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CD56 expression modulates NAD⁺ metabolic landscape and predicts sensitivity to anti-CD38 therapies in multiple myeloma

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Dear Editor,

In multiple myeloma (MM), monoclonal antibodies targeting CD38, such as Daratumumab (Dara) and Isatuximab (Isa), are standard-of-care drugs that produce good responses. However, it is likely that some patients will become resistant even to these innovative therapies. Recently, Robinette et al. [1] reported that CD56 levels on bone marrow plasma cells (BMPCs) predict the efficacy of Dara-based regimens, especially when combined with immunomodulatory drugs [2]. CD56, also known as NCAM1 (Neural Cell Adhesion Molecule 1), facilitates cell-cell adhesion and is deregulated in many tumors, including MM [3].

We found that low CD56 expression was significantly correlated with extramedullary disease ($p = 0.016$), while higher CD56 levels were associated with improved clinical outcomes ($p = 0.003$), as assessed by flow cytometry in 287 Newly Diagnosed MM (NDMM) patients diagnosed at our clinic after 2019 (Fig. S1A, B). Consistent with Robinette et al. [1], there was a positive correlation between CD56 and CD38 expression levels in our cohort and in the CoMMpass dataset (Fig. S1C, D). Importantly, we confirmed that CD56 expression predicts the efficacy of Dara-based therapies: higher CD56 levels on bone marrow plasma cells were associated with better overall response rates and deeper responses (\geq PR) following Dara monotherapy in relapsed/refractory multiple myeloma (RRMM) patients (Fig. S1E).

Dara kills MM cells through multiple mechanisms, including antibody-dependent cellular cytotoxicity (ADCC), which relies on immune cells to kill the antibody-coated tumor cells. Given the correlation between the expression of CD56 and CD38, we hypothesized that increased CD56 expression would lead to more efficient killing by anti-CD38 monoclonal antibodies. We first screened a panel of human myeloma cell lines (HMCLs) and categorized them as CD56-low or -high (Fig. S2A). Then we performed standard 3-hour ADCC assays using these cell lines as targets and natural killer (NK) cells from healthy donors as effector cells. Myeloma cells were stained with calcein-AM and co-cultured with NK cells at the indicated effector: target (E:T) ratios in the presence or absence of 1 μ g/mL Dara. We found that MM cell lines with higher surface expression of CD56 were, in general, more sensitive to Dara-mediated NK cell killing compared to MM cell lines with significantly lower surface expression of CD56 (Fig. S2B). Overall, our data confirm recent evidence on CD56 level as an important biomarker for sensitivity to Dara-based therapies [1, 4].

CD38 consumes NAD⁺ as a substrate to produce ADP-ribose and cyclic ADP-ribose, which are important for calcium signaling within cells [5]. Given that CD56 upregulates CD38 expression [1], we evaluated the impact of CD56 levels on NAD⁺ metabolism. We ectopically overexpressed CD56 (CD56 OE) in different MM cell lines and evaluated intracellular NAD⁺ content and cGDP-ribose

production from the NAD⁺ analog NGD⁺. CD56 OE MM cells produced higher levels of cGDP-ribose and lower levels of NAD⁺ than control cells (Figs. 1A and S3). Concordantly, this impaired NAD⁺-dependent enzymes, including SIRTuins, as suggested by increased H3K9 acetylation (Figs. 1B, S3 and S4); opposite effects were observed in CD56 knockdown (KD) cells (Fig. 1C and S4).

NAD⁺ supports processes like DNA repair and the oxidative stress response, which are essential for cell survival. NAD⁺ can be produced by the enzyme nicotinamide phosphoribosyltransferase (NAMPT), and inhibitors for this enzyme, such as FK866, have shown therapeutic promise. We recently found that CD38 surface levels affected MM cells' sensitivity to a wide range of NAMPT inhibitors; [6] accordingly, CD56-overexpressing MM cells were more vulnerable to these small molecules than control cells (Fig. 1D), whereas CD56 silencing did not modulate the response to these agents in two different cell lines (Fig. S5). To confirm that CD56 influences NAD⁺ consumption in a CD38-dependent manner, we modified CD56 expression in CD38-KD cells. As shown in Fig. 1E–G, CD56 overexpression led to mild NAD⁺ depletion, while silencing CD56 increased intracellular NAD⁺ levels, consistent with reduced CD38 enzymatic activity.

Focusing on translational applicability, we analyzed FK866-induced transcriptome changes described in GSE96636 [7] to assess MM patients included in the CoMMpass dataset. Enrichment analysis identified a group of patients exhibiting an FK866-specific signature (denoted as FK866-upregulated and FK866-downregulated, respectively), mirroring the transcriptional profiles of FK866-treated cells. These patients were designated as sensitive, while those with different gene expression patterns were classified separately (Fig. 2A), as previously reported [6, 8]. Clinical analyses revealed that FK866-sensitive patients tended to overexpress CD56 ($N = 175$ vs. 118; $p = 0.001$), and among these patients, those who overexpressed CD56 demonstrated improved median progression-free survival (mPFS, 1176 vs. 776 days, $p = 0.024$). (Fig. 2B, C) Collectively, these data support the clinical benefits of lower NAD⁺ in MM patients with higher levels of CD56. Specific genetic abnormalities confer poor prognosis and shorter response to currently available anti-MM therapies [9]. Notably, in the CoMMpass dataset, a greater number of CD56-overexpressing patients was observed in every screened chromosomal abnormality; t(4;14) emerged as the most enriched abnormality among CD56-overexpressing MM patients (87% high vs 13% low), while other categories were almost unaffected by CD56 level status (Fig. 2D). Consistent with these findings, we analyzed HMCLs included in the Genomics of Drug Sensitivity in Cancer (GDSC1) database, comparing FK866 IC50 values in t(4;14)-positive versus t(4;14)-negative cells. While there was a trend toward enhanced drug sensitivity in t(4;14)-positive cells, the differences were not statistically significant, likely due to the limited number of tested cell lines (4 vs. 9, respectively); similar results were observed in our panel of MM cell lines (Fig. S6). Importantly, Isa-based approaches described in the IKEMA and ICARIA clinical trials have shown

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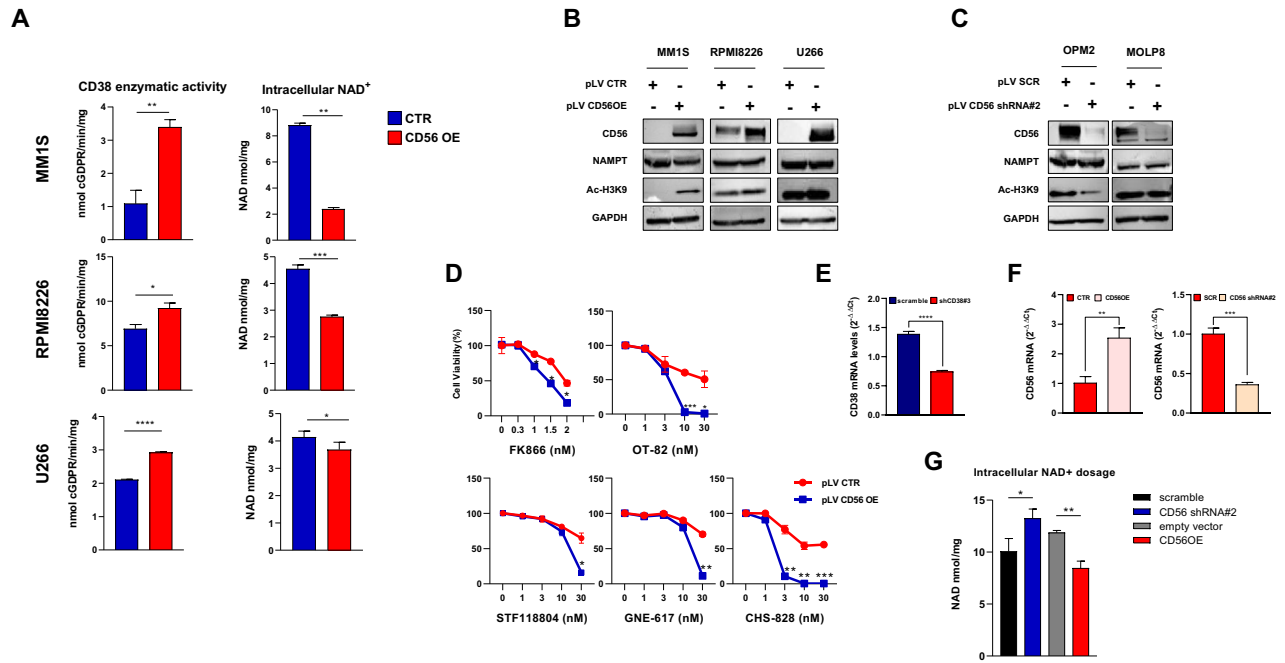


Fig. 1 CD56 levels on bone marrow plasma cells enhance anti-MM activity of NAD⁺-depleting agents by affecting CD38 enzymatic activity. **A** MM1S, RPMI 8226, and U266 cells infected with CD56 expression lentiviral plasmid (CD56 OE), or empty vector, were assayed for their CD38 enzymatic activity (GDP-ribosyl cyclase) and intracellular NAD⁺ content. Data are presented as mean \pm S.D. ($*p < 0.05$, $**p < 0.01$, $***p = 0.0003$; $****p < 0.0001$; unpaired *t* test). **B, C** Cell lysates from control and CD56 OE (**B**) and KD (**C**) MM cells were subjected to WB analysis using the indicated Abs and GAPDH as loading control. One representative blot of at least two independent experiments performed is shown. **D** MM1S cells ectopically overexpressing CD56 (pLV CD56 OE) or empty vector (pLV CTR) were treated with increasing doses of FK866 (0–2 nM), OT-82 (0–30 nM), STF118804 (0–30 nM), GNE-617 (0–30 nM) and CHS-828 (0–30 nM) for 96 h. Cell viability was measured with MTS assay and presented as a percentage of cell viability from untreated cells. Data are presented as mean \pm S.D ($n = 3$) ($*0.05 < p \leq 0.005$, $**0.005 < p \leq 0.001$, $***0.0009 \leq p \leq 0.0007$; unpaired *t* test). **E** MM1S cells infected with a lentiviral plasmid carrying short hairpin RNA targeting CD38 (shRNA#3) or a scramble control were assayed for CD38 mRNA expression. **F** CD38-silenced cells were subsequently infected with lentiviral plasmids either overexpressing (OE) or downregulating (KD) CD56 levels. **G** Finally, intracellular NAD⁺ levels in these cells were measured and expressed as nmol/mg. Data in (**E–G**) are presented as mean \pm SD; $*p = 0.02$; $**0.019 \leq p \leq 0.003$; $***p = 0.0002$; $****p < 0.0001$ (unpaired *t* test).

improved outcomes in high-risk MM, particularly in t(4;14) patients, whereas Dara-based strategies lack sufficient data on this subgroup [10–12]. Overall, our data, while requiring further investigation in larger cohorts, support NAD⁺-related metabolism as a potential actionable vulnerability in these patients and align with evidence suggesting increased metabolic dependencies in more aggressive disease [2, 10].

Among the anti-CD38 therapies, only Isa inhibits CD38 enzymatic activity [13–15]. Whether blocking the NADase activity is beneficial or harmful for immunotherapy efficacy remains uncertain. To explore this further, we assessed the ability of MM cells to generate cGDPR from the NAD⁺ analog NGD⁺, alongside measuring intracellular NAD⁺ levels, following exposure to Dara or Isa. We found that Isa significantly outperforms other anti-huCD38 monoclonal antibodies, including Dara, in inhibiting CD38 cyclase activity. This, in turn, leads to enhanced intracellular NAD⁺ levels, confirming the superior enzymatic inhibitory activity of Isa compared to Dara. (Fig. 2E).

Although CD56 expression positively correlates with CD38 levels, enhancing target availability on the cell surface, the increased CD38 ecto-enzymatic activity observed in CD56-overexpressing cells suggests that Isa-based regimens may provide comparable or even greater benefits than Dara-based approaches. We then assessed Dara- and Isa-mediated killing of CD38-overexpressing MM cells engineered with a lentivirus carrying a short hairpin RNA (shRNA) targeting CD56 or a scramble control.

We first overexpressed CD56 in H929 cells, which naturally express high levels of CD38, and analyzed their metabolic profile.

As shown in Fig. 3A, this genetic modification led to increased cGDPR levels and reduced NAD⁺ content compared to control cells. Next, we confirmed that CD56 surface expression was significantly downregulated in CD38-overexpressing cells transduced with CD56 shRNA compared to the scramble control (Fig. 3B). Finally, we observed that Dara-mediated ADCC against MM cells was consistently higher than that of Isa across all effector-to-target (E:T) ratios tested in CD56-silenced cells (Fig. 3C). These findings indicate that CD38 is the primary mechanism through which CD56 influences these drugs response. Consistent with these findings, we observed in our cohort of relapsed/refractory MM (RRMM) patients ($N = 20$) that combinations involving Isa resulted in extended progression-free survival (PFS) among patients with high CD56 levels compared to those with low CD56 expression (median PFS 369 vs. 129 days; Fig. 3D). Although limited by the small samples size, this analysis suggests a trend that contrasts with a previous report [1].

Overall, this study underscores the importance of routine immunophenotypic analysis of bone marrow plasma cells, thus paving the way for a precision medicine-driven approach. Therefore, an accurate phenotypic analysis of plasma cells would provide clear indications for the most suitable treatment strategy for each patient affected by MM. While these findings require validation in larger clinical trials, they lay the groundwork for adopting metabolism-targeted strategies, including NAD⁺-depleting agents and monoclonal antibodies targeting CD38 enzymatic activity, particularly in MM patients with elevated levels of CD56 on the surface of bone marrow plasma cells.

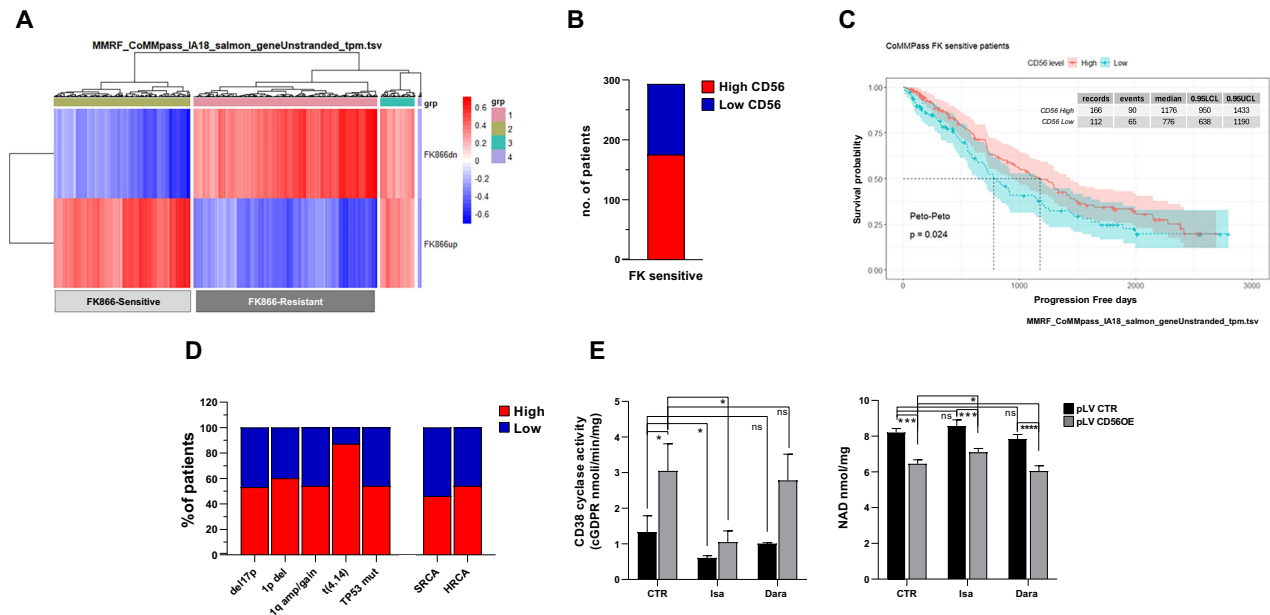


Fig. 2 CD56 levels in MM patients predict outcome following metabolism-targeting agents. **A** Heatmap showing FK866 activity signature expression in MM patients derived from the MMRF_CoMMpass_IA18_salmon_geneUnstranded_tpm.tsv dataset grouped by GSEA method as “FK866 sensitive” (patients with gene expression in accordance with FK866 treatment) and “FK866 resistant” (patients with non-overlapping profiles). **B** Number of MM patients from CoMMpass study exhibiting an FK866-sensitive signature, separated according to CD56 expression levels: high vs. low ($N = 118$ vs. 175 ; p value = 0.001 ; exact binomial test). **C** Kaplan–Meyer curves of the progression-free survival probability for FK866-sensitive patients based on their CD56 expression level ($p = 0.024$; Peto-Peto test). **D** Bar graph showing the percentage of indicated chromosomal abnormalities in MM patients included in MMRF CoMMpass, according to their CD56 expression levels (high vs. low). SRCA indicates standard risk, and HRCA indicates patients with ≥ 2 CA. **E** CD38 enzymatic activity (GDP-ribosyl cyclase) and intracellular NAD⁺ content in MM1S CD56-overexpressing cells compared to control cells at baseline and after treatment with indicated $1 \mu\text{g}/\text{mL}$ of anti-CD38 MoAbs for 12 h. Data are presented as mean \pm S.D. ($n = 3$). (ns not significant, $*p < 0.05$, $***p < 0.001$, $****p < 0.0001$; unpaired t tes).

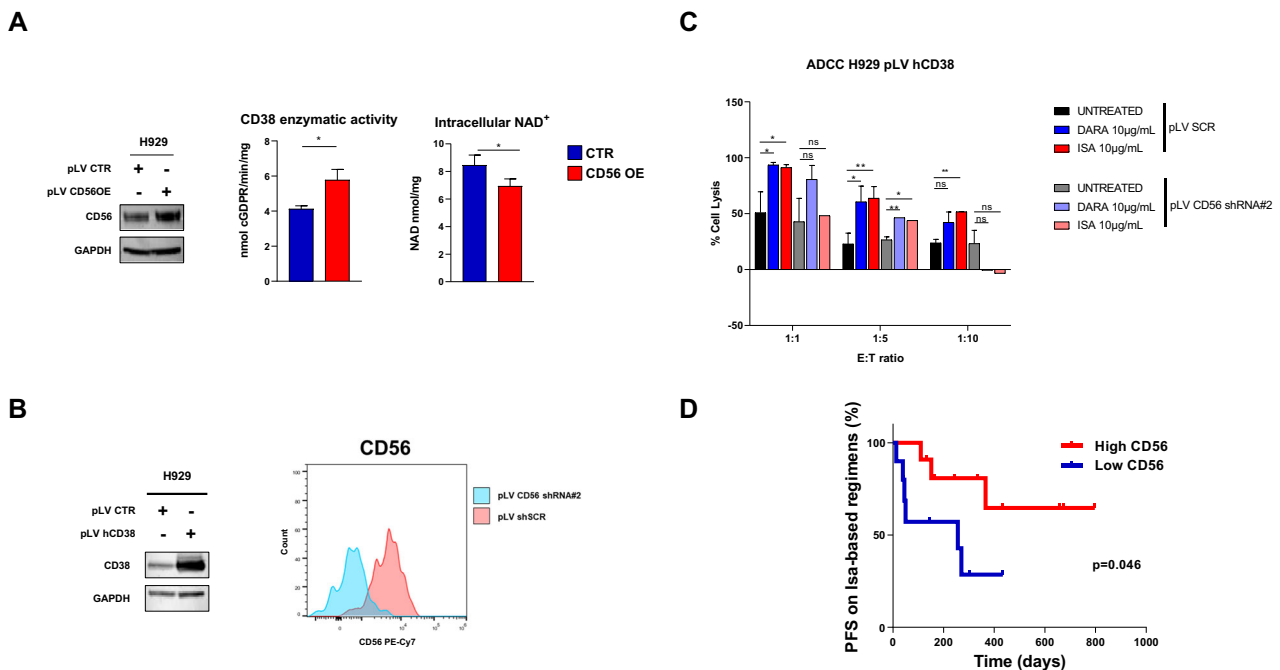


Fig. 3 CD56 modulation in CD38 high-expressing cells modulates ADCC response to anti-CD38 Monoclonal Antibodies. **A** H929 cells infected with CD56 expression lentiviral plasmid (CD56 OE) or empty vector. On the left, western blot analysis (WB) shows CD56 expression in H929 control cells (pLV CTR) and CD56 OE cells (pLV CD56 OE); GAPDH was used as loading control. On the right, cells were assayed for their CD38 enzymatic activity (GDP-ribosyl cyclase) and intracellular NAD⁺ content. Data are presented as mean \pm S.D ($n = 3$) ($*p < 0.05$; unpaired t test). **B** On the left, western blot analysis (WB) shows CD38 expression in H929 control cells (pLV CTR) and CD38 high-expression cells (pLV hCD38); GAPDH was used as loading control. On the right, surface expression level of CD56 in H929 cells overexpressing CD38 (pLV hCD38) transduced with CD56 knockdown (CD56 pLV shRNA#2) or scramble control (pLV SCR). **C** ADCC assays were conducted using H929 CD38OE cells transduced with scramble control (pLV SCR) or shRNA targeting CD56 (pLV CD56 shRNA#2). Effector-to-Target (E:T) ratios were evaluated using NK cells from healthy donors, with treatment conditions including Daratumumab (DARA, $10 \mu\text{g}/\text{mL}$) and Isatuximab (ISA, $10 \mu\text{g}/\text{mL}$). **D** Progression-free survival (PFS) from the first day of Isa-based therapies (11 Isa-PD and 9 Isa-KD) in our cohort of RRMM patients exhibiting high (red) or low (blue) levels of CD56 on their bone marrow plasma cells. Log rank test; $p = 0.046$.

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AUTHOR CONTRIBUTIONS

GG, FM, and CM designed the research and wrote the manuscript; MME and FD performed flow cytometry analysis; SD, BP, TI, LF, UCRM, SA, FNJ, performed the experiments; TD and LF performed the statistical and bioinformatic analyses; BA, RS, and BS performed biochemistry analysis; NA provided NAMPT inhibitors; RG, CA, GF, and NA provided patient samples; MNC, LRM, FM, and CM revised the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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