	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	+	-	-	2+
8	+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	-	-	2+
9	+	0	+	+	0	0	0	+	0	0	+	0	+	+	+	+	+	0	+	W	+	0	+	+	+	+	+	-	-	2+
10	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	0	+	0	+	0	-	-	2+
Patient's cells																												-	-	2+

Practical approach

Before Daratumumab treatment.

- Type patient for Kell antigens

After Daratumumab treatment.

- Use DTT treatment to screen for RBC antibodies
- Provide Kell negative blood

Assays affected

Red cell antibody screening

Anti Neutrophil Antibody testing

Anti platelet antibody testing

Platelet crossmatch

And others that use secondary anti human antibodies

Other strategies to overcome interference

- soluble CD38 antigen
- anti-idiotype antibody
- F(ab')$_2$ fragments of DARA
- blocking mouse anti CD38 antibody
- Cord blood testing

All are expensive and/or commercially not available

Conclusions

- Daratumumab is a humanized antibody for treatment of multiple Myeloma that can cause interference due to not specific binding.
- The most affected assay is red cell antibody screen, but other assays that use secondary anti human antibody can be affected as well.
- The accepted approach to overcome interference is pre-treatment of the cells with DTT that destroys CD38. Other approaches has been tried, but not currently commercially available or accepted

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Disclosures/Potential Conflicts of Interest

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