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Synergistic Antitumor Effect of Selinexor, a Selective Inhibitor of Nuclear Export (SINE) Compound and Panobinostat in a Mouse Model of Multiple Myeloma

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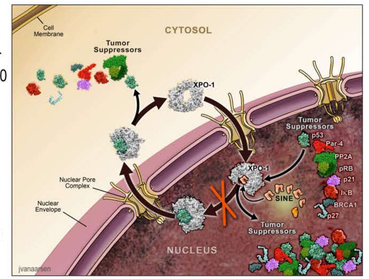
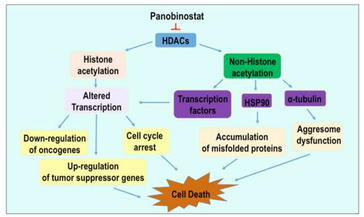
ABSTRACT

Introduction: Selinexor is an oral, first-in-class SINE compound that binds to the primary nuclear exporter XPO1/CRM1. XPO1 exports over 200 cargos, including major tumor suppressor proteins (TSPs) leading to their inactivation.
Inhibition of XPO1 results in nuclear retention of TSPs and restores their normal functions. XPO1 also mediates the export of key signaling molecules in multiple myeloma (MM) including c-MYC mRNA and NF-κB. Selinexor is currently being investigated in phase 2 clinical trials in MM in combination with dexamethasone, pomalidomide, lenalidomide, bortezomib and Carfilzomib.



In preclinical studies selinexor has been shown to synergize broadly with these anti-MM drugs making it an excellent candidate partner for combination therapies in MM. To identify additional synergistic pairings, we investigated the use of selinexor in combination with panobinostat, a pan-histone deacetylase inhibitor (HDAC) recently approved by the FDA in combination with bortezomib for 3rd line treatment of MM, as a potential treatment for MM both *in-vitro* and *in-vivo*.



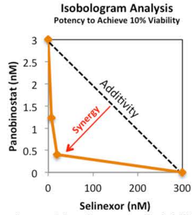
Methods: The effects of selinexor and panobinostat alone or in combination on cell viability were tested on MM1.S cells using MTT assays. Total RNA and whole protein cell lysates were extracted and analyzed by qPCR and by immunoblots. *In-vivo*, MM.1S cells were used to derive a xenograft mouse model. Mice were treated with sub-therapeutic doses of selinexor and panobinostat alone or in combination and with the therapeutic dose of selinexor. Tumor growth was monitored for 17 days and % Tumor Growth Inhibition (%TGI) was determined. Xenografts were harvested and analyzed microscopically and by immunohistochemestry (IHC).

Results: Selinexor-panobinostat combination was highly effective both *in-vitro* and *in-vivo*. In MTT assays, combination treatment showed synergistic effect on MM1S cells viability. Gene expression and western blot analyses showed that the combination treatment leads to a synergistic reduction in c-MYC mRNA and protein levels. In addition, an overall reduction in nuclear expression of the anti-apoptotic signaling molecule NF-κB and the proliferation marker KI67 and an increase of expression of pro-apoptotic molecules including cleaved caspase 3, P21 and PUMA were observed. Importantly, *in-vivo*, while %TGI of sub-therapeutic doses of selinexor and panobinostat measured 58% and 52% respectively, the combination showed a synergistic effect, measuring 93%. Importantly, this high %TGI exceeded the effect of the therapeutic dose of selinexor alone 79%.

RESULTS

Selinexor-Panobinostat Combination Treatment Shows Synergistic Effect on MM1S Cells Viability

MM.1S	Viability (%) Panobinostat (nM)						
Selinexor (uM)	100	33	11	3.7	1.2	0.4	0
10	3	2	3	2	4	4	5
3.3	2	1	1	1	1	2	4
1.1	2	0	1	0	1	1	6
0.37	2	1	1	1	1	1	9
0.12	2	1	1	1	1	2	18
0.04	2	1	1	0	4	7	36
0.01	2	1	1	1	9	16	48
0.005	2	1	2	1	13	23	75
0	3	1	2	6	25	39	100

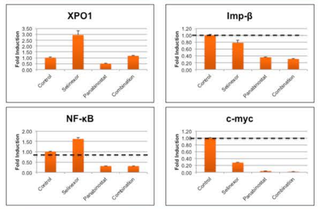


The effects of selinexor and panobinostat alone or in combination on cell viability were tested on MM.1S cell line using the MTT assay.

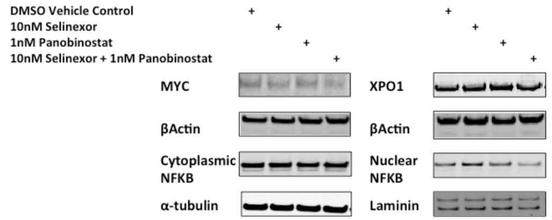
Combination Treatment Results in Synergistic/ Additive Effect on c-MYC and Overall Reduction in XPO1, NFκB and IMP-β mRNA Expression

MM1.S cells were treated with vehicle, 200nM selinexor, 50nM panobinostat or the combination of both. Gene expression was analyzed by Taqman qPCR

Synergistic reduction in c-myc mRNA. XPO1, IMP-β and NFκB mRNA expression reduced

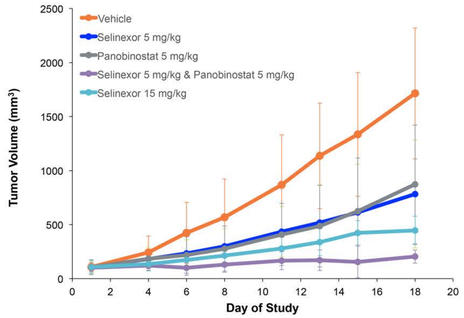


Reduction in XPO1, MYC and Nuclear NFκB Proteins in Combination Treated MM1S Cells



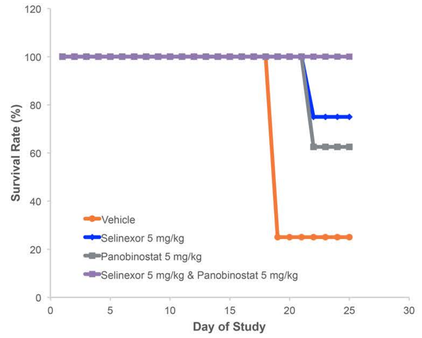
The effects of selinexor and panobinostat alone or in combination on XPO1, MYC and NFκB proteins level and localization in MM1.S were determined. Total protein cell lysates, nuclear and cytoplasmic fractions were extracted and analyzed by Western blot analysis.

Selinexor-Panobinostat Combination Treatment is Synergistic in MM1S–Derived Mouse Xenografts

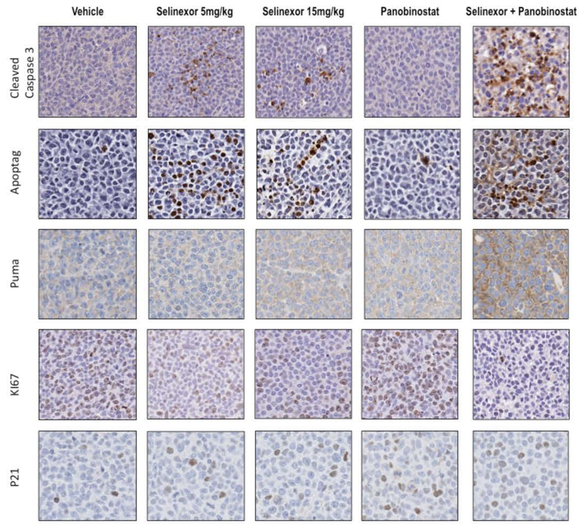


Treatment	TGI%
KPT-330 5 mg/kg MWF	58.0
Panobinostat 5 mg/kg QDx5	52.5
KPT-330 15 mg/kg MWF	78.9
KPT-330 5 mg/kg MWF & Panobinostat 5 mg/kg QDx5	94.0

Selinexor-Panobinostat Combination Treatment Prolongs Survival in MM1S-Derived Mouse Xenografts



Reduced Proliferation and Increased Apoptosis in Combination Treated MM1S-derived Mouse Xenografts



DISCUSSION AND CONCLUSIONS

The NFκB and c-MYC transcription factors play key roles in the survival and proliferation of MM cells. NFκB is activated by classical and/or alternative pathways and c-MYC transcriptional deregulation is achieved via at least three different mechanisms including translocation, point mutations and amplification. Therefore, simultaneous inhibition of NFκB and c-MYC, ideally with a combination of drugs that will target multiple activation mechanisms of these transcription factors, is an attractive therapeutic approach for MM. Selinexor and panobinostat are potent drugs with unique mechanisms of action, affecting multiple cellular processes. Here we show that the combination of these drugs results in synergistic reduction in MM cell viability, decrease in c-MYC mRNA and protein levels and an overall decrease in NFκB mRNA and nuclear protein levels. Interestingly, while the immediate cellular response to selinexor is induction of XPO1 expression, panobinostat treatment leads to inhibition of XPO1 expression. This result may be the basis for the synergistic effect observed when combining selinexor with panobinostat in-vitro and in-vivo and suggests a potential way to overcome possible development of mechanisms of resistance to selinexor.

Conclusion: Selinexor-panobinostat combination synergizes to induce apoptosis in MM cells and amplifies anti-tumor effect in a MM xenograft model. These data provide rational support for study of selinexor/ panobinostat combination in clinical trials.

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