Comparison of CD38 antibodies *in vitro* and *ex vivo* mechanisms of action in multiple myeloma

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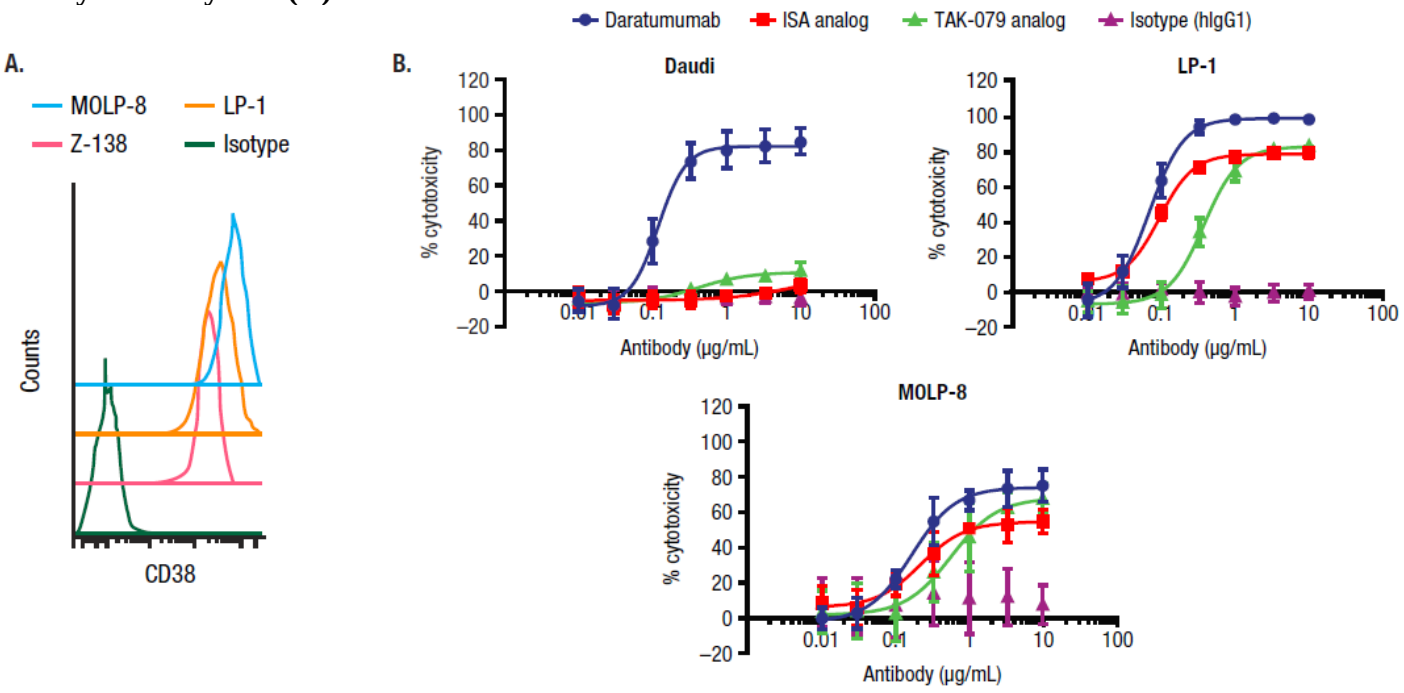
Supplementary Appendix

Supplementary Results

Supplementary Figure 1. (A) MOLP-8 cells showed higher CD38 expression levels compared with LP-1 and Z-138 cells; Daratumumab demonstrated higher CDC activity than comparator CD38 mAbs in Daudi, LP-1, and MOLP-8 cells assessed by (B) percent cytotoxicity and (C) EC50.



C.

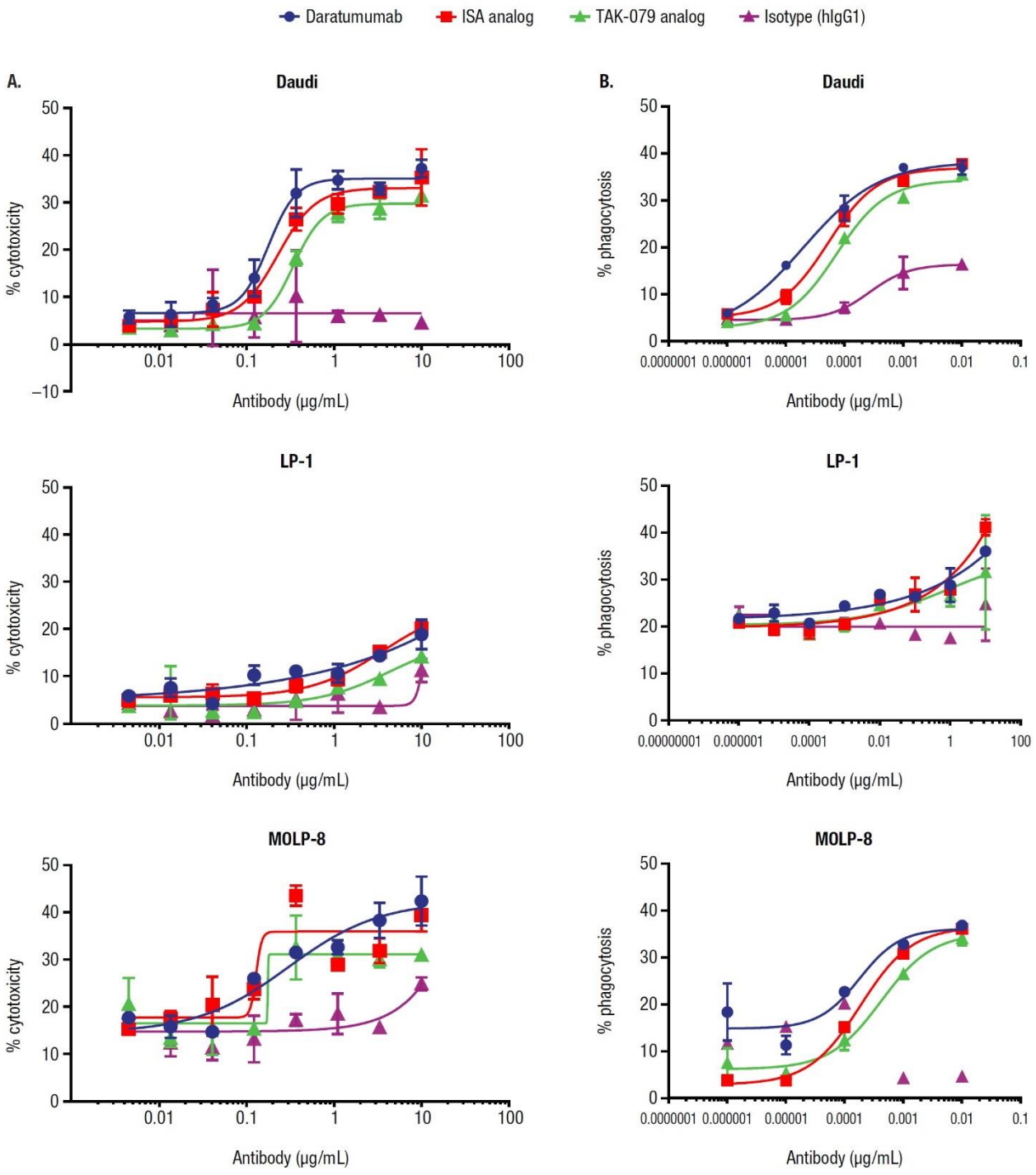
	Daratumumab	ISA analog	TAK-079 analog	Daratumumab vs ISA analog *P* value	Daratumumab vs TAK-079 analog *P* value
Daudi					
Max, µg/mL; 95% CI	81.82; 66.11-97.53	–	10.27; 4.04-16.50	–	–
EC50, µg/mL; 95% CI	0.14; 0.11-0.19	–	0.49; 0.23-1.01	–	–
LP-1					
Max, µg/mL; 95% CI	98.31; 97.07-99.55	77.98; 73.99-81.97	82.40; 77.20-87.61	<0.0001	<0.0001
EC50, µg/mL; 95% CI	0.09; 0.07-0.11	0.12; 0.09-0.15	0.42; 0.34-0.52	0.1339	0.0007
MOLP-8					
Max, µg/mL; 95% CI	73.33; 64.26-82.41	53.77; 46.25-61.29	67.69; 58.62-76.76	<0.0001	0.4983
EC50, µg/mL; 95% CI	0.22; 0.12-0.40	0.29; 0.13-0.66	0.81; 0.34-1.96	0.3054	0.0069

CD38 expression was measured on MOLP-8, LP-1, and Z-138 cells using CD38 (clone HIIT2) PerCp-Cy5.5 (BioLegend, San Diego, CA, USA).
CDC was analyzed by a CellTiter Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA) in Daudi, LP-1, and MOLP-8 cells, according to the manufacturer's protocol. Cells were plated at 10,000 cells/well. Human complement (CompTech, Tyler, TX, USA) and diluted antibodies were added and incubated for 2 hours. CellTiter Glo reagent was added, luminescence was measured on a SpectraMax® M5 spectrophotometer (Molecular Devices, LLC, San Jose, CA, USA), according to the manufacturer's protocol. Percent cytotoxicity = [(experimental lysis – spontaneous lysis)/(max lysis – spontaneous lysis)] × 100.

[a]Data are a summary of 3 independent experiments.
[b]We were unable to model ISA analog in Daudi cells. Due to the high level of divergence between the daratumumab and TAK-079 analog modeled curves, *P* values were not able to be calculated. However, the 95% CIs of max and EC50 values that do not overlap and the large difference between max values indicate a statistically significant difference between the max and EC50 values of daratumumab and TAK-079 analog.

CDC, complement-dependent cytotoxicity; EC50, half maximal effective concentration; max, maximum; CI, confidence interval.

Supplementary Figure 2. All 3 CD38 mAbs demonstrated similar levels of (A) ADCC[a] and (B) ADCP[b] activity in Daudi, LP-1, and MOLP-8 cells.



A calcein-release assay using human peripheral blood mononuclear cells (PBMCs) from healthy donors as effector cells was used to assess ADCC of Daudi, LP-1, and MOLP-8 cells. Cells were labeled with calcein-acetoxymethyl (10 µM; Thermo Fisher, Waltham, MA, USA) and seeded at

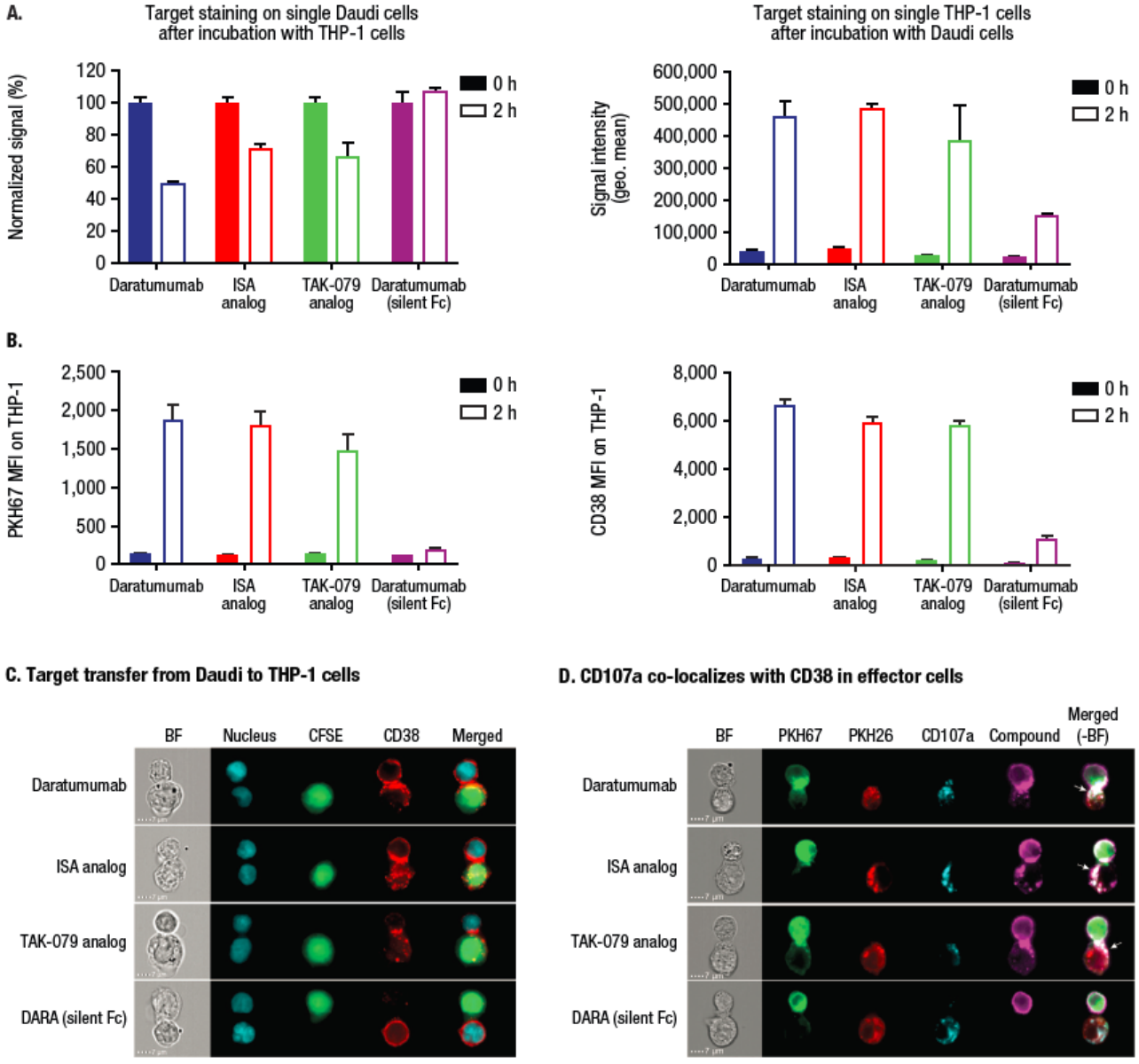
10,000 cells/well. Diluted antibodies were added to cells and incubated for 15 minutes at room temperature. PBMCs were obtained from 3 donors and seeded at an effector:target (E:T) ratio of 50:1. Calcein release was measured by fluorescence after 3 hours. Percent cytotoxicity = [(experimental lysis – spontaneous lysis)/(max lysis – spontaneous lysis)] × 100.

Macrophage phagocytosis of tumor cells was measured by ADCP using monocyte-derived macrophages as effector cells and pHrodo® Red (Life Technologies, Carlsbad, CA, USA)–labeled Daudi, LP-1, and MOLP-8 cells. Healthy donor $CD14^+$ monocytes were isolated from PBMCs (EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion, STEMCELL Technologies, Vancouver, BC, Canada) and differentiated into M2c macrophages by culturing with macrophage colony-stimulating factor (25 ng/mL; R&D Systems, Minneapolis, MN, USA) for 7 days and IL-10 (40 ng/mL; R&D Systems) for the final 3 days. Target cells were labeled with pHrodo Red and seeded with M2c macrophages at an E:T ratio of 4:1, along with mAbs. After 4 hours of incubation, cells were collected and stained with Live/Dead® and CD11b. ADCP was evaluated by flow cytometry (FACSCanto™; BD Biosciences, CA, USA). Percent phagocytosis = (pHrodo Red+CD11b+ cells/pHrodo Red+ cells) × 100.

[a]Three donors were tested in duplicate for all cell lines; data are shown as a representative experiment.
[b]4 assays for Daudi and 2 assays for LP-1 and MOLP-8; data are shown as a representative experiment.

mAbs, monoclonal antibodies; ADCC, antibody-dependent cellular-mediated cytotoxicity; ADCP, antibody-dependent cellular phagocytosis.

Supplementary Figure 3. All 3 CD38 mAbs showed comparable results, suggesting that all are participating in the same MOA, consistent with trogocytosis.
(A) CD38 signal on Daudi is lost in a time-, effector cell-, and FcR-dependent manner. Data are normalized to the 0-hour timepoint and demonstrate percent reduction. **(B)** Membrane dye is transferred in addition to CD38. **(C)** Comparable efficiency of target transfer from Daudi to THP-1 cells among the comparators. **(D)** CD107a co-localizes with CD38 in effector cells, suggesting that CD38 is degraded after trogocytosis.



Human monocytic THP-1 cells were labeled with carboxyfluorescein succinimidyl ester (Thermo Fisher) or PHK26 proliferation dye (Sigma-Aldrich, St. Louis, MO, USA). Daudi cells were coated with Alexa Fluor 647–conjugated daratumumab, ISA analog, or TAK-079 analog (10 µg/mL). Cells were cultured at an E:T ratio of 1:1 for 2 hours and washed and stained with

Hoechst dye (Thermo Fisher) and CD107a (BioLegend). Data were acquired using ImageStream®X Mark II flow cytometer and INSPIRE® software (both Amnis, Seattle, WA, USA). Three-thousand events (60× resolution) were collected to evaluate signal intensity in cell populations. Image analysis was performed in IDEAS 6.2 (Amnis). Data were normalized to 0-hour incubation to account for labeling efficiency differences.

mAb, monoclonal antibody; MOA, mechanism of action; MFI, mean fluorescence intensity; BF, bright field; CFSE, carboxyfluorescein succinimidyl ester.