SUPPLEMENTARY INFORMATION

Anti-CD38 monoclonal antibody interference with blood compatibility testing: Differentiating isatuximab and daratumumab via functional epitope mapping

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MATERIALS AND METHODS

1- Immunostaining reagents

Mouse anti-human CD38 antibodies (clone HB-7 and AT13/5) and control mouse immunoglobulin G1 (mIgG1) were purchased from Santa Cruz Biotech (Dallas, TX). Phycoerythrin (PE)-conjugated HB-7, PE-iso control mIgG, and fluorescein isothiocyanate (FITC)-conjugated goat anti-mIgG/IgM antibody were obtained from Becton Dickinson Bioscience (San Jose, CA). Human immunoglobulin G1 (hIgG1) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Daratumumab (Darzalex®, Janssen Biotech, Inc., Horsham, PA) was purchased from the drugstore. Isatuximab and CD38 inhibitor (ara-F-nicotinamide adenine dinucleotide [NAD], an irreversible inhibitor of CD38 ectoenzymatic activity)[1] were produced by Sanofi. Allophycocyanin (APC)-conjugated goat anti-hIgG and FITC-conjugated goat anti-mIgG were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). 16% paraformaldehyde was purchased from Thermo Fisher Scientific (Waltham, MA).

2- Confocal imaging

Washed RBCs (100 µL) were pre-treated with 0.1 µg of mIgG or HB-7 for 20 minutes (min) at room temperature (RT), then, incubated with 10 µg/mL of hIgG1) isatuximab or daratumumab for 30 min. After three washes, cells were blocked with 10% normal goat serum (Thermo Fisher Scientific, Waltham, MA) for 15 min at RT before staining with detection antibodies, Alexa Fluor

405 goat anti-mIgG (H+L), Alexa Fluor488 goat anti-hIgG (H+L) or CellMask Red (all from Thermo Fisher Scientific, Waltham, MA) for 30 min at RT. Cells were fixed with 1% paraformaldehyde/phosphate buffered saline (PBS) after washing three times with PBS. Confocal fluorescent images were acquired on an Opera QEHS (Perkin Elmer, Waltham, MA) automated microscope with a 20x/0.7NA UApo/340 water immersion objective (Olympus, Tokyo, Japan). Sequential excitation was carried out with 488 nm and 561 nm solid state lasers in combination with 543/22 and 600/40 emission filters. Images were acquired in four replicate wells per treatment condition with eight images captured in each well. and analyzed using the Perkin Elmer Columbus system. Briefly, RBCs were segmented using a texture-based machine learning algorithm, followed by a spot detection module for identifying labeled anti-CD38 antibodies within the RBC objects.

3- Tandem mass spectrometry

All reagents, unless otherwise stated, were purchased from Thermo Fisher Scientific (Waltham, MA).

Two groups of RBCs (~$6E^9$ cells/mL) were incubated with either 3 µg/mL HB7 antibody or control (1x PBS, pH 7.4) for 30 min at RT. Then 10 µg/mL of control hIgG1, isatuximab or daratumumab was added individually into each tube of these two groups and incubated for another 30 min at RT.

After incubation, RBCs were washed with 10 volumes of ice-cold PBS twice to remove unbound antibodies. Cell pellet was then resuspended with 4 volumes of ice-cold elution buffer (0.2 M glycine, 150 mM NaCl, pH 2.5). After sitting on ice for 2 min, 1 M Tris-HCL buffer (pH 9) was added for neutralization. Samples were then immediately centrifuged at 500 x g for 5 min. Affinity enrichment was performed by adding 50 µL of antibody affinity resins (CaptureSelect™ IgG1 [Hu] Affinity Matrix from Thermo Fisher Scientific, Waltham, MA) into each tube and equilibrated with 250 µL of 1x PBS twice. Samples were then mixed with the resins and

incubated for 1 hour at RT. After incubation, the matrix was then loaded onto the centrifuge column and washed with 500 µL PBS. Subsequently, bound antibodies were eluted with 250 µL of 0.1 M glycine (pH 3). The resulting eluent was neutralized with 1 M Tris-HCl (pH 8) and digested with LysC/Trypsin mixture overnight at 37˚C.

Liquid chromatography–mass spectrometry system consisting of the Thermo Oribtrap Elite mass spectrometer coupled to a nanoelectrospray ionization source and an AB Sciex NanoLC ultra performance liquid chromatography (UPLC) system (Framingham, MA) was utilized for analyzing the resulting peptides. The UPLC separation was performed with a trapping column (nanoACQUITY UPLC 2D Symmetry C18 Trap Column, 100 Å, 5 µm, 180 µm x 20 mm, Waters, Milford, MA) (150 µm i.d.) and a 200 mm analytical column (PicoFrit column, 75 µm internal diameter, packed with ACQUITY BEH C18, 1.7 µm, 130 Å, New Objective, Woburn, MA). Parallel reaction monitoring (PRM) analysis was adopted for monitoring 11 surrogate peptides from the constant region of hIgG1 based on precedent data dependent analysis. The spray voltage was set at 1.8 kV, and the capillary temperature at 275˚C. MS1 and MS2 scans were acquired by the Orbitrap mass analyzer at resolutions of 60,000 and 15,000, respectively. Raw files were searched by MaxQuant (version 1.5.3.30) and data was then processed by Skyline (version 3.5.0.9320). Peptide quantification was based on the total MS1 peak area.

SUPPLEMENTARY TABLE 1 PEG tube IAT tests done with samples containing RBC alloantibodies in the presence of 10 µg/mL isatuximab. Target RBC antigens on the screening cells are represented by yellow for single-antigen expression, orange for double-antigen expression, and no color for lack of expression of the cognate antigen.

Antibody-spiked plasma	Screening RBCs					
	Untreated			0.01 M DTT-treated		
	1	2	3	1	2	3
Anti-Jkb	2+	1+	2+	2+	0	2+
Anti-C	3+	1+	3+	3+	0	2+
Anti-C	2+	2+	2+	2+	0	1+
Anti-c	1+	2+	1+	0	2+	0
Anti-c	1+	2+	1+	0	1+	0
Anti-E	1+	2+	1+	0	1+	0
Anti-E	1+	2+	1+	0	1+	0
Anti-e	3+	1+	3+	3+	0	3+
Anti-e	2+	1+	1+	1+	0	1+
Anti-D	4+	4+	4+	4+	4+	4+
Anti-D	2+	2+	2+	2+	2+	2+
Anti-Dia	1+	1+	2+	0	0	1+

Abbreviations: DTT, dithiothreitol; IAT, indirect antiglobulin test; PEG, polyethylene glycol; RBC, red blood cell.

SUPPLEMENTARY TABLE 2 Baseline demographic characteristics for patients in the isatuximab arms with IAT during the on-treatment period (randomized population).[a]

	ICARIA-MM All IATs negative post baseline (n=32)	ICARIA-MM At least one positive IAT post baseline and a negative/missing baseline (n=67)	IKEMA All IATs negative post baseline (n=59)	IKEMA At least one positive IAT post baseline and a negative/missing baseline (n=101)
Age (years)				
Median (range)	66.5 (50–80)	69.0 (47–83)	65.0 (37–81)	64.0 (43–86)
Age group (years), n (%)				
<65	14 (43.8)	20 (29.9)	29 (49.2)	52 (51.5)
65-74	15 (46.9)	33 (49.3)	26 (44.1)	41 (40.6)
≥75	3 (9.4)	14 (20.9)	4 (6.8)	8 (7.9)
Sex, n (%)				
Female	12 (37.5)	29 (43.3)	29 (49.2)	41 (40.6)
Male	20 (62.5)	38 (56.7)	30 (50.8)	60 (59.4)
Race, n (%)				
White	23 (71.9)	50 (74.6)	40 (67.8)	78 (77.2)
Black or African American	0	0	1 (1.7)	3 (3.0)
Asian	5 (15.6)	12 (17.9)	10 (16.9)	12 (11.9)
Native Hawaiian or other Pacific Island	0	2 (3.0)	0	0
Multiple	0	0	2 (3.4)	1 (1.0)
Missing/Not reported	4 (12.5)	3 (4.5)	6 (10.2)	7 (6.9)
Ethnicity, n (%)				
Hispanic or Latino	0	3 (4.5)	2 (3.4)	9 (8.9)
Not Hispanic or Latino	27 (84.4)	57 (85.1)	46 (78.0)	80 (79.2)
Unknown	1 (3.1)	0	6 (10.2)	3 (3.0)
Not Reported	4 (12.5)	7 (10.4)	5 (8.5)	9 (8.9)
Geographical region[b], n (%)				
Europe	16 (50.0)	27 (40.3)	35 (59.3)	43 (42.6)
America	1 (3.1)	2 (3.0)	4 (6.8)	15 (14.9)

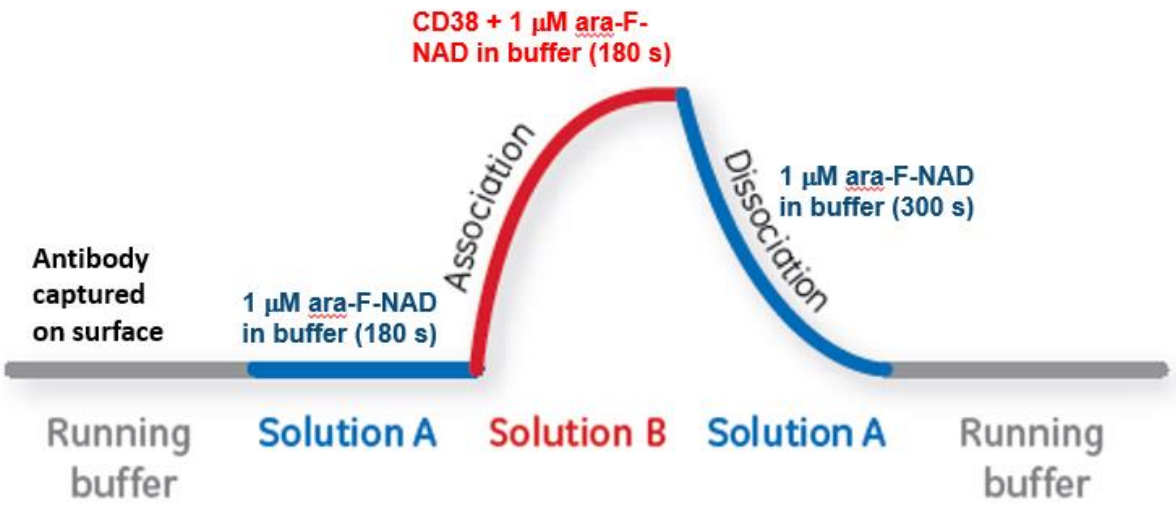
Asia	5 (15.6)	12 (17.9)	10 (16.9)	12 (11.9)
Other Countries	10 (31.3)	26 (38.8)	10 (16.9)	31 (30.7)

[a] All patients with a signed informed consent and who have been allocated a randomization number by the interactive response technology, regardless of whether the patient was treated or not.

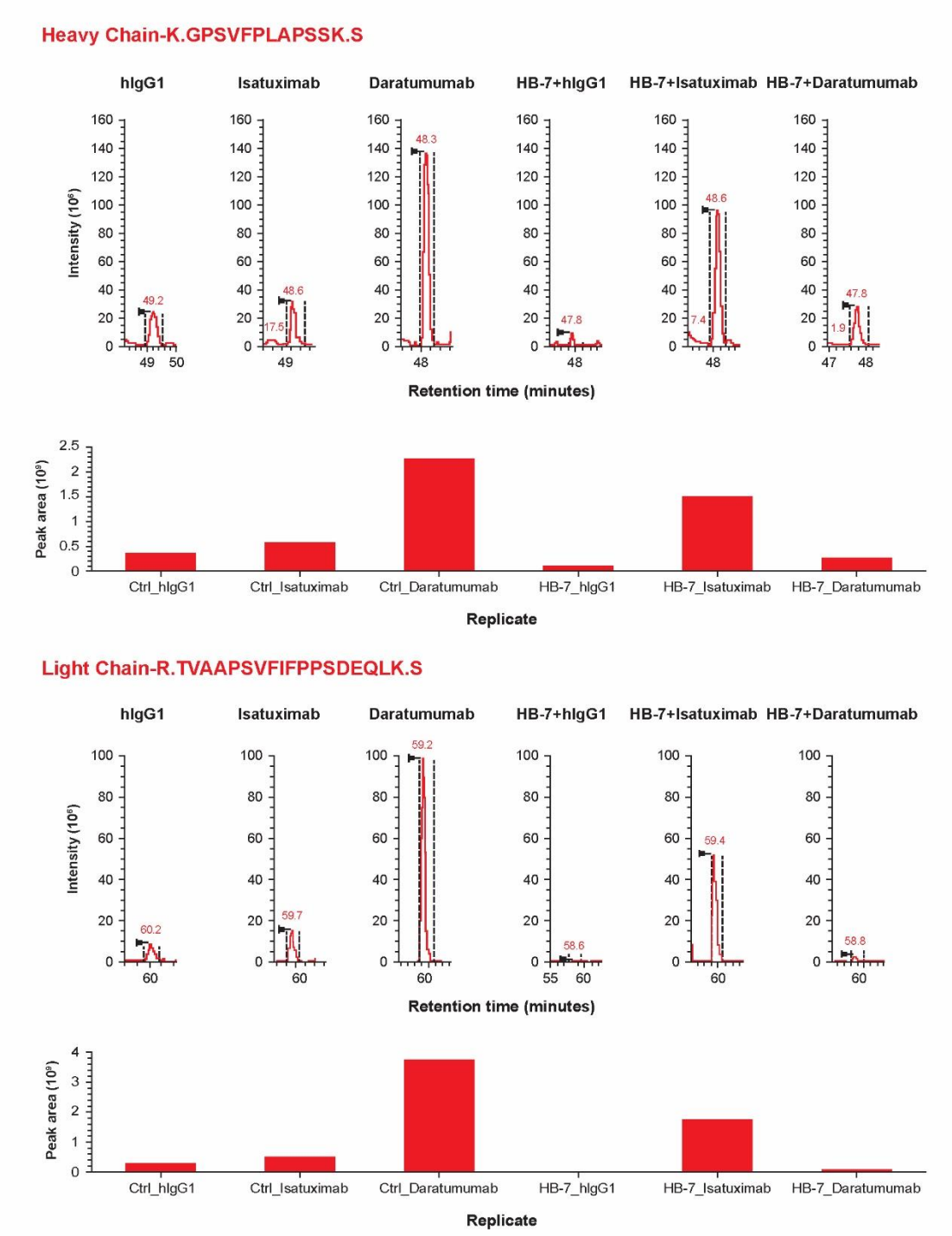
[b] Other countries were Australia, New Zealand, Turkey and Russia.

Abbreviations: IAT, indirect antiglobulin test; Isa, isatuximab; d, dexamethasone; K, carfilzomib; P, pomalidomide.

SUPPLEMENTARY FIGURE 1. Bicore A-B-A Injection mode. 1 µM ara-F-NAD was present during surface equilibration phase, CD38 association phase, and CD38 dissociation phase to ensure equilibrium condition between CD38 and ara-F-NAD. *Abbreviations:* ara-F-NAD, ara-F-nicotinamide adenine dinucleotide.



SUPPLEMENTARY FIGURE 2 (A) Detection of surface-bound anti-CD38 monoclonal antibodies on RBCs by tandem mass spectrometry (MS/MS). RBCs were treated with hIgG1, isatuximab or daratumumab at 10 µg/mL followed by acid elution. Quantification of eluted antibody peptides (heavy chain or light chain) from cell surface was based on the total MS1 peak area. A representative peptide from the heavy chain is shown “K.GPSVFPLAPSSK.S”, whereas light chain is shown as “R.TVAAPSVFIFPPSDEQLK.S”. *Abbreviations:* Ctrl, control; hIgG1, human immunoglobulin G1; min, minutes; RBC, red blood cell.



References

1. Sauve AA, Schramm VL. Mechanism-based inhibitors of CD38: a mammalian cyclic ADP-ribose synthetase. Biochemistry 2002;**41**: 8455-63.
2. Attal M, Richardson PG, Rajkumar SV, San-Miguel J, Beksac M, Spicka I, Leleu X, Schjesvold F, Moreau P, Dimopoulos MA, Huang JS, Minarik J, Cavo M, Prince HM, Macé S, Corzo KP, Campana F, Le-Guennec S, Dubin F, Anderson KC. Isatuximab plus pomalidomide and low-dose dexamethasone versus pomalidomide and low-dose dexamethasone in patients with relapsed and refractory multiple myeloma (ICARIA-MM): a randomised, multicentre, open-label, phase 3 study. Lancet 2019;**394**: 2096-107.