Evaluation of a Human Fab Anti-Idiotypic Anti-Daratumumab to Resolve Pan Reactions in Pre-Transfusion Testing due to Monoclonal Anti-CD38 Therapy

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Introduction

Monoclonal antibody therapies are used to treat a variety of pathologic conditions, including solid and hematologic cancers. Daratumumab, a monoclonal anti-CD38 antibody, has been used for several years and has been shown to be efficient for the treatment of multiple myeloma patients. This monoclonal antibody specifically targets the antigen CD38, which is overexpressed on multiple myeloma cells. However, CD38 is also expressed at various degrees on red blood cells. When a patient is treated with daratumumab, pan reactions are observed in antibody screening using the Indirect Antiglobulin Test (IAT), which may mask underlying clinically significant antibodies. Several solutions have been developed, such as soluble recombinant proteins or treating the reagent red blood cells with dithiothreitol or trypsin, but they present limitations in the detection of some red cell alloantibodies.

Objective

The objective of this study was to confirm, in two different immunohematology laboratories, the efficacy of a new candidate in neutralizing daratumumab interferences in pretransfusion testing. Another objective was to demonstrate that this candidate has no impact on the detection of underlying red cell antibodies.

Table 1. Neutralization efficiency on 50 samples with various daratumumab titers

		Daratumumab Titers											
		8	16	32	64	128	512	1024	2048	4096	8192	16384	Total
Neutralization Efficiency with Various Anti-Daratumumab Quantities	10% (v/v)	100% (1/1)	100% (1/1)	100% (3/3)	100% (1/1)	100% (2/2)	100% (6/6)	100% (5/5)	80% (4/5)	86% (12/14)	22% (2/9)	0% (0/3)	74% (37/50)
	20% (v/v)								100% (5/5)	100% (14/14)	78% (7/9)	33% (1/3)	92% (46/50)
	30% (v/v)										100% (9/9)	100% (3/3)	100% (50/50)

Table 2. Neutralization efficiency of the anti-daratumumab on 40 samples with weak underlying alloantibodies

				Daratumumab Neutralization		Alloantibody Reaction (per Red Blood Cell)		
Sample	Daratumumab Titer	Alloantibody	Alloantibody Titer*	10%	20%	Double Dose	Single Dose	Negative
1	4096	Anti-D	4	N	Y	Positive	Positive	Negative
2	64	Anti-E	4	Y	/	Positive	Positive	Negative
3	256	Anti-C	4	Y	/	Positive	Positive	Negative
4	1024	Anti-c	4	Y	/	Positive	Positive	Negative
5	256	Anti-Jk^a	4	Y	/	Positive	Positive	Negative
6	16	Anti-Fy^a	4	Y	/	Positive	Positive	Negative
7	1024	Anti-s	4	Y	/	Positive	Positive	Negative
8	4096	Anti-S	4	N	Y	Positive	Positive	Negative
9	512	Anti-K	4	Y	/	/	Positive	Negative
10	256	Anti-D	4	Y	/	Positive	Positive	Negative
11	32	Anti-E	4	Y	/	Positive	Positive	Negative
12	256	Anti-C	4	Y	/	Positive	Positive	Negative
13	512	Anti-c	4	Y	/	Positive	Positive	Negative
14	1024	Anti-Jk^a	4	Y	/	Positive	Positive	Negative
15	8	Anti-Fy^a	4	Y	/	Positive	Positive	Negative
16	4096	Anti-s	4	Y	/	Positive	Positive	Negative
17	256	Anti-S	4	Y	/	Positive	Positive	Negative
18	2048	Anti-K	4	Y	/	/	Positive	Negative
19	2048	Anti-D	4	Y	/	Positive	Positive	Negative
20	2048	Anti-K	4	Y	/	/	Positive	Negative
21	8192	Anti-E	2	Y	/	Positive	Positive	Negative
22	512	Anti-Jk^a	1	Y	/	Positive	Positive	Negative
23	4096	Anti-Fy^a	4	Y	/	Positive	Positive	Negative
24	2048	Anti-D	1	Y	/	Positive	Positive	Negative
25	1024	Anti-Kp^a	4	Y	/	/	Positive	Negative
26	4096	Anti-D	1	Y	/	Positive	Positive	Negative
27	64	Anti-E	4	Y	/	Positive	Positive	Negative
28	4096	Anti-C^w	1	Y	/	/	Positive	Negative
29	2048	Anti-K	4	Y	/	/	Positive	Negative
30	2048	Anti-Fy^a	4	Y	/	Positive	Positive	Negative
31	512	Anti-C^w	4	Y	/	/	Positive	Negative
32	4096	Anti-Fy^a	2	Y	/	Positive	Positive	Negative
33	2048	Anti-Fy^a	4	Y	/	Positive	Positive	Negative
34	2048	Anti-D	4	Y	/	Positive	Positive	Negative
35	4096	Anti-E	4	Y	/	Positive	Positive	Negative
36	256	Anti-Fy^a	4	Y	/	Positive	Positive	Negative
37	4096	Anti-K	4	Y	/	/	Positive	Negative
38	512	Anti-Lu^a	2	Y	/	/	Positive	Negative
39	2048	Anti-K	4	Y	/	/	Positive	Negative
40	1024	Anti-e	4	Y	/	Positive	Positive	Negative

* Against single dose cell

Methods

The neutralizing solution was provided ready-to-use. The daratumumab relative concentration in patient samples was estimated by antibody titration. To assess the neutralization efficiency, 50 daratumumab samples (25 samples per site) were titrated and neutralized, starting with 10% v/v of neutralizing solution and then with an increasing concentration if the neutralization was not achieved at 10% (i.e., 20% and then 30% v/v of neutralizing solution). No preincubation was required (Table 1). Thereafter, 40 samples (20 samples per site) were prepared to contain a weak alloantibody (titer equal to or smaller than 4 against single dose cell) and daratumumab at a known titer. Specificities were selected to represent a broad spectrum of clinically significant antibodies (Table 2). These positive samples were tested using the same neutralization protocol, starting with 10% and so on if needed (Figure 1). Respective dilution controls using a titration buffer at 10%, 20%, or 30% were used at each step to validate the neutralization tests.

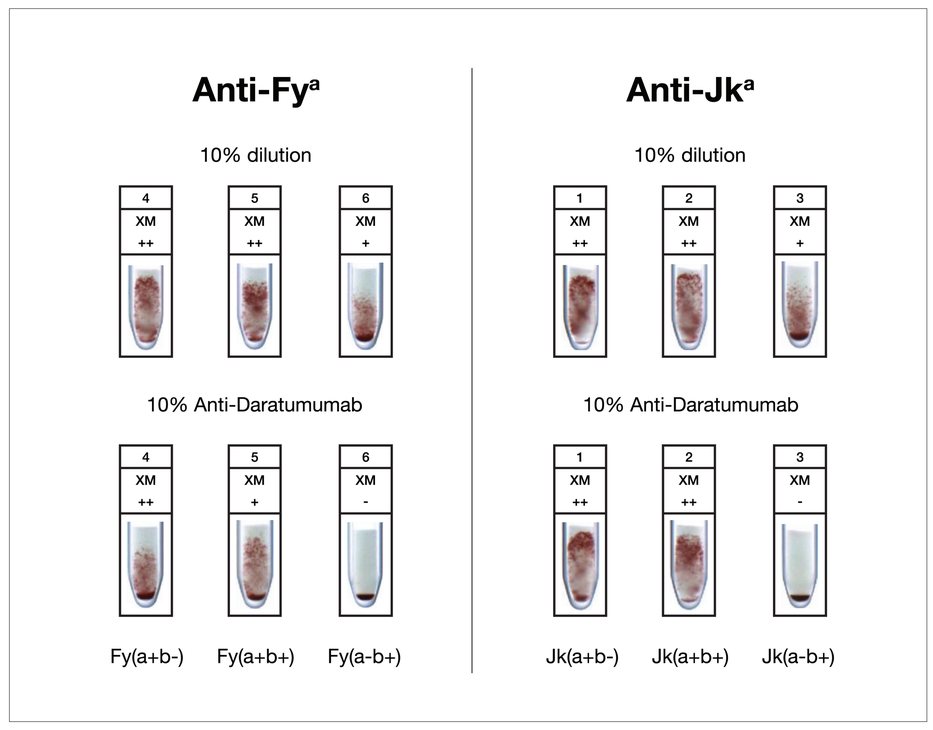

Figure 1. Examples of neutralization with weak alloantibodies

Results

At both sites, daratumumab titers ranged between 8 and 16,000. Overall, 74% of the samples (37/50) were neutralized using 10% of anti-daratumumab, 92% (46/50) with 20%, and 100% with 30% (Table 1). All underlying clinically significant alloantibodies were detected after the efficient neutralization of daratumumab (Table 2). Of 40 positive samples, 38 (95%) were neutralized with 10% and 2 required 20% of anti-daratumumab, but it did not affect the detection of weak underlying antibodies (Table 2). This ready-to-use anti-daratumumab did not impact the turnaround time as there was no pre-incubation required.

Conclusion

This human Fab anti-idiotypic anti-daratumumab demonstrated very efficient neutralization, while allowing the detection of weak underlying red cell antibodies. Using a 10% concentration, the neutralization was successful in 83% of samples (75/90), and gradual increase achieved a complete neutralization. This user-friendly reagent could become a new candidate for daratumumab neutralization in pre-transfusion testing.

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