Synergistic interaction between HDAC and MCL-1 inhibitors through downregulation of BCL-X1 in multiple myeloma

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Supplement figure 1.
Concurrent MCL-1 and HDAC inhibition synergistically kills MM cells *in vitro.*
Cell viability of indicated human MM cell lines 96h after treatment with S63845 (A)-(H) and venetoclax (I)-(N) alone or in combination with either panobinostat (A),(C),(E),(G),(I),(K),(M) or ricolinostat (B),(D),(F),(H),(J),(L),(N). Results are presented relative to 0.1% DMSO control. Combination index (CI) values of <0.8, 0.8–1.2, and >1.2 were interpreted as synergistic, additive, and antagonistic drug activity, respectively. (O)-(P) Assessment of cleaved caspase 3 and cleaved PARP was performed via flow cytometry 48 or 72 hours post treatment induction, respectively. (Q)-(R) BAK activation was determined by staining with antibodies against its active form. Graphs show the mean +/- standard deviation of the mean (SDM) of triplicate experiments. Differences between groups were calculated with one-way ANOVA, corrected for multiple comparison with Bonferroni-Holm correction, where **** denotes $P<0.0001$, ** denotes $P<0.001$ and * denotes $P<0.05$

Supplement figure 2.
S63845 in combination with HDACi downregulates BCL-X$_L$ and BCL-X$_L$ overexpression reduces apoptosis by sequestering BIM and BAK. (A-B) Immunoblot analysis of the indicated Bcl-2 family members was performed 24 h post treatment initiation. One representative experiment of three independent biological replicates is shown. (C-E) Cell cycle analysis was performed 24h after treatment initiation with either single-agent or combination treatment of S63845 and HDACi. Differences between groups were calculated with three-way ANOVA, corrected for multiple comparison with Bonferroni-Holm correction. (FG) MM cells transduced with pcW57.1 EGFP (left panel) or pcW57.1 BCL-X$_L$ (right panel) were treated for 24h with 0.5 µg/ml doxycycline to induce protein overexpression and afterwards exposed to the indicated treatments. Apoptotic cells were assessed 24 hours post treatment induction. Results indicate the mean +/- SDM of three independent experiments. Differences between groups were calculated with one-way ANOVA, corrected for multiple comparison with Bonferroni-Holm correction, where **** denotes $P<0.0001$, ** denotes
P<0.001 and * denotes P <0.05 (H–I) Co-immunoprecipitation experiments in U266 cells transduced with either pcW57.1-EGFP (right panels) or pcW57.1-BCL-XL (left panels) were performed after 24h pretreatment with 0.5µg/ml doxycycline to induce protein overexpression and subsequent drug exposure for 24 hours.

Supplement figure 3.
Ricolinostat promotes the activity of S63845 independent of HDAC6 inhibition. (A–D) Co-immunoprecipitation experiments in MM cells transduced with either pcW57.1-EGFP (right panels) or pcW57.1-BCL-XL (left panels) were performed after 24 h pretreatment with 0.5 µg/ml doxycycline to induce protein overexpression and subsequent drug exposure for 24 hours. (E) KMS-12-BM and (F) U266 cells were either treated with panobinostat or ricolinostat for 24h, then whole-cell lysates were blotted for the indicated proteins. (G) Single cell clones of U266 cells harboring a Tet-on miR-E vector expressing either a shRNA targeting Renilla (control) or two different shRNAs targeting HDAC6 were exposed to 0.3 µg/ml doxycycline for 48 h and whole-cell lysates were blotted for the indicated proteins. Western blots are representative for three independent experiments. (H–J) U266 cells were pretreated with doxycycline for 48 h and viability was assessed after an additional 48 h treatment with increasing concentrations of S63845 as indicated in the figure. Results show the mean +/- SD of three independent experiments performed in triplicates.
Supplement figure 1.

A. U266 Combination index - Range
   Panobinostat [nM] 0 250 500 1000 1500 2000 2500 3000 3500 4000
   S63845 [nM] 0 250 500 1000 1500 2000 2500 3000 3500 4000

B. U266 Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

C. KMS-12-BM Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

D. KMS-12-BM Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

E. OPM-2 Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

F. OPM-2 Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

G. NCI-H929 Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

H. NCI-H929 Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

I. KMS-12-BM Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

J. KMS-12-BM Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

K. OPM-2 Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

L. OPM-2 Combination index - Range
   Panobinostat [nM] 0 5 10 20 40 80 160 320 640 1280
   S63845 [nM] 0 5 10 20 40 80 160 320 640 1280

M. RPMI Combination index - Range
   Panobinostat [nM] 0 7 9 10 12 25 50 100 200 500
   S63845 [nM] 0 7 9 10 12 25 50 100 200 500

N. RPMI Combination index - Range
   Panobinostat [nM] 0 7 9 10 12 25 50 100 200 500
   S63845 [nM] 0 7 9 10 12 25 50 100 200 500

O. U266 Combination index - Range
   Panobinostat [nM] 0 250 500 1000 1500 2000 2500 3000 3500 4000
   S63845 [nM] 0 250 500 1000 1500 2000 2500 3000 3500 4000

P. KMS-12-BM Combination index - Range
   Panobinostat [nM] 0 7.5 15 22.5 30 60 90 270 450 675
   S63845 [nM] 0 7.5 15 22.5 30 60 90 270 450 675

Q. U266 Combination index - Range
   Panobinostat [nM] 0 250 500 1000 1500 2000 2500 3000 3500 4000
   S63845 [nM] 0 250 500 1000 1500 2000 2500 3000 3500 4000

R. KMS-12-BM Combination index - Range
   Panobinostat [nM] 0 7.5 15 22.5 30 60 90 270 450 675
   S63845 [nM] 0 7.5 15 22.5 30 60 90 270 450 675

Supplement figure 1.
