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 reversible drugs that bind covalently to Exportin 1 (XPO1/BRE) and inhibit nuclear export. This results in nuclear retention of major tumor suppressor proteins (TP53, p21, p27, etc.) as well as other XPO1 cargos compared to the single agent treatment.

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

In vivo: Selinexor treatment in combination with proteasome inhibitors showed synergistic therapeutic outcome in cancer patients.

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

Figure 1: SINE resistant HT1080 (Fremosona) cells were selected by continued exposure of sensitive parental cells in the presence of SINE compound. Parental and resistant HT1080 cells were treated with 100 nM of selinexor for 4 hours. Nuclear retention of key tumor suppressor proteins including IκB-α was tested by immunostaining and shown to be insufficient in resistant cells.

Figure 2: Two-dimensional HT1080, (Fremosona), selinexor-resistant HT1080, and ASPS-KY cells were treated with selinexor and bortezomib at various concentrations for 24 hours. The combination of selinexor and bortezomib increased nuclear retention of IκB-α as well as other XPO1 cargos compared to the single agent treatment.

Proteasome Inhibitors Sensitize Selinexor Resistant Cells to SINE Compounds

Figure 3: U2OS cells (Osteosarcoma) and IM-9 cells (Multiple Myeloma) were transfected with siRNA targeting the p65 (NF-κB) subunit. The siRNA knockdown was tested by cytoplasmic-nuclear fractionation. The IF and fractionation results show that the combination treatment is predicted to result in synergistic therapeutic outcome in cancer patients.

Figure 4: HT1080 resistant cells were treated with 1 µM of selinexor and/or 100 nM bortezomib for 12 hours. The cells were then treated with DMSO or 100 nM bortezomib for 4 hours. The combination treatment of selinexor and bortezomib increased nuclear retention of IκB-α as well as other XPO1 cargos compared to the single agent treatment.

Figure 5: HT1080 resistant cells were pretreated with or without bortezomib and/or 100 nM selinexor for 4 hr before exposure induced NFκB transcriptional activity by ELISA assay. The results shows that higher NFκB transcriptional activity is correlated with lower IC50 value.

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

Figure 6B: Selinexor and Bortezomib were more cytotoxic than any one of the single agents.

Figure 7: MM-1S, 126.6, H929 (Multiple Myeloma) cells with different sensitivity to selinexor were treated for 24hrs. Combination treatment of selinexor and bortezomib was tested in all the 3 cell lines. The protein levels of IκBα and NFκB p65 subunit were reduced by the combination suppressing degradation of NFκB activity.

Summary of Results and Conclusions

- Selinexor resistant HT1080 and ASPS-KY cells treated with vehicle, carfilzomib (1.5 µg/kg or 3.0 µg/kg), selinexor/KPT-335 (1.5 µg/kg or 10 µg/kg) or selinexor plus carfilzomib. Carfilzomib was given on 2 consecutive days at the beginning of each week by IV injection, while selinexor was given via PO on Monday, Wednesday and Friday (SWW) schedule. The group treated with 100 nM bortezomib and 100 nM of selinexor showed statistically significant reduction in tumor growth (94 %±10%) compared to the control untreated group at the end of Day 20. In contrast, all the other groups treated with carfilzomib or carfilzomib showed statistically significant reduction in tumor growth (88 %±20%). The reduction in tumor growth rate in these groups was 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).

- selinexor Bortezomib treatment in combination with proteasome inhibitors showed synergistic antitumor activity in a Multiple Myeloma xenograft mouse model.

- The combination treatment is predicted to result in synergistic therapeutic outcome in cancer patients.

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