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

Shih-Syuan Cheng[1#], Jing-Gu Jiang[2#], Shih-Chiang Lin[2], Yueh-Hsiang Yu[3]*, Tse Wen Chang[1,3], Carmay Lim[1,3]*, Yuan-Bin Yu[2,4,5]*, and Hsing-Mao Chu[1]

[1]T-E Meds, Inc., Taipei, Taiwan
[2]Department of Medicine, Division of Hematology and Oncology, Far Eastern Memorial Hospital, New Taipei City, Taiwan
[3]Immunwork, Inc., Taipei, Taiwan
[4]Faculty of Medicine, School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan
[5]Graduate Institute of Medicine, Yuan Ze University, Taoyuan, Taiwan

[#]SSC and JGJ contributed equally as first authors

*Corresponding authors:
Carmay Lim - carmaylim0830@gmail.com
Yuan-Bin Yu - fishie.yu@gmail.com

Disclosures

TWC, founder of Immunwork, Inc. and T-E Meds, Inc.; SSC and HMC, employees of T-E Meds, Inc.; YHY, employee of Immunwork, Inc., and CL, scientific advisor to Immunwork, Inc. and T-E Meds, Inc., hold stock or have stock options in T-E Pharma Holding.

Contributions

YHY, TWC, and HMC designed experiments; SSC and JGJ performed experiments and data and statistical analysis; JGJ, SCL, and YBY contributed patient samples and provided clinical correlations; CL analyzed and interpreted results and wrote the manuscript.

Data sharing statement

YHY can be contacted for more information.

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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, have 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 site-specifically conjugated to six lenalidomide molecules (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] TE-1146's design 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 TE-1146's *ex vivo* efficacy against primary MM cells from 14 patients, categorized as untreated newly diagnosed MM (NDMM; n=5) and relapsed/refractory MM without (RRMM1; n=4) and with (RRMM2; n=5) prior daratumumab/lenalidomide exposure. TE-1146 showed markedly enhanced anti-myeloma activity 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*/*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 (Supplementary Table S1), after obtaining informed consent and approval by the Research Ethics Review Committee (IRB No. 112088-F) of Far Eastern Memorial Hospital, New Taipei City, Taiwan. 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 TE-1146's drug-to-antibody ratio. TE-1146 showed potent cytotoxicity against MM cells from 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] TE-1146's enhanced efficacy is due to its direct intracellular delivery of lenalidomide, a mechanism unavailable to Dara+Lena. This finding supports the rationale behind TE-1146's design and aligns with our previous study showing that TE-1146 retains Fc-mediated immune activity similar to daratumumab, but exhibits enhanced tumor inhibition efficacy than 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 with 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 vs. 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 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 patient groups 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 remained TE-1146-insensitive, likely due to co-expression of high CD138, whereas an NDMM patient with the lowest CD38, but also the lowest CD56/CD138, remained TE-1146-sensitive (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 TE-1146 efficacy. 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 TE-1146 sensitivity.

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

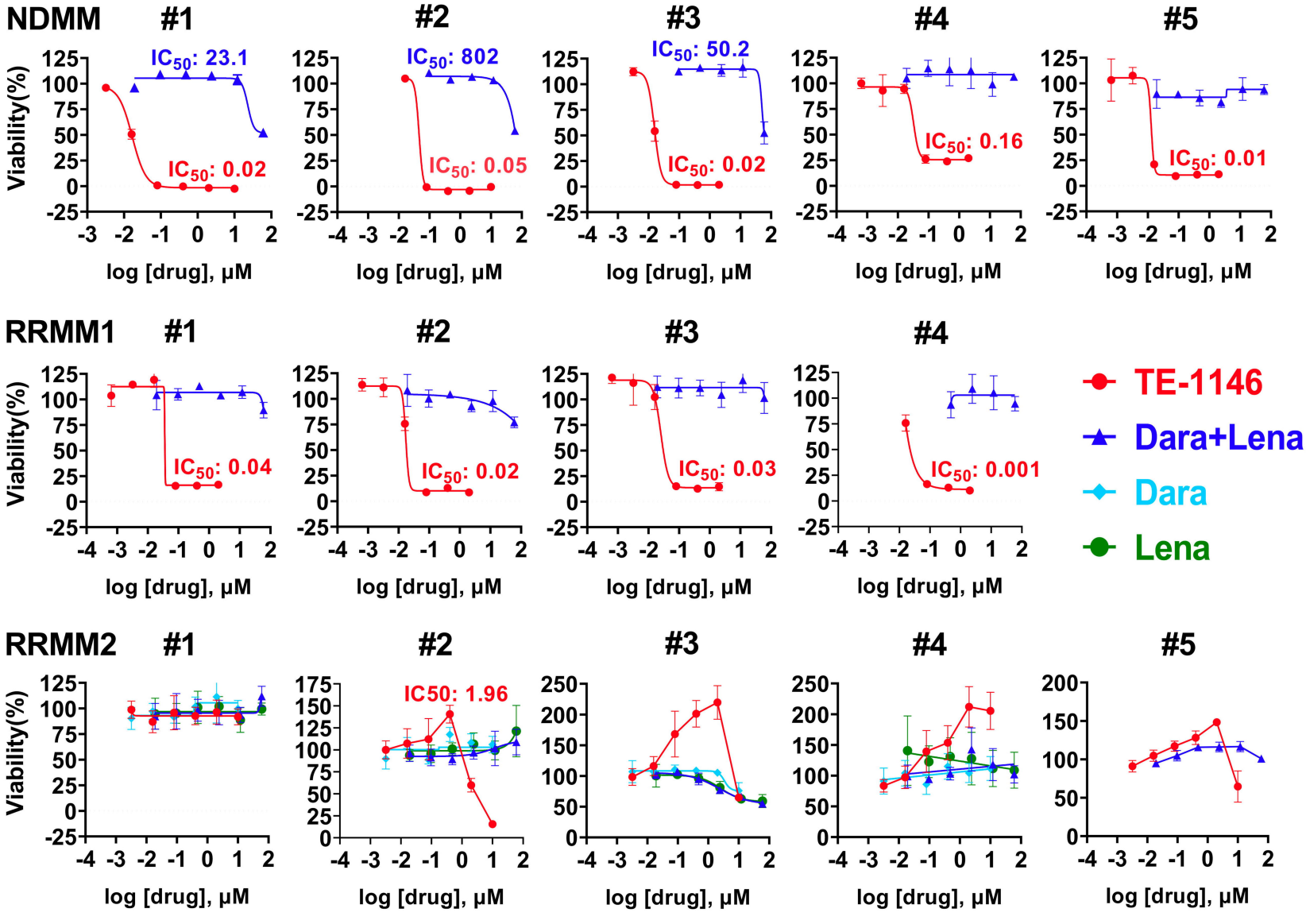
Group	Patient	Prior Therapies[a]	CD138$^+$		MFI[d]			$IC_{50,}$ μM[e]	
			% purity[b]	% CD38^{+c}	CD38	CD56	CD138	TE-1146	Dara+ Lena
NDMM	#1		97	99.8	2347	9289	13108	0.017	23.05
	#2		83	99.4	3543	783	304	0.046	801.6
	#3		76	99.9	3241	845	986	0.016	50.19
	#4		72	99.4	*976*	*280*	*81*	0.163	–
	#5		95	99.9	4159	320	138	0.013	–
RRMM1	#1	Thalidomide, VTd, RT, ESHAP, Auto-SCT	79	97.7	3277	1324	2147	0.036	–
	#2	VTd	95	97.7	3466	1064	1330	0.017	–
	#3	VTd, ESHAP, Auto-SCT	84	98.0	2351	462	2971	0.025	–
	#4	VTd, VT	77	99.7	5347	852	2408	0.001	–
RRMM2	#1	Auto-SCT, bortezomib, carfilzomib, CTX, Dd, Id, Rd	84	99.5	1472	5115	4780	> 100	> 100
	#2	Auto-SCT, bortezomib, lenalidomide, VRd	85	99.5	4586	2710	9794	1.957	> 100
	#3	Auto-SCT, DRd, RT, VTd	95	99.8	2306	4518	5298	–	> 100
	#4	DRd, Vd, VTd,	97	99.9	1573	**18403**	**18330**	–	> 100
	#5	DRd, VTd	59	92.2	**9544**	709	3893	–	> 100

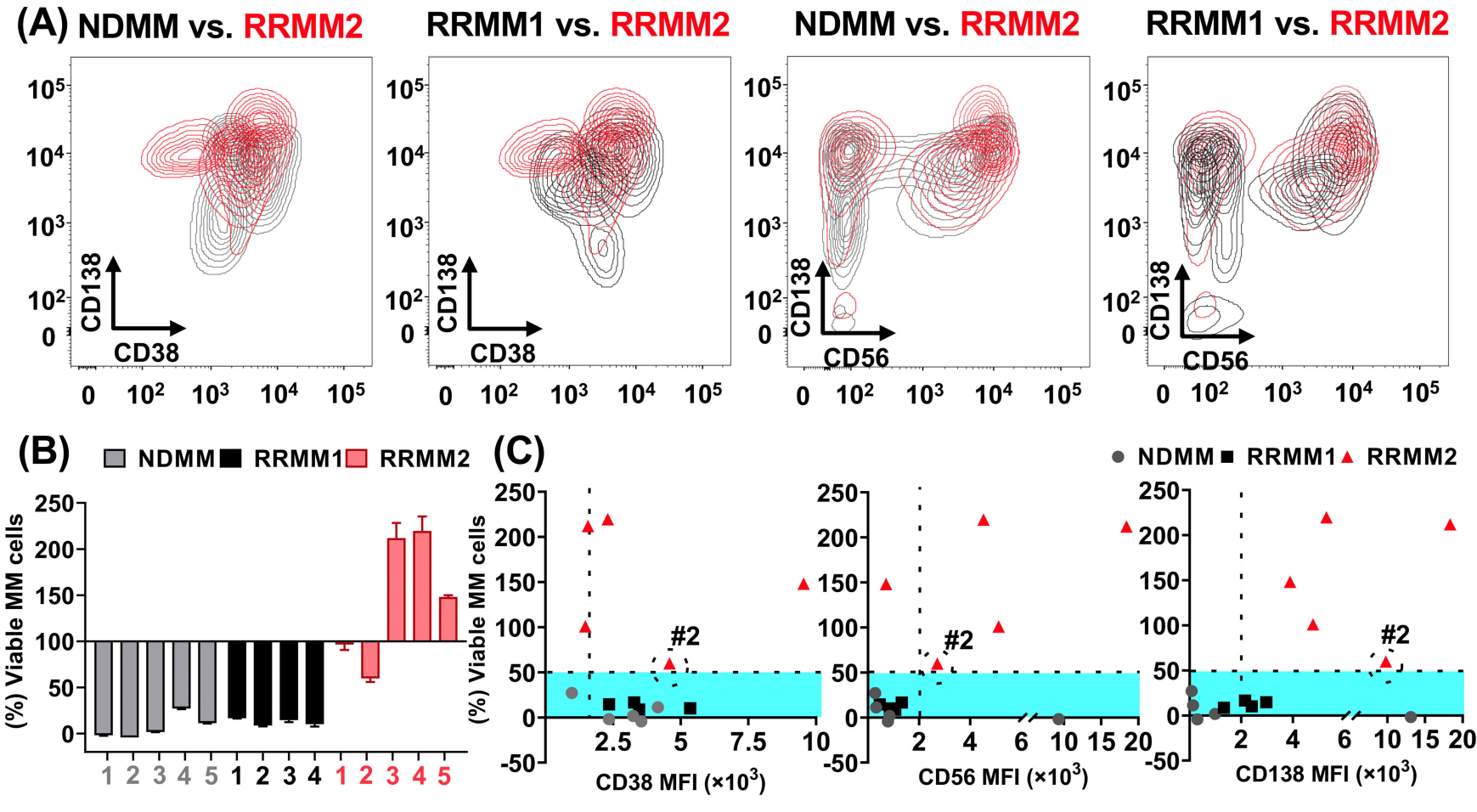
[a]Auto-SCT: Autologous stem-cell transplantation; CTX: Cyclophosphamide; Dd: Daratumumab and dexamethasone; DRd: Daratumumab, lenalidomide, and dexamethasone; ESHAP: Etoposide, methylprednisolone, cytarabine, and cisplatin; Id: Ixazomib and dexamethasone; Rd: Lenalidomide and dexamethasone; RT: Radiotherapy; Vd: Bortezomib and dexamethasone; VRd: Bortezomib, lenalidomide, and dexamethasone; VT: Bortezomib and thalidomide; VTd: Bortezomib, thalidomide, and dexamethasone. [b]Purity of CD138$^+$ cells after CD138$^+$ magnetic bead isolation. [c]Percentage of CD38$^+$ cells within 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 is in bold and the lowest in italics. [e]IC_{50}: the drug concentration required to inhibit CD138$^+$ cell viability by 50%; a dash indicates no inhibitory effect.

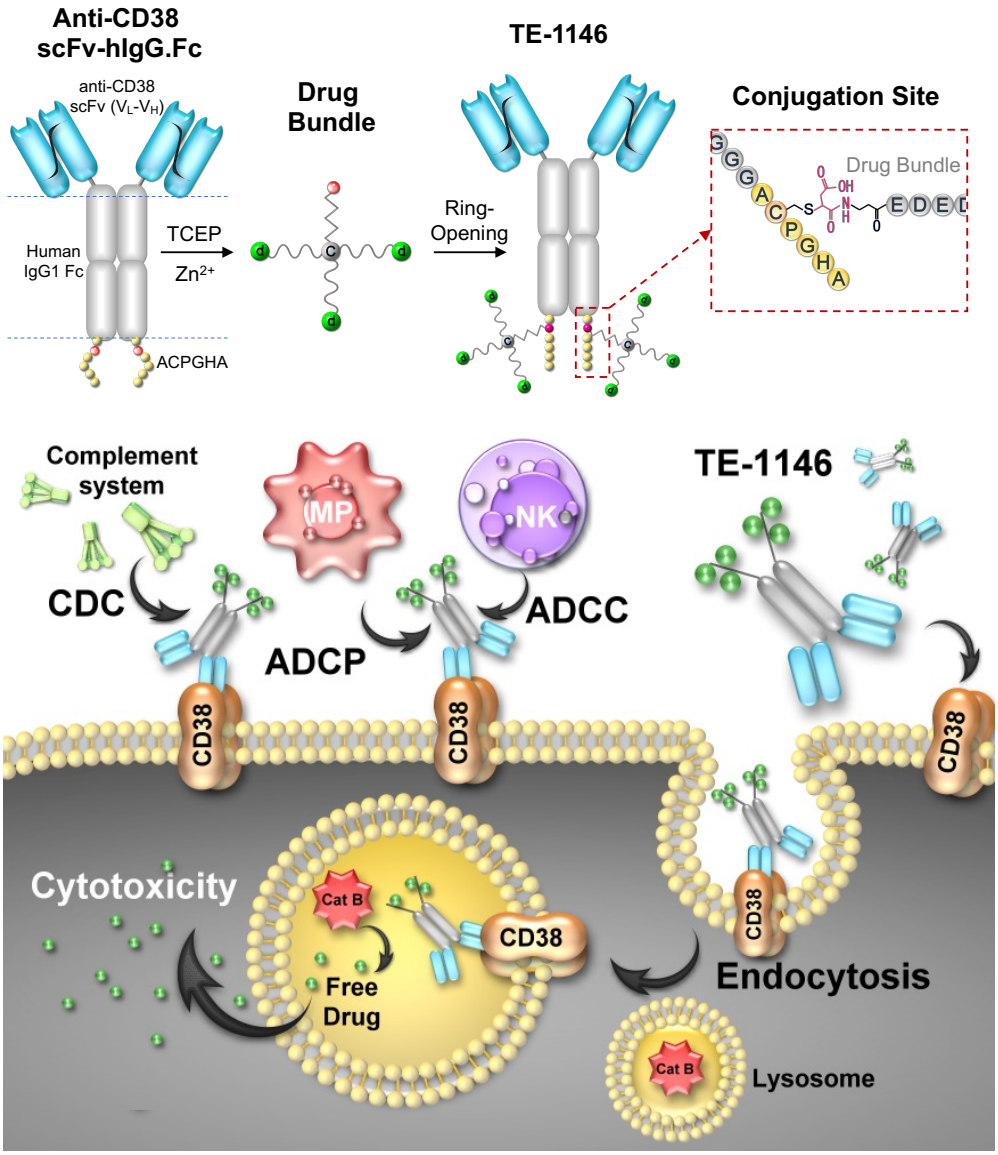
Figure Legends

Figure 1. *Ex vivo* cytotoxicity of TE-1146 vs. Dara+Lena in primary MM cells. Purified $CD138^+$ cells (5×10^3 – 2×10^4) from each patient group (except one $CD138^-$ RRMM1 patient) were treated with increasing concentrations of TE-1146 (red) and 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 ELISA multimode microplate reader (BioTek Synergy H1). The % of viable $CD138^+$ cells was calculated as [OD(treated sampled) − OD(background)]/[OD(untreated sampled) − OD(background)] X 100. Data points represent mean ± SD from experiments performed 2 to 4 times. Dose-response curves were generated using nonlinear regression. IC_{50} is the drug concentration required to inhibit cell viability by 50%.

Figure 2. **TE-1146 resistance in RRMM2 patients correlates with surface marker expression.** (A) Density contour plots comparing CD138 vs. CD38 (left) and CD138 vs. 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 $CD138^+$ MM cells and the mean fluorescence intensities (MFI) of CD38, CD56, and CD138 markers on $CD138^+$ cells prior to drug exposure. The circled red triangle corresponds to the RRMM2 patient with partial response. TE-1146 sensitivity (< 50% viability, aqua background) generally correlated with elevated CD38 coupled with lower CD56/CD138 expression.


NDMM
#1
#2
#3
#4
#5
RRMM1
#1
#2
#3
#4
RRMM2
#1
#2
#3
#4
#5
Viability(%)
log [drug], μM
IC50: 23.1
IC50: 0.02
IC50: 802
IC50: 0.05
IC50: 50.2
IC50: 0.02
IC50: 0.16
IC50: 0.01
IC50: 0.04
IC50: 0.02
IC50: 0.03
IC50: 0.001
IC50: 1.96
TE-1146
Dara+Lena
Dara
Lena


(A) NDMM vs. RRMM2
RRMM1 vs. RRMM2
NDMM vs. RRMM2
RRMM1 vs. RRMM2
CD138
CD38
CD56
(B)
NDMM
RRMM1
RRMM2
(%) Viable MM cells
(C)
#2
CD38 MFI (×10^3)
CD56 MFI (×10^3)
CD138 MFI (×10^3)



Supplementary Figure S1. TE-1146 preparation and proposed mechanism of action. (Top) Two drug bundles, each containing 3 lenalidomide molecules (green spheres), were prepared using a multi-arm linker and site-specifically conjugated to the engineered cysteines (pink) at the C-termini of a 2-chain α-CD38 single-chain variable fragment (scFv, blue)–human IgG1.Fc (gray)–A**C**PGHA (beads) fusion protein via S^{-}-maleimide reaction, followed by irreversible hydrolysis of the maleimide group to prevent premature drug release. (Bottom) The dual mechanisms of action of TE-1146: (i) direct cytotoxicity via internalization of the CD38/TE-1146 complex, cleavage by lysosomal cathepsin B, releasing cytotoxic lenalidomide, and (ii) Fc-mediated immune effector functions (complement-dependent cytotoxicity, CDC; antibody-dependent cellular phagocytosis, ADCP; and antibody-dependent cell-mediated cytotoxicity, ADCC). MP denotes macrophage and NK, natural killer cells.

Supplementary Table S1. Summary of patient cohort characteristics

Parameter	NDMM	RRMM1	RRMM2
# of patients[a]	5	5	5
Median age at diagnosis (range)	64 (45-90)	65 (42-77)	63 (54-63)
Gender (Male/Female)	(2/3)	(4/1)	(1/4)
Prior Therapy[b] (mAb, PI, IMiD, Others)	(0, 0, 0, 0)	(0, 4, 4, 2)	(4, 5, 5, 3)
ISS stage (I, II, III)[c]	(0, 3, 2)	(2, 2, 1)	(0, 2, 3)
M-Protein Subtype (IgA, IgG, IgM, LCD)	(2, 2, 0, 1)	(1, 1, 2, 1)	(0, 5, 0, 0)
MNCs[d] ($\times 10^6$, mean ± SD)	3.4 ± 3.1	4.1 ± 2.1	16.0 ± 13.2
$CD138^+$ cells[e] ($\times 10^6$, mean ± SD)	1.8 ± 2.6	2.5 ± 1.4	2.1 ± 0.9
$CD38^+$ cells[f] (mean ± SD)	2324 ± 991	4445 ± 542	5045 ± 4730
CD38 MFI[g] mean ± SD (range)	2853±1236 (976–4159)	3610±1256 (2351–5347)	3896±3398 (1472–9544)
CD56 MFI[g] mean ± SD (range)	2303±3914 (280–9298)	926±364 (462–1324)	6291±6986 (709–18403)
CD138 MFI[g] mean ± SD (range)	2923±5705 (81–13108)	2214±682 (1330–2971)	8419±5991 (3893–18330)

[a]15 MM patients were recruited between September 2023 and May 2024 after obtaining informed consent and approval by the Research Ethics Review Committee (IRB No. 112088-F) of Far Eastern Memorial Hospital, New Taipei City, Taiwan. However, only 14 MM patients were analyzed as 1 RRMM1 patient was $CD138^-$. [b]mAb: monoclonal antibodies, PI: proteasome inhibitors, IMiD: immunomodulatory drugs. [c]ISS: International Staging System classifying MM patients into 3 stages, with stage I denoting the best prognosis and stage III the worst, which was present in all three patient groups. [d]The mean number of mononuclear cells (MNCs) isolated via density gradient centrifugation using Ficoll-Paque 1.077 (GE Healthcare, Munich, Germany) from bone marrow aspirates (8–10 mL) obtained from each patient in the group. The mean counts of MNC cells did not differ significantly among the groups ($p > 0.05$), but RRMM2 patients showed a wider range of MNC counts. [e]The mean number of $CD138^+$ cells isolated from freshly

obtained MNCs using magnetic-activated cell sorting (Miltenyi Biotec), except for one $CD138^-$ RRMM1 patient. [f]The mean number of $CD38^+$ cells among total MNCs in MM patients, evaluated by flow cytometry using homemade anti-CD38-APC. RRMM2 patients showed significant variability with one patient exhibiting lower $CD38^+$ cell percentage (2%), and two patients higher proportions (21 and 54%), compared to the $CD38^+$ cell percentage in NDMM (5–13%) or RRMM1 (5–17%) patients. [g]MFI: mean fluorescence intensity of CD38, CD56, or CD138 marker on $CD138^+$ cells in patient samples prior to drug exposure, characterized by flow cytometry using fluorophore-conjugated antibodies: anti-CD38-APC (homemade), anti-CD138-PE (Biolegend), and anti-CD56-SB436 (eBioscience). Flow cytometry was conducted using an LSR Fortessa™ Cell Analyzer (BD Biosciences, San Jose, CA). Immunophenotypic data were analyzed using FlowJo v10 software (FlowJo LLC).