Selective Inhibitor of Nuclear Export (SINE™) Compounds Show Synergistic Anti-Tumor Activity in Combination with Dexamethasone in Multiple Myeloma

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Abstract

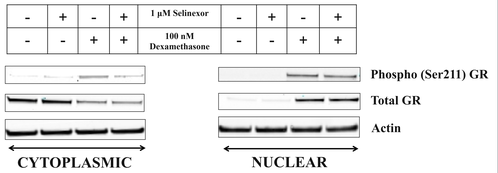
Background: Dexamethasone is a known activator of Glucocorticoid Receptor (GR) and GR is a cargo of the nuclear export protein Exportin 1 (XPO1/CRM1). SINE compounds are a family of covalent, oral selective inhibitors of XPO1. Inhibition of XPO1 results in the retention of major tumor suppressor proteins (TSPs) such as p53, FOXO, pRB and IκB and subsequently in 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. This study was aimed at the evaluation of the combinatory effects of selinexor and dexamethasone on the expression of GR-regulated anti-tumor proteins in multiple myeloma (MM) tumors.

Methods: Total RNA and whole protein cell lysates from MM cell lines treated with selinexor with or without dexamethasone were analyzed by quantitative PCR and by immunoblots. Localization of GR was evaluated by immunofluorescence. GR and NFκB transcriptional activity was analyzed using ELISA assays (Thermo Scientific). MM.1S Multiple Myeloma xenograft model in NOD-SCID mice were treated with selinexor (5 mg/kg) and dexamethasone (1 mg/kg) alone or in combination and tumor growth was evaluated for 17 days.

Results: Dexamethasone, but not selinexor, induced phosphorylation of GR resulting in GR nuclear localization. Selinexor prevented nuclear export of phosphorylated GR, leading to the synergistic induction of GR-dependent transcriptional activity. RNA levels of GR regulated genes such as MNK2 were induced by this combination treatment. Interestingly, between the two MNK2 isomers, the combination treatment increased the expression of MNK2a, which is a tumor suppressor protein but not the MNK2b isoform. NFκB transcriptional activity was inhibited additively by this treatment. The combination treatment of selinexor with dexamethasone showed synergistic cytotoxic effects on MM cells, which expresses Glucocorticoid Receptor. *In vivo*, the combination of the two drugs inhibited MM.1S tumor growth by 94% compared to 59% and 47% by selinexor and dexamethasone respectively.

Conclusion: Selinexor forced and enhanced the nuclear retention of GR, which is initiated by dexamethasone. Increased GR transcriptional activity induced expression of tumor suppressor genes like MNK2a and repressed NFκB activity. Cytotoxic effects of the combination were superior to the single agents. These data suggest that such a combination treatment is predicted to result with synergistic therapeutic outcome in cancer patients.

Dexamethasone, but not Selinexor, Induces Phosphorylation of Glucocorticoid Receptor (GR) Resulting in GR Nuclear Localization



1 μM Selinexor	-	+	-	+
100 nM Dexamethasone	-	-	+	+

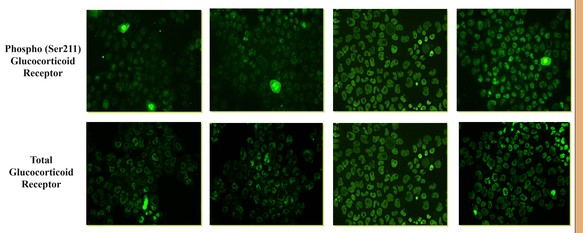


Figure 1: MM.1S (Multiple Myeloma) cells were treated with 1 μM of selinexor and/or 100 nM of dexamethasone for 4 hours. The cells were then either used for Phospho (Ser211) glucocorticoid receptor (GR) immunofluorescence (IF) study or cytoplasmic-nuclear fractionation. The results from IF and fractionation shows that dexamethasone, but not selinexor, induced phosphorylation of GR at serine 211 resulting in GR nuclear localization.

Selinexor Synergizes with Dexamethasone to Induce the Transcriptional Activity of the Glucocorticoid Receptor

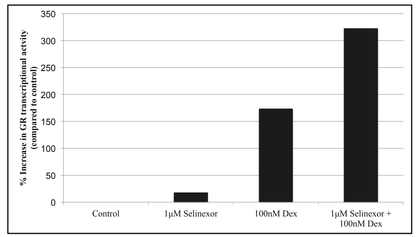


Figure 2: MM.1S (Multiple Myeloma) cells were treated with 1 μM of selinexor and/or 100 nM of Dexamethasone for 4 hours. Glucocorticoid Receptor (GR) transcriptional activity was measured by ELISA. The concomitant treatment of selinexor and dexamethasone increased GR transcriptional activity by 4 folds compared to 2.5 folds increase by dexamethasone alone. Selinexor by itself did not affect GR transcriptional activity.

Combination of Selinexor and Dexamethasone Attenuates NFκB Transcriptional Activity Better than the Single Agents

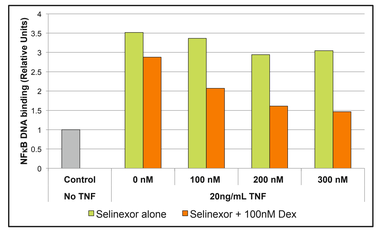
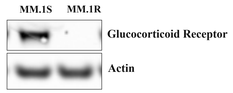


Figure 3: MM.1S cells were pre-treated with 3 different concentrations (100nM, 200nM, 300nM) of selinexor and/or 100nM dexamethasone for 2 hours and then exposed to 20ng/mL TNFα for 4 hours in serum free media. TNFα exposure induced NFκB transcriptional activity up to 3.5 folds as measured by Transcription Factor Assay kit (Thermo Scientific, Catalog# 89859). The combination of the highest concentration of selinexor and dexamethasone reduced the activity by 60% compared to ~15% inhibition by each of the single agents.

The Synergistic Effects by Selinexor and Dexamethasone are Glucocorticoid Receptor Dependent



Cell Type	Selinexor IC_{50}	Dexamethasone IC_{50}	Selinexor IC_{50} (in the presence of dexamethasone)	
			6.25 nM Dexamethasone	100 nM Dexamethasone
MM.1S	40 nM	26 nM	11 nM	< 0.15 nM
MM.1R	50 nM	>30 μM	62 nM	57 nM

Figure 4: Glucocorticoid receptor (GR) expression is vital for dexamethasone response and synergism with selinexor. Dexamethasone at 6.25 nM (IC_{20}) increased selinexor cytotoxic effects in MM.1S (GR positive) by 5.6 folds and by almost 400 folds at 100 nM (IC_{65}). Whereas, MM.1R (GR null) cells are resistant to dexamethasone and do not show any additive cytotoxic effects when treated in combination with selinexor.

Combination of Selinexor and Dexamethasone Additively Induces / Reduces the Expression of Glucocorticoid Receptor Dependent Genes

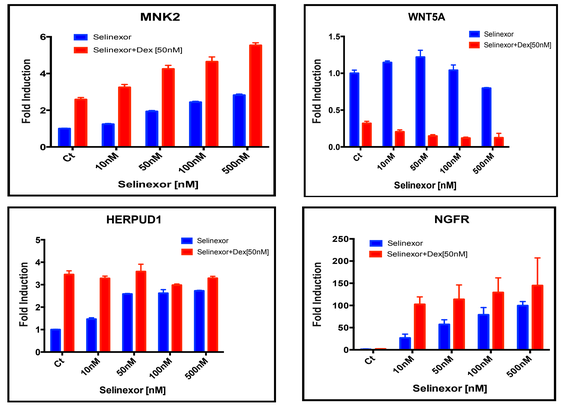


Figure 5. MM1.S cells were treated for 24hrs with increasing concentrations of selinexor with and without 50 nM dexamethasone. GR regulated gene expression was analyzed by quantitative PCR . Expression of four genes; MNK2, Wnt5a, HERPUD1 and NGFR were up or down regulated in an additive manner as a result of combination treatment.

Selinexor / Dexamethasone Combination Upregulates Alternative Splicing of MNK2 Towards the MNK2a Tumor Suppressor Isoform

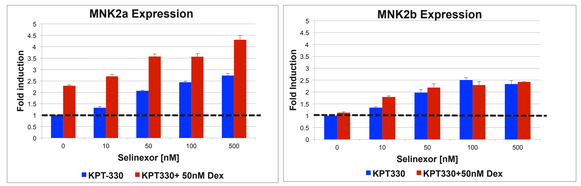
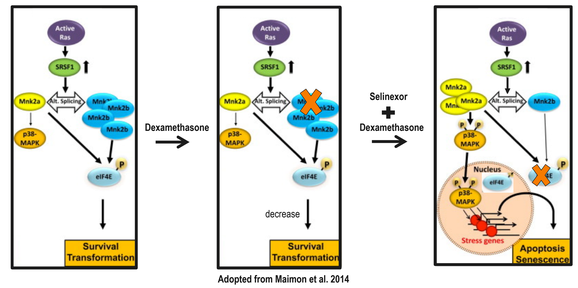


Figure 6. Selinexor/dexamethasone combination treatment additively induced MNK2a but not MNK2b isoform. MM1.S cells were treated with increasing concentrations of selinexor with and without 50 nM dexamethasone for 24hrs. MNK2a/b gene expression was analyzed by quantitative PCR.

Selinexor and Dexamethasone Upregulate MNK2A and Selinexor Traps eIF4E in the Nucleus Leading to Apoptosis and Senescence



Adopted from Maimon et al. 2014

Figure 7. Selinexor traps the translation factor eIF4E in the cell nucleus and abrogates the expression of key proteins that governs cellular proliferation such as c-Myc and cyclins. Selinexor in combination with dexamethasone shifts the alternative splicing of MNK2 from oncogenic MNK2B towards tumor suppressor MNK2A which modulates the p38-MAPK pathway to induce cell death.

Reduction of H929 Tumor Xenografts by Combined Selinexor-Dexamethasone Treatment Exceeds Effects of the Single Agents

Figure 9-A

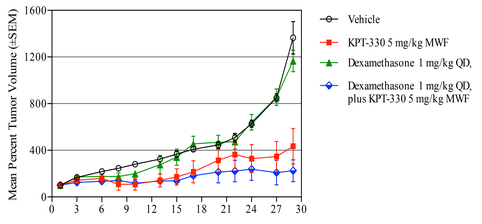


Figure 9-B

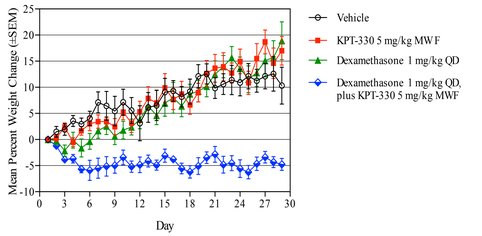


Figure 9: A. NOD-SCID mice inoculated with H929 (Multiple Myeloma) cells were treated with vehicle, 1mg/kg dexamethasone, 5 mg/kg of selinexor/KPT-330 or selinexor plus dexamethasone. Selinexor was given via oral gavage on a Monday-Wednesday-Friday schedule (MWF), while dexamethasone was given via IP injection daily. In the groups that received single-agent therapy, no significant reduction in tumor growth was seen compared to the group receiving combination therapy. The combination group experienced a significant reduction in tumor growth (p=0.0022) (Figure 9-A). The group receiving the combination experienced a statistically significant inhibition in weight gain (p=0.0022) (Figure 9-B).

Summary of Results and Conclusions

- Dexamethasone phosphorylates Glucocorticoid Receptor (GR) at serine 211 and promotes nuclear localization of GR where it acts as a transcriptional factor.
- Selinexor causes nuclear retention of phosphorylated GR since GR is an XPO1cargo.
- Combination of selinexor and dexamethasone increases transcriptional activity of GR.
- Selinexor and dexamethasone treated together inhibit the transcriptional activity of NFκB better than the single agents.
- Cancer cytotoxic effects of selinexor and dexamethasone combination are dependent on the expression of GR. MM.1R cells which do not express GR are resistant to dexamethasone and show no additive/synergistic effects of combining selinexor with dexamethasone.
- The mRNA levels of genes transcribed by GR are regulated in an additive manner by the combination of selinexor and dexamethasone.
- MNK2 RNA is processed by alternative splicing to form two isoforms: MNK2a and MNK2b. Interesting, combination treatment induces the tumor suppressor MNK2a isoform.
- In vivo studies of H929 MM model system in mice showed that the combination treatment of selinexor and dexamethasone led to statistically significant reduction in tumor growth
- These studies demonstrate that selinexor plus dexamethasone combination are synergistic in MM preclinical models, decipher possible mechanism of action and suggest that such a combination treatment will result with synergistic therapeutic outcome in cancer patients.

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