Journal name: Targeted Oncology

Article title: Evaluation of the Therapeutic Potential of the Novel Isotype Specific HDAC Inhibitor 4SC-202 in Urothelial Carcinoma Cell Lines

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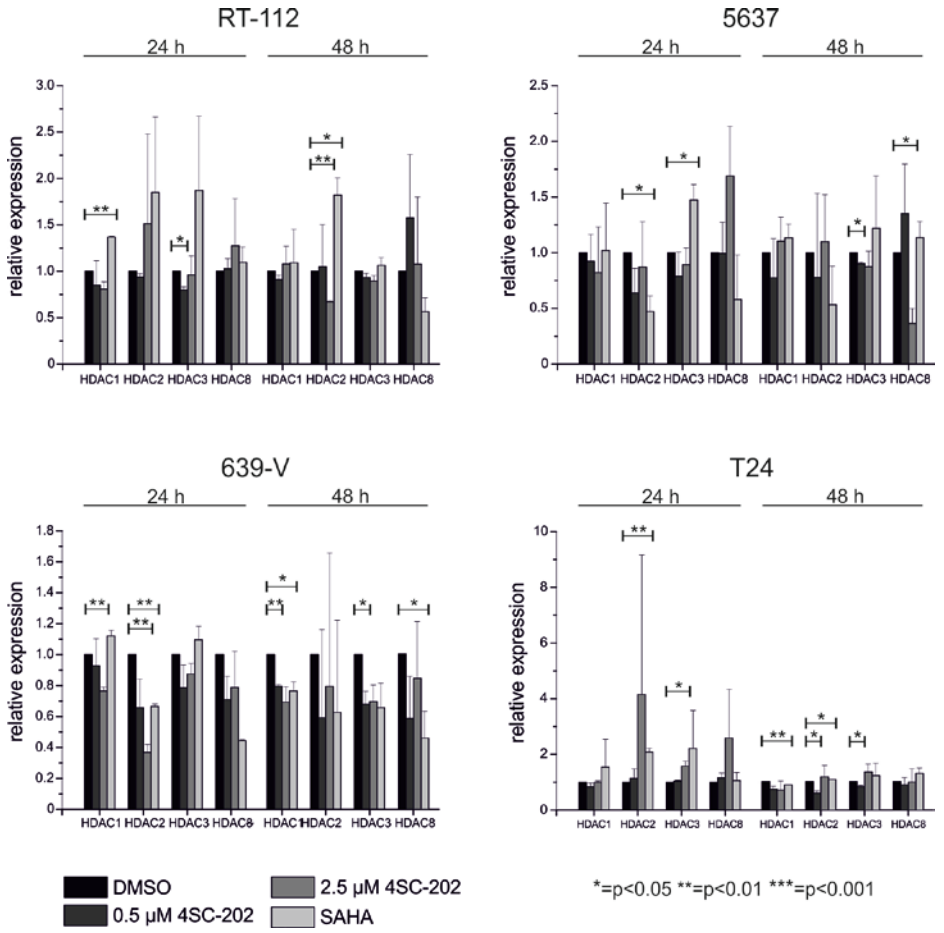
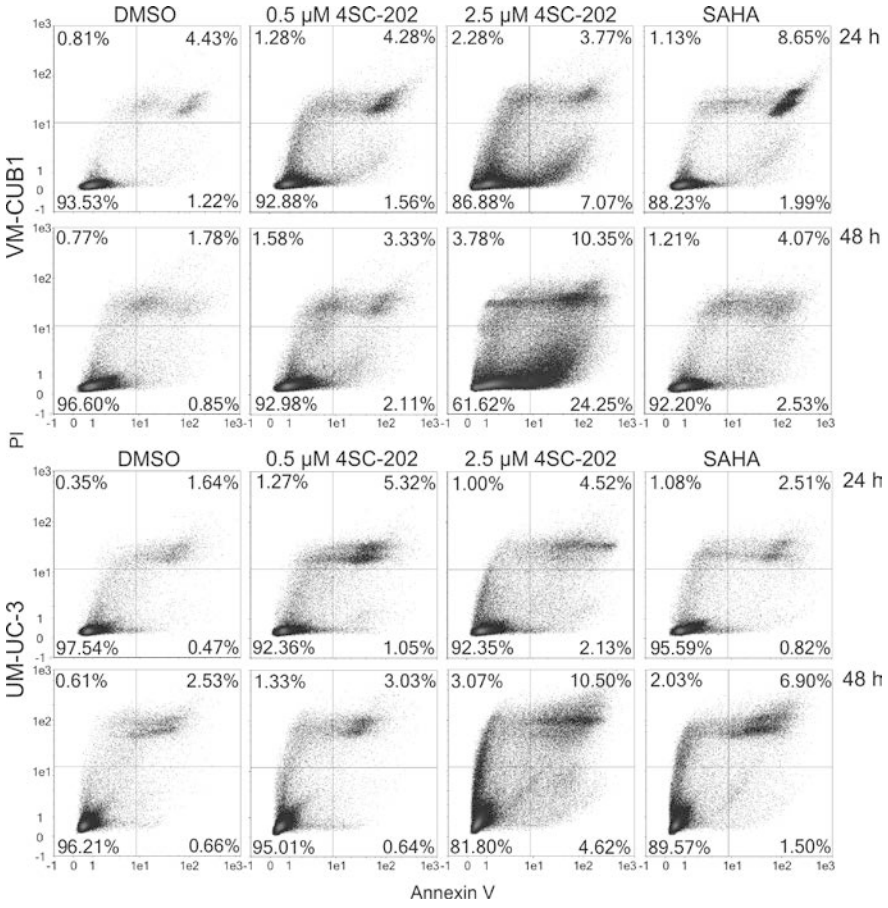
**Supplementary Fig S1. Impact of 4SC-202 treatment on class I HDAC expression in epithelial and mesenchymal UC cells.**

Effects of 4SC-202 and SAHA treatment (24/48 h) on HDAC1, HDAC2, HDAC3 and HDAC8 mRNA expression were measured via quantitative real-time PCR in the two epithelial-like UCCs RT-112 and 5637 and in the two mesenchymal-like UCCs 639-V and T24. The calculated significances refer to the DMSO solvent control.



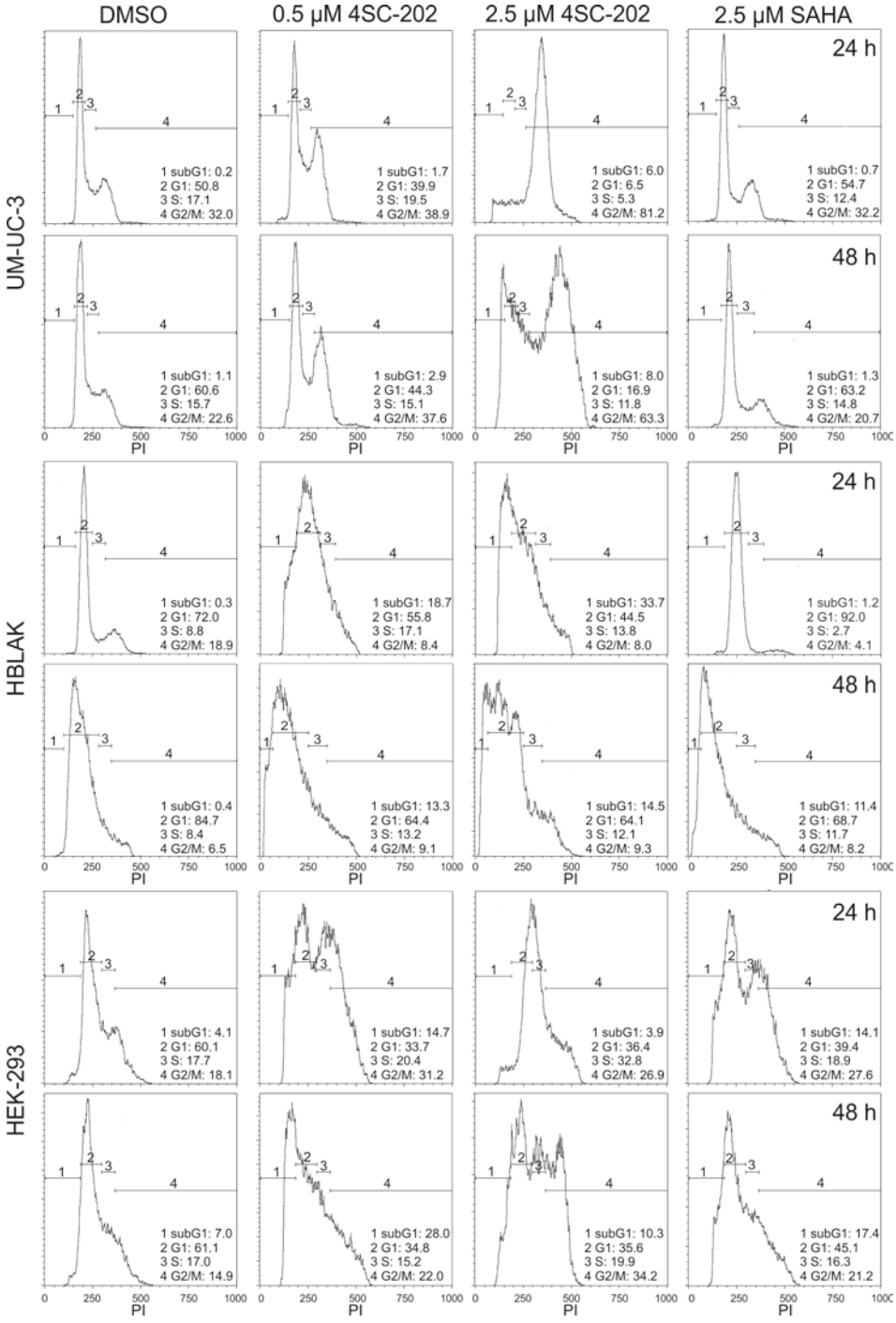
**Supplementary Fig S2. Induction of necrosis and apoptosis in the UCCs VM-CUB1 and UM-UC-3.**

Combined Annexin V- and PI-staining was performed in the UCCs VM-CUB1 and UM-UC-3 after treatment with two different concentrations of 4SC-202 (0.5/2.5 µM) or SAHA for 24 and 48 h. Representative scatter plot diagrams are shown. The figure quantify viable cells (lower left), necrotic cells (upper left), early apoptotic cells (lower right) and late apoptotic/necrotic cells (upper right).



**Supplementary Fig S3. 4SC-202 induced changes in cell cycle distribution in UM-UC-3, HBLAK and HEK-293 cells.**

Similar to figure 5A, 4SC-202 (0.5/2.5 µM) or 2.5 µM SAHA treated UM-UC-3, HBLAK and HEK-293 cells were measured by cell cycle analysis using flow cytometry in comparison to DMSO treatment (24/48 h).



**Supplementary Fig S4. LSD1 and HDAC1/2 inhibitor combination therapy vs. 4SC-202 treatment in UM-UC-3 cells.**

Similar to main figure 8 different parameters were measured in UM-UC-3 cells following treatment with LSD1i/HDACi combinations (3 nM romidepsin, 0.5 µM SP2509, 3 nM romidepsin + 0.5 µM SP2509) in comparison to two different concentrations of 4SC-202 (0.5 and 2.5 µM). Changes in viability (CellTiter-Glo®, A), clonogenicity (Giemsa-staining of grown colonies, B), cell cycle distribution and amount of apoptotic cells (as sub G1fraction; cell cycle analysis using flow cytometry, C) and morphology (D) are shown after 24 and 48 h treatment. Phase-contrast images are shown with a 20 x magnification.