CORRESPONDENCE

F(ab')$_2$ Fragments to Overcome Daratumumab Interference in Transfusion Tests

TO THE EDITOR: Daratumumab is an antibody directed against CD38 for the treatment of multiple myeloma.[1] CD38 is also expressed on red-cell membranes, so that free daratumumab in patient serum can bind to test erythrocytes during pretransfusion blood-compatibility testing. This results in panreactive agglutination in the standard indirect antihuman globulin (Coombs') test used for antibody screening and cross-matching.[2] Consequently, clinically relevant red-cell alloantibodies may not be recognized in patients who have received daratumumab.

Several methods have been proposed to overcome this interference. These methods include pretreatment of red cells with dithiothreitol (which inhibits daratumumab binding by reducing CD38 disulfide bridges), the use of anti-idiotypic antibodies against daratumumab, supplementation of soluble CD38 to bind daratumumab in patient serum,[3] and the use of red cells from newborns as test cells, since they express low levels of CD38.[4] Each of these methods has inherent limitations: they are time consuming, destroy red-cell antigens, and are insufficiently standardized for use in routine laboratories.

We developed an easy-to-use method to block the interference of daratumumab with pretransfusion antibody screening and red-cell compatibility testing. We prepared F(ab′)$_2$ fragments of daratumumab by digestion with pepsin. The F(ab′)$_2$ fragments and intact daratumumab bind to the same epitope on CD38; thus, these fragments block binding to red cells by free daratumumab in serum. This method allows for standard pretransfusion testing without interference by daratumumab.

We obtained plasma or serum specimens containing known clinically relevant red-cell alloantibodies (anti-D, anti-C, anti-E, anti-e, anti-K, anti-Fy^a, anti-Lu^a, anti-S, or anti-M) from patients. We then adjusted these specimens to the lowest detectable concentration in the indirect antihuman globulin test performed with a gel card technique. Since daratumumab in vivo rarely exceeds plasma concentrations of 0.5 mg per milliliter, we tested the ability of daratumumab F(ab′)$_2$ fragments to block interference of this daratumumab concentration during the indirect antihuman globulin test (technical details are provided in the Supplementary Appendix, available with the full text of this letter at NEJM.org). All antibodies were reproducibly detected with the same sensitivity as the undiluted specimens (Fig. 1).

We conclude that the use of F(ab′)$_2$ fragments may solve the problem of daratumumab interference with standard types of blood-compatibility tests. The widespread use of F(ab′)$_2$ fragments would require commercial availability and further validation with other antihuman globulin

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	Serum without Anti–Red-Cell Antibodies			Anti-D			Anti-K			Anti-E			Anti-Fy^a		
Red-Cell Phenotype				D	D	dd	Kk	Kk	kk	Ee	Ee	ee	Fy^a+	Fy^a+	Fy^a–
Daratumumab	–	+	+	–	+	+	–	+	+	–	+	+	–	+	+
F(ab')$_2$ Fragments	–	–	+	–	+	+	–	+	+	–	+	+	–	+	+
Result of Indirect Antihuman Globulin Test	Negative	1+	Negative	1+	1+	Negative	1+	1+	Negative	1+	2+	Negative	1+	1+	Negative

Figure 1. Results of Indirect Antihuman Globulin Tests of Plasma Specimens Containing Various Red-Cell Alloantibodies.

Results of indirect antihuman globulin (Coombs') tests of red-cell antibody–positive plasma specimens in the presence (plus sign) or absence (minus sign) of daratumumab and F(ab´)$_2$ fragments are shown. Plasma or serum specimens obtained from patients with known red-cell alloantibodies were adjusted to the lowest detectable level of the antibodies, then spiked with a final concentration of daratumumab (0.5 mg per milliliter) and tested. Results in four different plasma samples containing anti-D, anti-K, anti-E, or anti-Fy^A are shown. A solid pellet at the bottom of the tubes indicates a negative result, and suspended particles (red-cell agglutinates) within the gel matrix indicate a positive test result (either a 1+ or 2+ degree of agglutination).

test techniques. Preincubation of other therapeutic humanized monoclonal antibodies with F(ab´)$_2$ fragments could be a general solution to problems of interference with blood-compatibility testing.

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Immune Checkpoint Blockade in Advanced Renal-Cell Carcinoma

TO THE EDITOR: In the CheckMate 214 trial, Motzer et al. (April 5 issue)[1] found higher overall survival among intermediate- and poor-risk patients with previously untreated metastatic renal-cell carcinoma who received nivolumab plus ipilimumab than among those who received sunitinib. Programmed death ligand 1 expression of 1% or greater was associated with longer median overall survival among patients who received nivolumab plus ipilimumab than among those who received sunitinib (hazard ratio for death, 0.45; 95% confidence interval [CI], 0.29 to 0.71).

A subgroup analysis showed a significant benefit of nivolumab plus ipilimumab only in

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