TE-1146, a novel anti-CD38-antibody-lenalidomide conjugate, demonstrates potent *ex vivo* anti-myeloma activity

Current therapies for multiple myeloma (MM), a malignancy of plasma cells, are limited by acquired resistance and eventual relapse.[1] While the anti-CD38 antibody, daratumumab, both alone and in combination with the immune modulator, lenalidomide, has significantly improved progression-free survival in relapsed/refractory MM,[2,3] it is associated with significant toxicity and a high relapse rate.[4] To enhance efficacy and tolerability, we developed TE-1146, an antibody-drug conjugate comprising a reconfigured daratumumab specifically conjugated to six lenalidomide molecules (*Online Supplementary Figure S1*), designed to deliver cytotoxic lenalidomide directly to CD38-expressing myeloma cells, enhancing antitumor activity beyond what is achievable with lenalidomide or daratumumab alone or combined (Dara+Lena): free lenalidomide cannot easily enter cells,[5] while daratumumab alone relies on Fc-mediated immune effector mechanisms.[6] The design of TE-1146 was confirmed in our *in vitro* and *in vivo* studies[5] showing that TE-1146 effectively enters CD38-expressing tumor cells, releases lenalidomide, and leads to enhanced cell-killing effects compared to lenalidomide, daratumumab, or Dara+Lena. Here, we evaluated the *ex vivo* efficacy of TE-1146 against primary MM cells from 14 patients, categorized as having untreated newly diagnosed MM (NDMM; N=5) and relapsed/refractory MM without (RRMM1; N=4) or with (RRMM2; N=5) prior exposure to daratumumab/lenalidomide. TE-1146 showed markedly enhanced anti-myeloma activity

Table 1. Treatment history, CD138$^+$ cell characteristics, and drug sensitivity (IC_{50}) of each patient.

Group	Patient	Prior therapies[a]	CD138$^+$		MFI[d]			IC_{50}, µM[e]	
			% purity[b]	% CD38$^+$[c]	CD38	CD56	CD138	TE-1146	Dara+Lena
NDMM	#1	-	97	99.8	2,347	9,289	13,108	0.017	23.05
	#2	-	83	99.4	3,543	783	304	0.046	801.6
	#3	-	76	99.9	3,241	845	986	0.016	50.19
	#4	-	72	99.4	976°	280°	81°	0.163	-
	#5	-	95	99.9	4,159	320	138	0.013	-
RRMM1	#1	Thalidomide, VTd, RT, ESHAP, Auto-SCT	79	97.7	3,277	1,324	2,147	0.036	-
	#2	VTd	95	97.7	3,466	1,064	1,330	0.017	-
	#3	VTd, ESHAP, Auto-SCT	84	98.0	2,351	462	2,971	0.025	-
	#4	VTd, VT	77	99.7	5,347	852	2,408	0.001	-
RRMM2	#1	Auto-SCT, bortezomib, carfilzomib, CTX, Dd, Id, Rd	84	99.5	1,472	5,115	4,780	>100	>100
	#2	Auto-SCT, bortezomib, lenalidomide, VRd	85	99.5	4,586	2,710	9,794	1.957	>100
	#3	Auto-SCT, DRd, RT, VTd	95	99.8	2,306	4,518	5,298	-	>100
	#4	DRd, Vd, VTd	97	99.9	1,573	18,403*	18,330*	-	>100
	#5	DRd, VTd	59	92.2	9,544*	709	3,893	-	>100

[a]Prior therapies included VTd: bortezomib, thalidomide, and dexamethasone; RT: radiotherapy; ESHAP: etoposide, methylprednisolone, cytarabine, and cisplatin; Auto-SCT: autologous stem-cell transplantation; VT: bortezomib and thalidomide; CTX: cyclophosphamide; Dd: daratumumab and dexamethasone; Id: Ixazomib and dexamethasone; Rd: lenalidomide and dexamethasone; VRd: bortezomib, lenalidomide, and dexamethasone; DRd: daratumumab, lenalidomide, and dexamethasone; Vd: bortezomib and dexamethasone. [b]Purity of CD138$^+$ cells after CD138$^+$ magnetic bead isolation. [c]Percentage of CD38$^+$ cells within the CD138$^+$ cell fraction. [d]The mean fluorescence intensities (MFI) of CD38, CD56, and CD138 markers on CD138$^+$ cells characterized by flow cytometry using fluorophore-conjugated antibodies. *The highest MFI of each marker and °the lowest. [e]IC_{50}: the drug concentration required to inhibit CD138$^+$ cell viability by 50%; a dash indicates no inhibitory effect. NDMM: newly diagnosed multiple myeloma. RRMM1 relapsed/refractory multiple myeloma in patients not previously exposed to daratumumab/lenalidomide; RRMM2: relapsed/refractory multiple myeloma in patients with prior exposure to daratumumab/lenalidomide.

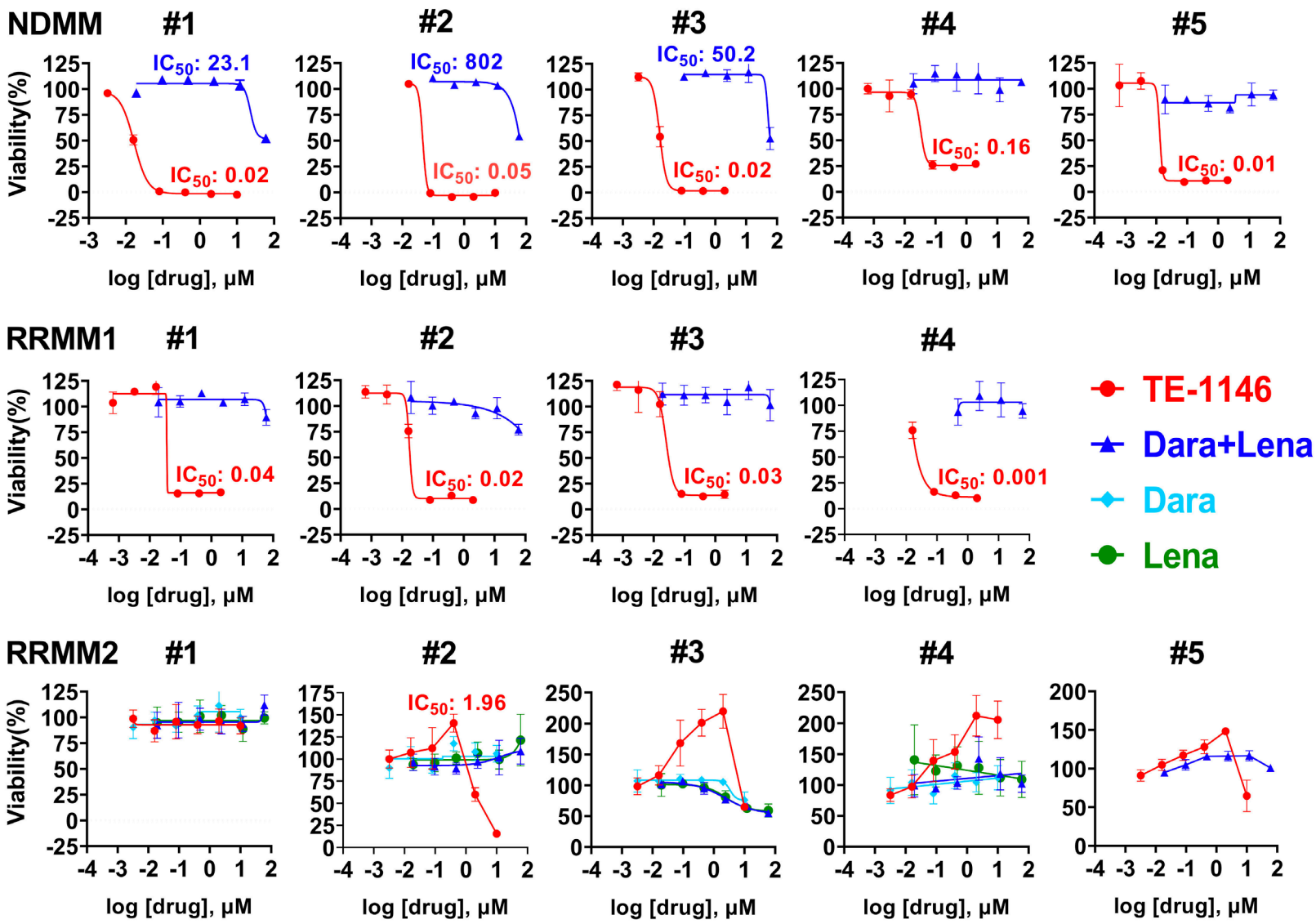

Figure 1. *Ex vivo* cytotoxicity of TE-1146 *versus* daratumumab plus lenalomide in primary multiple myeloma cells. Purified CD138$^+$ cells (5×10^3 - 2×10^4) from each group of patients (except one CD138$^-$ RRMM1 patient) were treated with increasing concentrations of TE-1146 (red) and daratumumab plus lenalidomide (Dara+Lena) (blue) at a 1:6 molar ratio for 4–6 days in a humidified incubator at 37°C with 5% CO_2. CD138$^+$ cells from RRMM2 patients were also treated with increasing concentrations of daratumumab (Dara, aqua) or lenalidomide (Lena, green). After incubating AlamarBlue™ (10 μL) with RPMI-1640 complete medium (100 μL) containing the purified CD138$^+$ cells for ≥1 hour, optical density (OD) was measured at excitation/emission wavelengths of 560/590 nm using an enzyme-linked immunosorbent assay multimode microplate reader (BioTek Synergy H1). The percentage of viable CD138$^+$ cells was calculated as [OD(treated sample) - OD(background)]/[OD(untreated sample) - OD(background)] X 100. Data points represent mean ± standard deviation from experiments performed two to four times. Dose-response curves were generated using nonlinear regression. IC_{50} is the drug concentration required to inhibit cell viability by 50%. NDMM: newly diagnosed multiple myeloma. RRMM1 relapsed/refractory multiple myeloma in patients not previously exposed to daratumumab/lenalidomide; RRMM2: relapsed/refractory multiple myeloma in patients with prior exposure to daratumumab/lenalidomide.

compared to Dara+Lena, particularly in NDMM and RRMM1 patients. Resistance to TE-1146 in daratumumab/lenalidomide-exposed RRMM2 patients correlated with lower CD38 and/or higher CD56/CD138 expression. These data, along with prior *in vitro*/*in vivo* findings, support TE-1146's potential for early-line use in daratumumab/lenalidomide-naïve patients. Since CD138 serves as a marker of mature plasma cells, including malignant MM cells, we isolated CD138$^+$ myeloma cells from bone marrow aspirates of 15 MM patients (*Online Supplementary Table S1*), after obtaining informed consent and approval from the Research Ethics Review Committee of Far Eastern Memorial Hospital, New Taipei City, Taiwan (Institutional Review Board N. 112088-F). However, one RRMM1 patient was CD138$^-$, hence only 14 MM patients were analyzed. The purity of the isolated CD138$^+$ cells exceeded 80% in most samples with >97% CD38$^+$ cells within this CD138$^+$ fraction (Table 1). CD138$^+$ cell viability was quantified by AlamarBlue™ assay after 4–6 days of incubation with TE-1146 or Dara+Lena at a 1:6 molar ratio to match the drug-to-antibody ratio of TE-1146. TE-1146 showed potent cytotoxicity, as determined by the half maximal inhibitory concentration (IC_{50}), against MM cells from patients in the NDMM (IC_{50} = 0.05±0.06 μM) and RRMM1 (IC_{50} = 0.02±0.01 μM) cohorts, achieving near-complete tumor cell eradication at higher concentrations. In contrast, Dara+Lena showed only modest/minimal effects (Figure 1). Since the *ex vivo* assay lacks immune effector cells required for daratumumab's Fc-mediated cytotoxicity,[6] the enhanced efficacy of TE-1146 is due to its direct intracellular delivery of lenalidomide, a mechanism unavailable to Dara+Lena. This finding supports the rationale behind the design of TE-1146 and aligns with our previous study showing that TE-1146 retains Fc-medi-

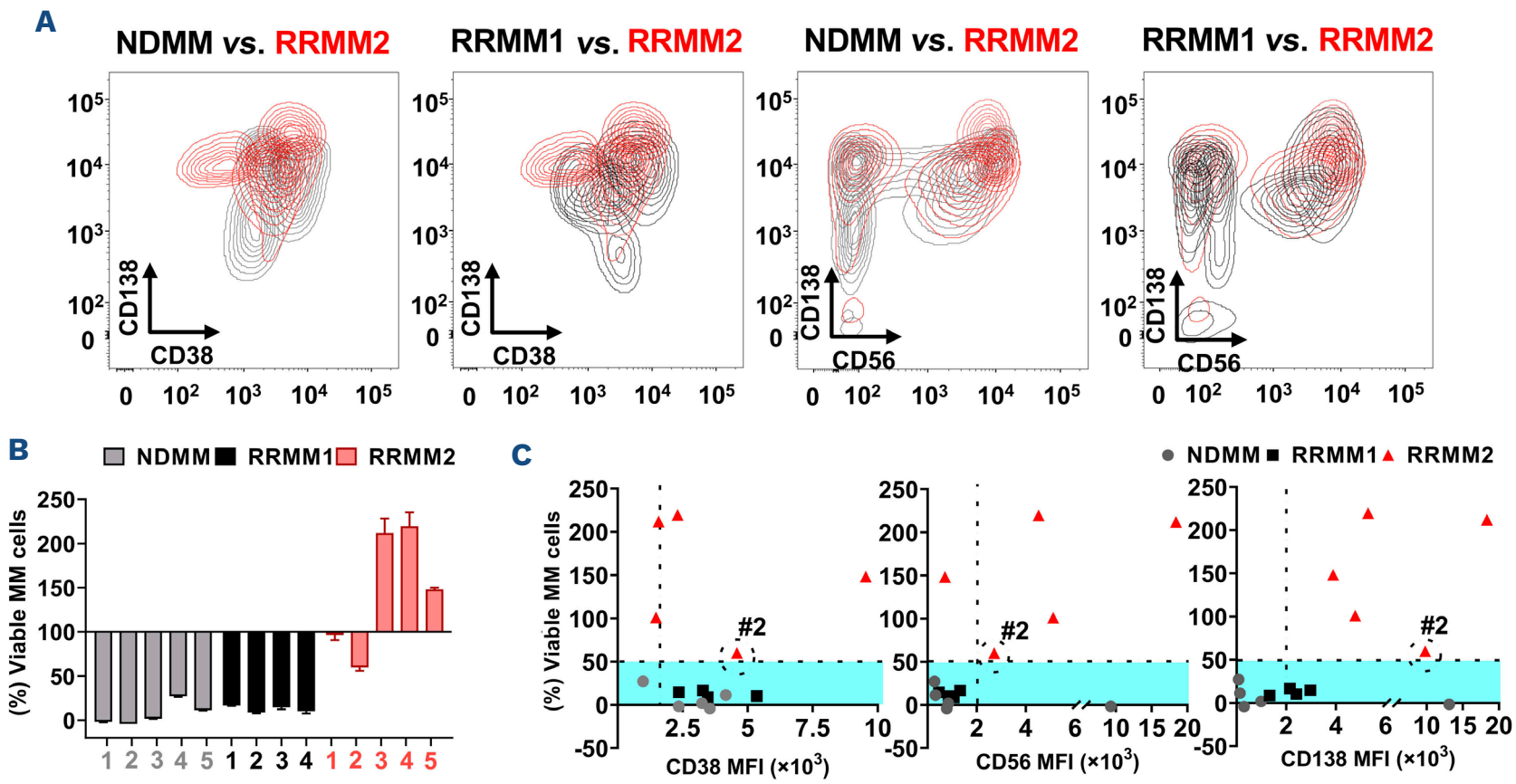

Figure 2. TE-1146 resistance in RRMM2 patients correlates with surface marker expression. (A) Density contour plots comparing CD138 *versus* CD38 (left) and CD138 *versus* CD56 (right) expression profiles between TE-1146-resistant RRMM2 patients (red contours) and TE-1146-sensitive NDMM/RRMM1 patients (gray/black contours). The contour lines represent the density of patient cell populations within specific expression ranges. (B) Waterfall plot showing the percentage of viable tumor cells remaining after treatment with 2 μM TE-1146. Each bar represents an individual patient from the NDMM (gray), RRMM1 (black), and RRMM2 (red) groups. (C) Relationship between post-treatment viability of CD138⁺ multiple myeloma cells and the mean fluorescence intensities of CD38, CD56, and CD138 markers on CD138⁺ cells prior to drug exposure. The circled red triangle corresponds to the RRMM2 patient with a partial response. TE-1146 sensitivity (<50% viability, aqua background) generally correlated with elevated CD38 coupled with lower CD56/CD138 expression. NDMM: newly diagnosed multiple myeloma. RRMM1 relapsed/refractory multiple myeloma in patients not previously exposed daratumumab/lenalidomide; RRMM2: relapsed/refractory multiple myeloma in patients with prior exposure to daratumumab/lenalidomide; MM: multiple myeloma; MFI: mean fluorescence intensity.

ated immune activity similar to daratumumab, but exhibits enhanced tumor inhibition efficacy compared to Dara+Lena in immune-competent mouse models, despite carrying only ~0.01% of the lenalidomide dosage used in Dara+Lena.[5] This implies that lenalidomide's efficacy depends on its ability to enter MM cells and bind to its target protein, cereblon, leading to the proteasomal degradation of proteins essential for MM cell survival.[7]

RRMM2 patients who had received extensive prior therapies were largely unresponsive to TE-1146, Dara+Lena, or daratumumab/lenalidomide alone, except for one daratumumab-naïve patient who showed partial sensitivity to only TE-1146 (Table 1, Figure 1). To investigate mechanisms underlying this resistance, we quantified the surface expression of CD38, CD56, and CD138 on CD138⁺ MM cells prior to drug exposure by flow cytometry (Table 1) and compared the CD138 *versus* CD38/CD56 expression profiles of TE-1146-resistant and sensitive MM cells. Compared to sensitive NDMM and RRMM1 cells, resistant RRMM2 cells displayed a clear phenotypic shift, characterized by a subpopulation with reduced CD38 expression and a higher proportion of MM cells with elevated CD56 and CD138 expression (Figure 2A). This translated into TE-1146 resistance (>50% CD138⁺ cell viability) in four of the five RRMM2 patients even at a high 2 μM dose (Figure 2B). TE-1146 resistance correlated with lower CD38 levels, a known escape mechanism from daratumumab therapy,[8] and/or higher expression of CD56 and/or CD138, markers associated with tumor cell survival (Figure 2C).[9-11] Furthermore, elevated CD56 expression has been shown to downregulate cereblon,[9] the target protein that underlies lenalidomide's cytotoxic effects against tumor cells.[7]

The differential efficacy of TE-1146 across these groups of patients highlights that drug sensitivity is determined by a complex interplay of factors beyond simple target expression. Resistance did not strictly correlate with CD38 density alone: an RRMM2 patient with the highest CD38 expression remained insensitive to TE-1146, likely due to high co-expression of CD138, whereas an NDMM patient with the lowest CD38 expression, but also the lowest CD56/CD138 expression, remained sensitive to TE-1146 (Table 1). These observations suggest that a favorable balance of *high* target density and *low* pro-survival marker expression supports TE-1146 sensitivity. Furthermore, the partial response in the single daratumumab-naïve RRMM2 patient suggests that the absence of prior selective pressure from CD38-targeted therapy may preserve the efficacy of TE-1146. For patients with low CD38

expression, strategies to restore CD38, such as extended drug washout periods[12] or combination with CD38-upregulating agents,[13,14] may help to recover sensitivity to TE-1146.
In summary, TE-1146 shows more potent cytotoxicity against primary MM cells from daratumumab/lenalidomide-naïve patients than the standard Dara+Lena combination due to its distinct mechanism of direct, intracellular payload delivery. While limited by a modest sample size and an *ex vivo* design that cannot fully recapitulate the bone marrow microenvironment,[15] our findings provide a strong rationale for our planned clinical evaluation of TE-1146 using *ex vivo* cytotoxicity assays to select patients for treatment.

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TWC, S-SC, and H-MC, Y-HY and CL hold stock or have stock options in T-E Pharma Holding.

Contributions
Y-HY, TWC and H-MC designed experiments. S-SC and J-GJ performed experiments and data and statistical analysis. JGJ, SCL and YBY contributed patients' samples and provided clinical correlations. CL analyzed and interpreted results and wrote the manuscript.

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Data-sharing statement
Y-HY can be contacted for more information.

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