Selinexor, a Selective Inhibitor of Nuclear Export (SINE), Acts Through NF-κB Deactivation and Combines with Proteasome Inhibitors to Synergistically Induce Tumor Cell Death.

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Abstract

Background: SINE compounds are a family of small-molecule bioavailable drugs that bind covalently to Exportin 1 (XPO1/CRM1) and inhibit nuclear export. This results in nuclear retention of major tumor suppressor proteins (TSPs) such as p53, FOXO, pRB and IkB, leading to specific cancer cell death. Selinexor is the clinical SINE compound currently in human phase I/II clinical trials in patients with solid and hematological malignancies. We found that NFκB transcriptional activity was induced in SINE resistant cells and in correlation with that silencing of IκB reduced sensitivity of cells to SINE cytotoxic effects. Therefore, we selected to test whether proteasome inhibitors, which have been shown to suppress NFκB activity, could reverse SINE resistance.

Methods: Whole protein cell lysates from solid and hematological cancer cell lines treated with selinexor with or without proteasome inhibitors were analyzed by immunoblots. IκB localization was evaluated by immunofluorescence. Cytotoxic effects of SINE compounds were evaluated in the presence or absence of proteasome inhibitors. NFκB transcriptional activity was analyzed using an ELISA assay (Thermo Scientific). Multiple Myeloma H929 xenografts in NOD-SCID mice were treated with selinexor (5 mg/kg) and carfilzomib (1.5 mg/kg) alone or in combination and tumor growth was evaluated for 24 days.

Results: The proteasome inhibitors, Bortezomib and Carfilzomib, inhibited NFκB transcriptional activity in SINE resistant cells. This was achieved by blocking the proteasome-mediated degradation of IκB. Combination of proteasome inhibitor and selinexor increased the accumulation of nuclear IκB compared to selinexor by itself. Increased nuclear IκB inhibited NFκB transcriptional activity better than the single agents. Silencing of IκB reduces sensitivity of solid and hematological cancer cell lines to selinexor up to 70 folds. Synergistic cytotoxic effects were seen with the combination of proteasome inhibitors and selinexor in cell lines resistant to selinexor. *In vivo*, selinexor (5 mg/kg) reduced xenograft tumor size of multiple myeloma (H929 cell line model) by 10% whereas carfilzomib by itself (1.5 mg/kg) had no effects on the tumor size. The combination treatment resulted in a 75% reduction in tumor size.

Conclusion: One mechanism of SINE resistance is dependent on increased NFκB transcriptional activity. Combination of proteasome inhibitors and selinexor increases the nuclear levels of IκB, which inhibits NFκB activity and eventually leads to cytotoxicity and decrease in tumor volumes in a synergistic manner. The findings suggest that the combination treatment is predicted to result with synergistic therapeutic outcome in cancer patients.

Nuclear Retention of XPO1 Cargos Including IκB-α is Impaired in SINE Resistant Cells

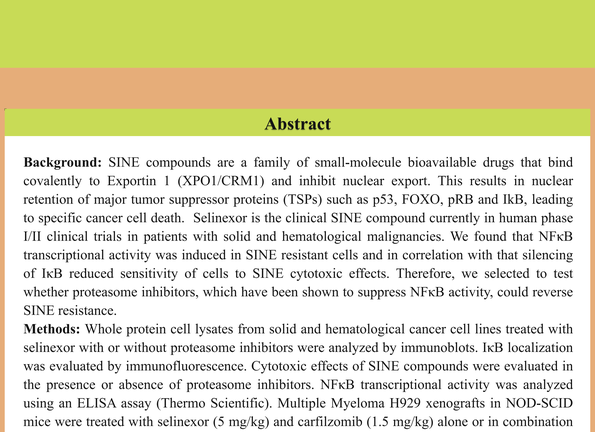


Figure 1: SINE resistant HT1080 (fibrosarcoma) cells were selected by continued exposure of sensitive parental cells in increasing concentration of SINE compound. HT1080 resistant cells, unlike parental cells, proliferate in the presence of SINE compound in the 8-day Clonogenic Assay. Parental and resistant HT1080 cells were treated with 1μM of selinexor for 4 hours. Nuclear retention of key tumor suppressor proteins including IκB-α was tested by immunofluorescence and shown to be ineffective in SINE resistant cells.

Basal NFκB Transcriptional Activity is Up-regulated in Selinexor Resistant Cells

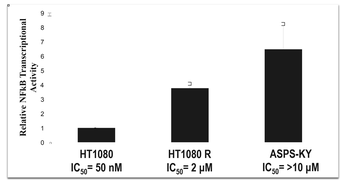
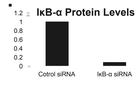


Figure 2: Parental-sensitive HT1080, (Fibrosarcoma), selinexor-resistant HT1080, and ASPS-KY (Alveolar Soft Part Sarcoma) cells were tested for NFκB transcriptional activity by ELISA assay. The results shows that higher NFκB transcriptional activity is correlated with lower sensitivity to the cytotoxic effects of selinexor.

IκB-α Silencing Reduces Selinexor Cytotoxic Effects in Solid and Hematological Cell Lines

U2OS cells	Selinexor IC_{50} (μM)	Fold Change
No transfection	0.027	1
Control siRNA	0.023	0.85
IκB-α siRNA	1.85	65.5



IM-9 cells	Selinexor IC_{50} (nM)	Fold Change
No transfection	87.2	1
Control siRNA	45.5	0.52
IκB-α siRNA	824.7	9.45

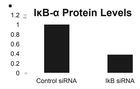


Figure 3: U2OS cells (Osteosarcoma) and IM-9 cells (Multiple Myeloma) were transfected with either 40nM IκB-α or Control siRNA. 24 hours post transfection specific IκB-α protein reduction is shown. Then 96 hours post-transfection, cells were assayed for the cytotoxic effect of selinexor. IκB-α knockdown in U2OS and IM9 cells reduces selinexor toxicity by 65 and 9 folds respectively, whereas Control siRNA showed no effects. Lower transfection efficiency in IM-9 cells resulted in lower reduction of IκB-α knockdown.

Bortezomib Enhances Selinexor Induced Nuclear Localization of XPO1 Cargos including IκB in HT1080 Resistant Cells

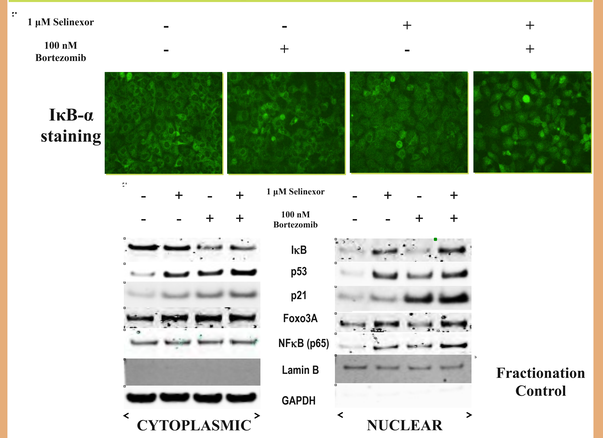


Figure 4: HT1080 resistant cells were treated with 1 μM of selinexor and/or 100 nM bortezomib for 12 hours. The cells were then tested by IκB-α immunofluorescence (IF) study or by cytoplasmic-nuclear fractionation. The IF and fractionation results show that the combinatory treatment of selinexor and bortezomib increased nuclear retention of IκB-α as well as other XPO1 cargos compared to the single agent treatment.

Combination of Selinexor and Bortezomib Inhibits NFκB Transcriptional Activity Better than the Single Agents

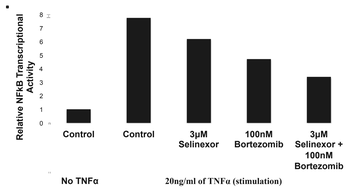


Figure 5: HT1080 resistant cells were pre-treated with 3μM of selinexor and / or 100nM bortezomib for 2 hours and then exposed to TNFα for 4 hours in serum free media. TNFα exposure induced NFκB transcriptional activity by 8 folds. The combination of selinexor and bortezomib reduced the activity by 60% compared to 20% and 40% by each single agent respectively.

Proteasome Inhibitors Sensitizes Selinexor Resistant Cells to SINE Compounds

6A

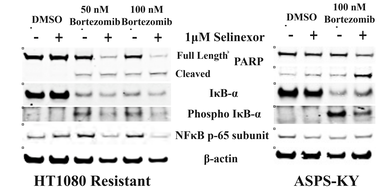


Figure 6A: Selinexor resistant HT0180-R and ASPS-KY cells were treated as indicated for 24 hours. The combination of Selinexor and Bortezomib was more cytotoxic than each one of the single agents.

6B

HT1080 Resistant Cells

TREATMENT	KPT-185 IC_{50} (μM)	Fold Change
KPT-185 alone	4.3	1
KPT-185 + 4.1 nM Bortezomib (IC_{50} – 5 nM)	0.11	39
KPT-185 + 4.1 nM Carfilzomib (IC_{50} – 8 nM)	0.28	15.4

ASPS-KY cells

TREATMENT	Selinexor IC_{50} (μM)	Fold Change
Selienxor alone	22	1
Selinexor + 1 μM Bortezomib (IC_{50} >1μM)	1.89	11.64

Figure 6B: HT0180 resistant and ASPS-KY cells were treated with serial dilutions of SINE compounds with/without proteasome inhibitors at a concentration lower than their IC_{50} for 72 hours. Proteasome inhibitors sensitized HT1080-R and ASPS-KY cells to SINE compounds by 39 and 12 folds respectively.

Synergistic Cytotoxic Effects of Selinexor and Bortezomib Visible in Multiple Myeloma Cell Lines with High and Low SINE Sensitivity

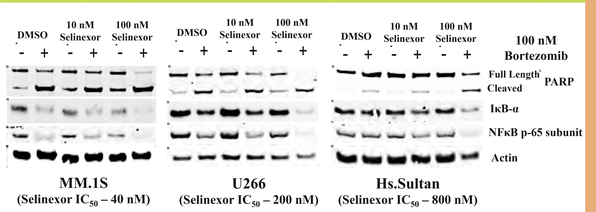


Figure 7: MM.1S, U266, Hs.Sultan (Mutiple Myeloma) cells with different sensitivity to selinexor were treated for 24hrs. Concomitant treatment of selinexor and bortezomib was toxic to all the 3 cell lines. The protein levels of IκB-α and NFκB p-65 subunit were reduced by the combination suggesting deactivation of NFκB activity.

Combination Treatment of Selinexor with Carfilzomib Results in Synergistic Effects in Multiple Myeloma Cancer Xenograft Model

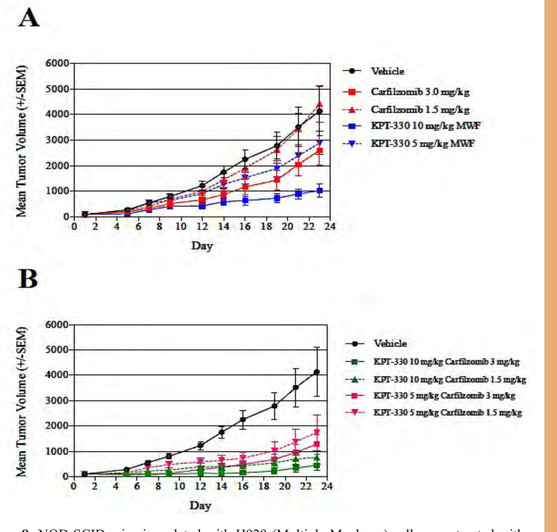


Figure 8: NOD-SCID mice inoculated with H929 (Multiple Myeloma) cells were treated with vehicle, carfilzomib (1.5 mg/kg or 3.0 mg/kg), selinexor/KPT-330 (5 mg/kg or 10 mg/kg) or selinexor plus carfilzomib. Carfilzomib was given on 2 consecutive days at the beginning of each week via IV injection, while selinexor was given via PO on Monday, Wednesday and Friday (MWF) schedule. The group treated with selinexor at 10mg/kg had a statistically significant reduction in tumor growth (**8A**: p-0.020). No other group with a single agent showed statistically significant reduction in tumor growth. All groups treated with selinexor and carfilzomib combinations showed statistically significant reductions in tumor growth (**8B**). The reduction in tumor growth seen in these groups were dose dependent for both selinexor and carfilzomib. Limited evidence of weight loss was observed in the group treated with the highest dose of one or both of the compounds (not shown).

Summary of Results and Conclusions

- Selinexor binds to XPO1 and induces nuclear entrapment of key tumor suppressor proteins and cell cycle regulators.
- The nuclear retention efficiency of selinexor is challenged in cells resistant to selinexor.
- Cells resistant to selinexor showed up-regulation of NFκB transcriptional activity.
- Knockdown of IκB-α, the inhibitor of NFκB decreased cell sensitivity to selinexor.
- The proteasome inhibitors (PI) bortezomib and carfilzomib were reported by others to inhibit NFκB activity.
- Combinations of PI and selinexor improved nuclear entrapment of IκB-α and resulted in better inhibition of NFκB transcriptional activity compared to the single agents.
- Combinations of PI with SINE compounds sensitized cancer cell death in both solid and hematological cell lines.
- Selinexor treatment in combination with proteasome inhibitors showed synergistic anti-tumor activity in a Multiple Myeloma xenograft mouse model.
- The combination treatment is predicted to result with synergistic therapeutic outcome in cancer patients.

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