Targeting B-cell maturation antigen increases sensitivity of multiple myeloma cells to MCL-1 inhibition

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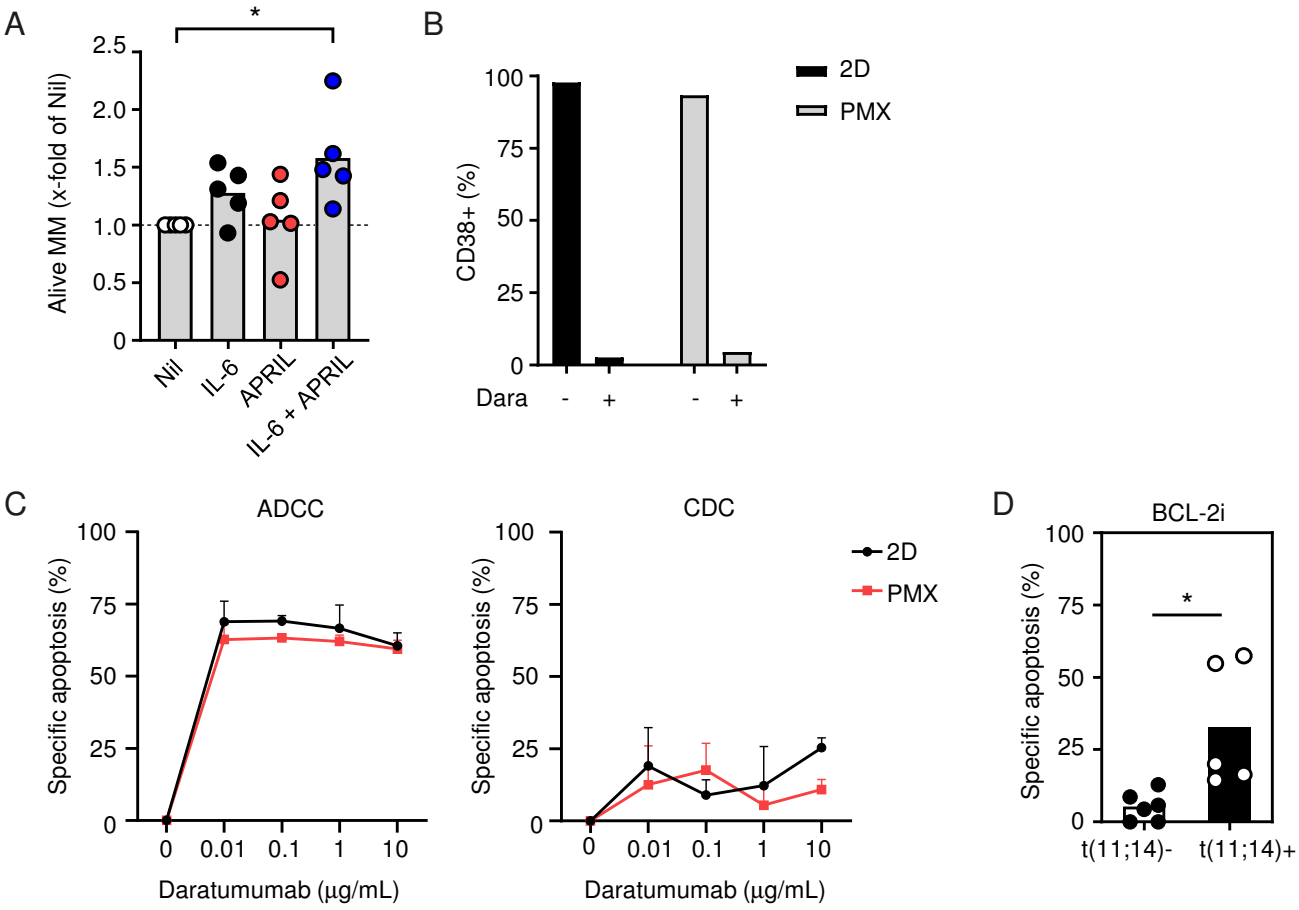
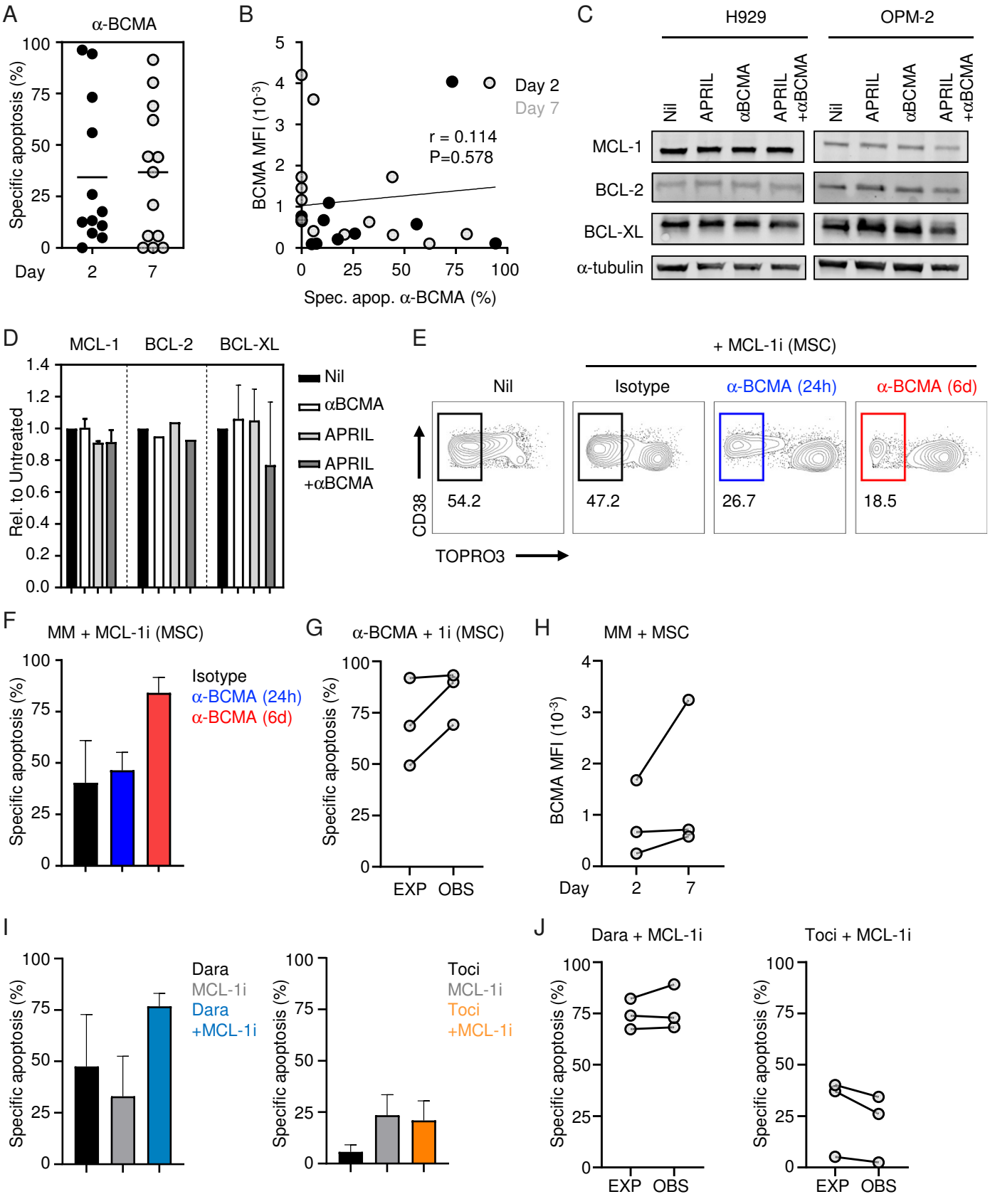
Supplementary Figure 1



Supplementary Figure 1. PMX hydrogel as a platform for *ex vivo* MM drug testing. (A) Primary MM samples (n=5) were cultured for 7 days in PMX hydrogel in the presence of 100 ng/mL IL-6, APRIL, or their combination. The graph shows the fold increase in the proportion (%) of alive MM cells relative to unstimulated controls. Statistical differences between groups were analyzed using a one-way ANOVA with Bonferroni's multiple comparison test. *, $p<0.05$. (B) H929 cells were cultured in 2D or PMX before being treated with 1 µg/mL Daratumumab (Dara) for 24h. For flow cytometry analysis, cells were stained with a PE-labeled anti-CD38 antibody (clone HIT2) that binds to the same epitope as Dara. Thus, if Dara is bound to the cell surface, cells appear CD38(HIT2)-. The graph shows the proportion (%) of CD38(HIT2)+ cells measured in each setting. (C) Antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) assays in PMX. L363 cells (MM cell line) were cultured for 24h in 2D or PMX before being treated with Dara at the indicated concentrations. 30 minutes after Dara addition, PBMCs (ratio PBMC:MM 10:1) or pooled human serum (25 µL/well in a final volume/well of 250 µL) were added to ADCC and CDC wells, respectively. Cell lysis was measured by flow cytometry 24h (ADCC) or 2h (CDC) after adding PBMC or serum. Error bars indicate Mean + SD. Data were pooled from 2 independent experiments. (D) Specific apoptosis in PMX-cultured primary MM samples harboring or not the translocation t(11;14), after a 24h treatment with 100 nM BCL-2 inhibitor (BCL-2i; ABT-199). Each dot represents an individual sample. Statistical differences between both groups were analyzed using an unpaired t-test. *, $p<0.05$.

Supplementary Figure 2



Supplementary Figure 2. Sensitivity of primary MM cells to BCMA targeting does not correlate with BCMA expression, and is maintained in the presence of MSC. (A) Specific apoptosis induced by 5 μg/mL anti-BCMA in primary MM samples (n=13) cultured in PMX. Anti-BCMA was added on day 1 or 6, and MM cell viability was measured 24h later (day 2 or 7, respectively). (B) Simple linear regression analysis relating BCMA mean fluorescence intensity (MFI) in untreated MM cells to specific apoptosis after 24h treatment with 5 μg/mL anti-BCMA at different time points in culture (day 2 and 7) (n=13). (C) Western blot analysis of MCL-1, BCL-2, and BCL-XL expression in H929 or OPM-2 MM cell lines after 24h treatment with APRIL (100 ng/mL), anti-BCMA (5 μg/mL), or their combination. (D) Quantification of protein expression as measured in (C), relative to expression in untreated cells. Bar graphs show averages of 2 independent experiments (+SEM). (E) Representative flow cytometry plots showing the proportion of alive MM cells (CD38+ TOPRO3-) after the indicated treatments. MM samples were co-cultured with mesenchymal stromal cells (MSC, 80.000/well) in PMX for 6 days in the presence of either 5 μg/mL isotype control antibody (black), anti-BCMA (red), or no antibody (blue). After this time, cells were treated with 100 nM MCL-1i (black, red) or co-treated with MCL-1i + anti-BCMA (blue). MM viability was analyzed 24h later (i.e., on day 7) by FACS. (F) Cumulative plots showing apoptosis induced by MCL-1i in the conditions specified in (E). Mean + SEM (n=3). (G) Plots comparing expected (EXP) to observed (OBS) specific apoptosis induced by combining anti-BCMA (5 μg/mL) and MCL-1i (100 nM) for 24h in the presence of MSC (n=3). (H) Mean Fluorescence Intensity (MFI) of BCMA in MM cells was measured by flow cytometry after 2 or 7 days in PMX + MSC co-culture (n=3). (I) MM cells were cultured in PMX and pre-treated with either daratumumab (0.1 μg/mL) or tocilizumab (5 μg/mL) before being exposed to MCL-1i (100 nM) for 24h. Bar graphs show specific apoptosis (Mean + SEM, n=3) induced by each individual agent and their combination. (J) Plots comparing expected (EXP) to observed (OBS) specific apoptosis induced by combining daratumumab or tocilizumab with MCL-1i in the settings described in (I). Statistical differences between 2 groups (G, H, J) were analyzed using paired t-tests. Statistical differences between 3 or more groups (D, F, I) were analyzed using a one-way ANOVA with Bonferronis' multiple comparison test. (A, B, G, H, J) Each dot represents an individual sample.

Supplementary Table 1

Sample code	ND/RR	Cytogenetics
MM1	RR	t(11;14), amp(1q)
MM2	SMM	t(11;14)
MM3	RR	HD
MM4	SMM	t(11;14)
MM5	ND	either t(14q32;?) or trisomy 14; del(13q)
MM6	ND	
MM7	ND	HD, t(14;?), del(13q)
MM8	ND	no abnormalities
MM9	RR	
MM10	ND	HD
MM11	ND	HD, del(13q), del(16q)
MM12	RR	t(11;14)
MM13	RR	no abnormalities
MM14	RR	HD, del(1p), amp(1q)
MM15	ND	del (13q), del(17p), del(16q), amp(11q)
MM16	ND	no abnormalities
MM17	ND	t(11;14), del(13q)
MM18	ND	1q
MM19	ND	HD
MM20	ND	13q
MM21	ND	1q + 13q + t(4;14)
MM22	ND	1q
MM23	ND	HD
MM24	ND	HD
MM25	ND	1q + HD
MM26	ND	13q + HD
MM27	ND	1q + 13q + t(11;14) + HD
MM28	ND	1q + 13q + t(11;14)
MM29	ND	HD, del(13q)
MM30	ND	1q + t(11;14)
MM31	ND	t(14)

Supplementary Table 1. Clinical characteristics of MM patients (n=31). ND, newly diagnosed; RR, relapsed/refractory; SMM, smoldering multiple myeloma; HD, hyperdiploidy.