LETTER TO THE EDITOR

HDAC6 inhibitors sensitize resistant t(11;14) multiple myeloma cells to a combination of bortezomib and BH3 mimetics

Authors

Cristina Florean,[1] Manon Lernoux,[1] Anne Lorant,[1] Helene Losson,[1] Guy Bormans,[2] Michael Schnekenburger[1] and Marc Diederich[3]

[1]Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Luxembourg, Luxembourg; [2]Laboratory for Radiopharmaceutical Research, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium and [3]Research Institute of Pharmaceutical Sciences & Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul, Republic of Korea

Correspondence:
M. DIEDERICH - marcdiederich@snu.ac.kr
M. SCHNEKENBURGER - michael.schnekenburger@lbmcc.lu

https://doi.org/10.3324/haematol.2024.286279
Received: August 7, 2024.
Accepted: September 30, 2024.
Early view: October 10, 2024.

Supplementary Materials

HDAC6 inhibitors sensitize resistant t(11;14) multiple myeloma cells to a combination of bortezomib and BH3 mimetics

Cristina Florean[1], Manon Lernoux[1], Anne Lorant[1], Helene Losson[1], Guy Bormans[2], Michael Schnekenburger[1,*], Marc Diederich[3,*]

Supplementary Figure Legends

Supplementary Figure 1. MAKV-15 is Effective in a Panel of multiple myeloma (MM) Cell Lines Without Correlation with histone deacetylase (HDAC)6 Expression Levels. A) Differential gene expression and pathway enrichment analysis in high vs low HDAC6 score patients. The left panel shows a volcano plot of differentially expressed genes between the high and low tertiles of HDAC6 activity scores in MM patients. Genes with a |log2 fold change > 0.3 and a false discovery rate (FDR) < 0.05 were considered significantly different. The middle panel depicts enriched hallmark pathways associated with high HDAC6 scores. The left panel depicts pathways enriched for genes negatively influencing MM cell line proliferation from whole genome CRISPR screening. **B)** Western blot analyses for ac-α-tubulin and ac-H4 in a panel of MM cell lines treated with 5 μM MAKV-15 for up to 48h. β-actin and Histone 1 (H1) were used as loading controls. Suberoylanilide hydroxamic acid (SAHA; 2 μM) was used as a reference pan-HDACi. **C)** Western blot analyses for acetyl (ac)-α-tubulin and ac-Histone 4 (H4) in U-266 cells treated for 24h with increasing concentrations of MAKV-15, tubastatin A, or ACY-1215. **D)** Kinetic western blot analysis of ac-α-tubulin and ac-H4 in MOLP-8 cells treated for up to 72h with MAKV-15 or tubastatin A. **E)** Western blot analysis of HDAC6 expression levels in the panel of MM cell lines used. Immunoblots are representative of three independent experiments. Abbreviations in the figure: OXPHOS (oxidative phosphorylation).

Supplementary Figure 2. Effect of MAKV-15, Tubastatin A, and ACY-1215 on MM Cell Viability. A) The viability of MOLP-8 and U-266 cells was assessed by nuclear morphology analysis upon treatment with the indicated concentrations of MAKV-15, tubastatin A, and ACY-

1215 for up to 72h. **B)** Non-proliferative and proliferative peripheral blood mononuclear cells (PBMCs) were treated with the drugs alone or in combinations as described in the figures. After the indicated incubation time, cell viability was assessed using a Trypan blue-exclusion analysis. PBMCs and P_PBMCs were used with the approval of the National Research Ethics Committee of Luxembourg. PBMCs were isolated from blood obtained from the Red Cross (Luxembourg, Luxembourg) under the authorization LBMCC-2019-0002: "Assessment of toxicity of new drugs or drug combinations in preclinical development in nonproliferating peripheral blood mononuclear cells (systemic acute toxicity)". P_PBMCs were generated from blood obtained from the Red Cross under the authorization LBMCC- 2019-0001: "Assessment of differential toxicity of new drugs or drug combinations in preclinical development in *ex-vivo* proliferating peripheral blood mononuclear cells vs. proliferating cancer cells." **C)** Nuclear morphology analysis of MOLP-8 cells treated with the indicated compounds for 72h. **D)** Western blot analysis of caspase-3, poly (ADP-ribose) polymerase (PARP)-1 cleavage in MOLP-8 treated with the indicated drugs. **E)** Nuclear morphology analysis (upper panel) and Western blot analysis of caspase 3, PARP-1, and Bcl-2 family protein members (lower panel) in KMS-28-BM cells treated with the indicated drugs for 72h. Images are representative of three independent experiments. Graphs correspond to the mean ± SD of three independent experiments. The selected statistical analysis reports the significance of triple treatments compared to all the corresponding double treatments. **F)** FACS analysis of B-cell maturation antigen (BCMA) surface expression levels in MM.1S cells. Cells were co-treated with the gamma-secretase inhibitor RO4929097 and SAHA or MAKV-15 for 24 hours. Graphs represent the mean ± SD of BCMA median fluorescence intensity (MFI) levels from three independent experiments. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. One-way ANOVA with Holm-Šidák's multiple comparison test was used to compare each sample; statistical significance was determined for comparisons with the control sample and indicated pairs of samples.

Supplemental Table 1: Molecular features and key genetic aberrations in multiple myeloma cell lines. [1-3]

Cell Line	Relative HDAC6 Expression	Relative Mcl-1 Expression	Bcl-2 Expression	t(11;14) Translocation	Origins and Characteristics
AMO-1	++	++++	High	unknown	Ascitic fluid of a 64-year-old woman with plasmacytoma (IgAkappa); coexpression of a CD4 antigen and plasma cell antigens (CD38 and PCA-1)
KMS-12-PE	+++	+++	High	+	Pleural effusion of a 64-year-old woman with refractory, terminal multiple myeloma
KMS-34	+++	+++	Low	unknown	Pleural effusion
MM.1S	++	++	High	-	B lymphoblast cell that was isolated from the peripheral blood of a Black, 42-year-old, female patient with immunoglobulin A lambda myeloma
MOLP-8	++	+++	Low	+	Peripheral blood of a 52-year-old man with multiple myeloma (stage IIIA, type IgD lambda)
JJN-3	+	++	High	-	Bone marrow of a 57-year-old woman with plasma cell leukemia (IgA1kappa)
KMS-28-BM	++++	+	High	-	Plasma Cell Myeloma
OPM-2	+++	++	High	-	Peripheral blood of a 56-year-old woman with multiple myeloma (IgG lambda) in leukemic phase (relapse, terminal)
MM.1R	++	++	High	-	B lymphoblast cell line that was isolated in 1990 from the peripheral blood of a 42-year-old, Black female with multiple myeloma who had become resistant to steroid-based therapy
U-266	++	+++	High	+	Peripheral blood of a 53-year-old man with IgE-secreting myeloma (refractory, terminal)

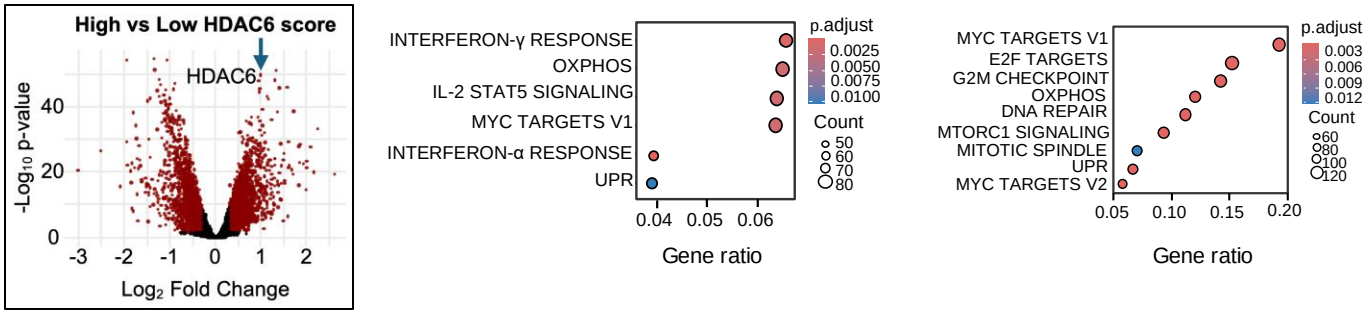
Supplementary References

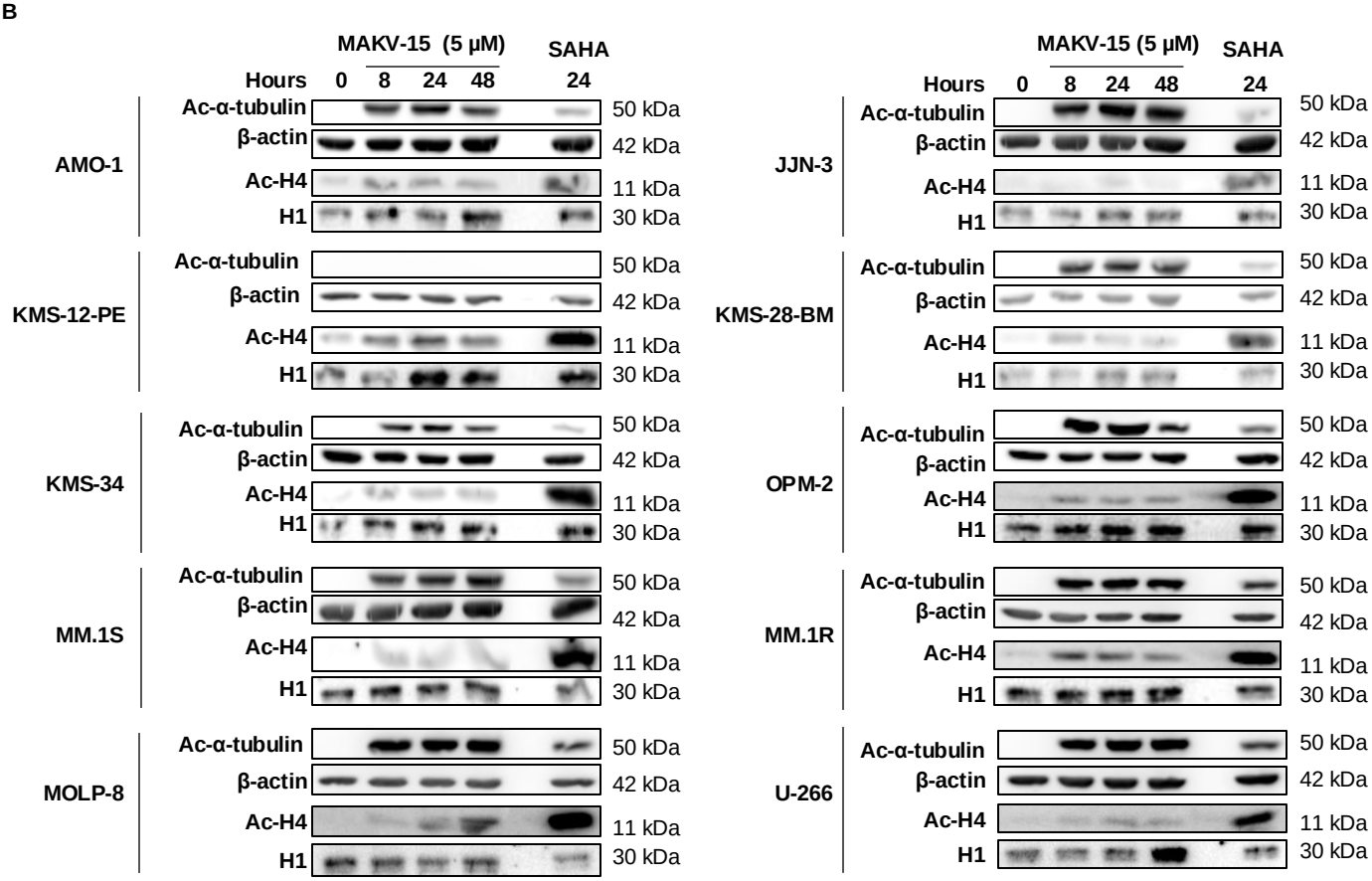
1. Punnoose EA, Leverson JD, Peale F, et al. Expression Profile of BCL-2, BCL-XL, and MCL-1 Predicts Pharmacological Response to the BCL-2 Selective Antagonist Venetoclax in Multiple Myeloma Models. Mol Cancer Ther. 2016;15(5):1132-1144.

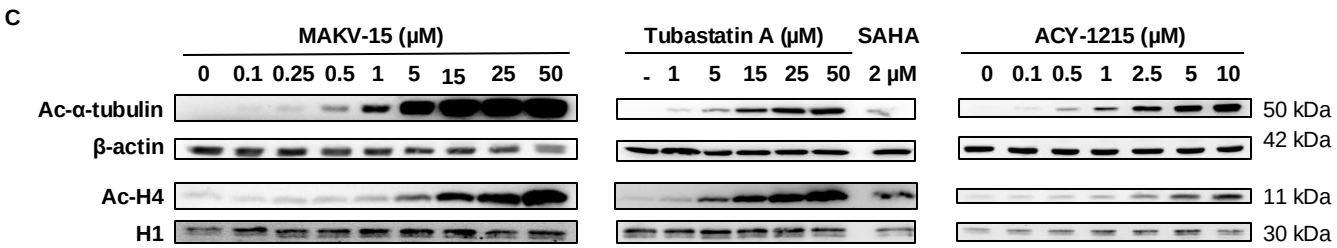
2. Trudel S, Li ZH, Rauw J, Tiedemann RE, Wen XY, Stewart AK. Preclinical studies of the pan-Bcl inhibitor obatoclax (GX015-070) in multiple myeloma. Blood. 2007;109(12):5430-5438.

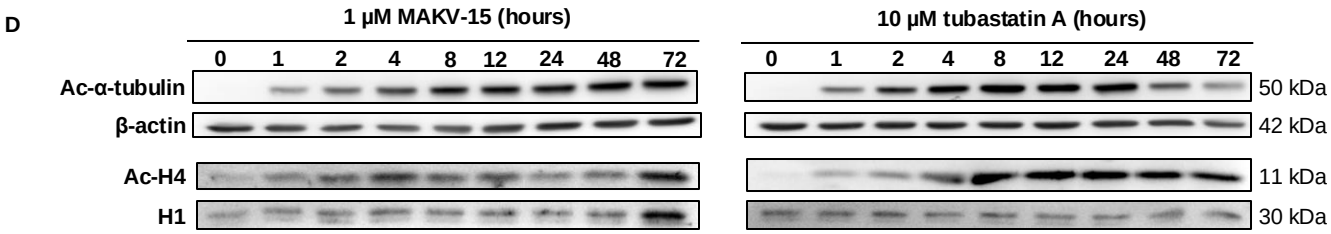
3. Jernberg-Wiklund H. Multiple Myeloma Cell Lines. Human Cell Culture, 2006:81-155.

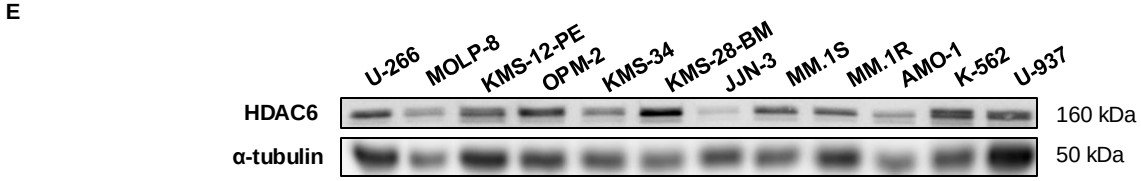
Supplementary Figure 1











Supplementary Figure 2