Selinexor or KPT-8602 Mediated XPO1 Inhibition Synergizes with Dexamethasone to Repress Convergent Pathways in the mTORC1 Signaling Network and Drive Cell Death in Multiple Myeloma

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ABSTRACT

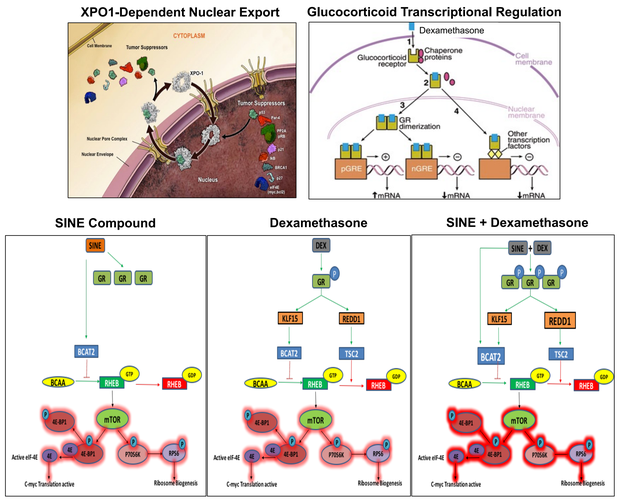
Background: Dexamethasone (DEX), a synthetic glucocorticoid (GC), is administered to nearly all multiple myeloma (MM) patients as a single agent and in combination with other chemotherapies or targeted agents. DEX and other GCs bind to glucocorticoid receptors (GR) in the cytoplasm, induce nuclear translocation and regulate GR-dependent gene expression networks in the nucleus. Selective Inhibitor of Nuclear Export (SINE) compounds (selinexor and KPT-8602) exhibit potent anti-tumor activity in MM especially when combined with DEX. SINE compounds enhance nuclear localization of tumor suppressor proteins (TSPs) through inhibition of the nuclear export protein, XPO1. We discovered that inhibition of the mechanistic Target of Rapamycin Complex 1 (mTORC1) pathway is a primary driver of the combination effect. Here we further elucidate the molecular mechanism of action of the SINE-DEX synergy in MM.

Methods: GR^{+} MM.1S and GR^{null} MM.1R MM cell lines were treated with SINE compounds and/or DEX for 24 hours. Whole cell lysates were subjected to SDS-PAGE and western blot analysis. Gene expression and GR transcriptional activity was analyzed using qPCR and ELISA, respectively. Cell viability was examined using the Celltiter-Fluor assay.

Results: We found that MM cell lines treated with SINE compounds (selinexor or KPT-8602) have increased basal GR protein levels. Consistent with these results, the SINE-DEX combination shows enhanced GR transcriptional activity. Several GR-DEX target genes are known to inhibit the GTPase, Ras homolog enriched in brain (RHEB), which is required for mTORC1 activation. We discovered that the SINE-DEX combination not only reduces RHEB protein but also induces the RHEB inhibitory pathways containing REDD1 and the KLF15-BCAT2 axis. Although SINE compound-mediated inhibition of mTORC1 (i.e. reduced phosphorylation of S6K1 and 4E-BP1) is GR independent, SINE-DEX inhibition is more robust in GR^{+} MM.1S cell line when compared to the GR^{null} MM.1R cells. The combination resulted in the selinexor IC_{50} in MM.1S cells shifting from 40 nM to 11 nM in the presence of low dose DEX. As expected, DEX did not modulate the IC_{50}s in MM.1R cells.

Conclusion: We show that SINE compound inhibition of MM cell viability is enhanced with DEX. Our results indicate that this combinatorial effect is due to convergent suppression of mTORC1 signaling by GR targets. The findings provide mechanism of action data around the SINE-DEX combination in MM with suggestive biomarkers (REDD1, KLF15, BCAT2 and GR) that may predict best response to the combination. Therefore, these data may translate directly to the current clinical development of SINE compounds.

Selinexor Plus Dexamethasone Mechanism



SINE Compounds Increase Expression of Glucocorticoid Receptor

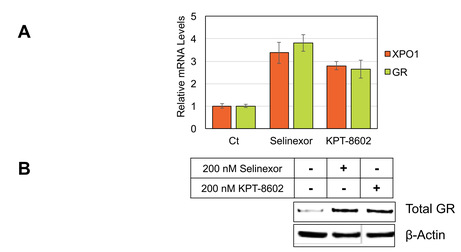


Figure 2: MM.1S (multiple myeloma) tumor cells were treated with 200 nM of selinexor or KPT-8602 for 24 hr. **(A)** Real time PCR indicated that SINE compounds increased glucocorticoid receptor (GR) mRNA expression. **(B)** Immunoblots of whole cell lysates show induction of GR protein levels following SINE compound treatment.

Selinexor Plus Dexamethasone Combination Causes A Synergistic Increase in GR Transcriptional Activity

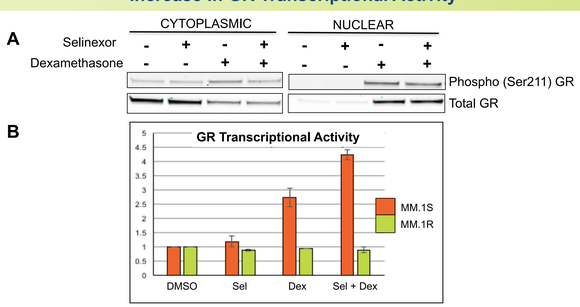


Figure 3: MM.1S and MM.1R cells were treated with 1 μM selinexor and 100 nM dexamethasone (DEX) for 4 hours. **(A)** DEX treatment results in phosphorylation and induction of nuclear (active) glucocorticoid receptor. **(B)** The combination of selinexor plus DEX resulted in a 4.5-fold increase in basal GR activity, compared to 3-fold increase by DEX treatment alone. GR^{null} MM.1R cells had no change in basal GR activity in the presence of the either compound alone or in combination.

Selinexor Plus Dexamethasone Inhibition of the mTOR Pathway in GR^{wt} and GR^{null} MM Cell Lines

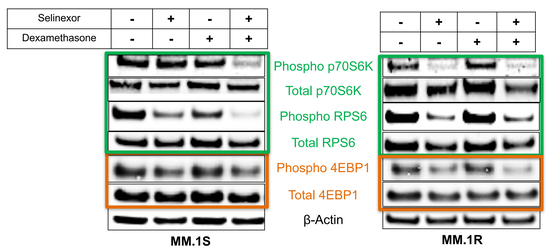


Figure 4: MM.1S (GR^{wt}) and MM.1R (GR^{null}) cells were treated with 200 nM selinexor and 100 nM DEX for 24 hrs. In MM.1S cells, selinexor alone had little to no effect on the mTOR pathway (p70S6K and p4EBP1). However in combination with DEX, a significant reduction in p70S6K and 4EBP1 phosphorylation resulted. In MM.1R cells the effect of selinexor on the mTOR pathway was significant in the absence of DEX.

Selinexor Plus Dexamethasone Modulates the Expression of GR Downstream Targets That Negatively Regulate mTOR

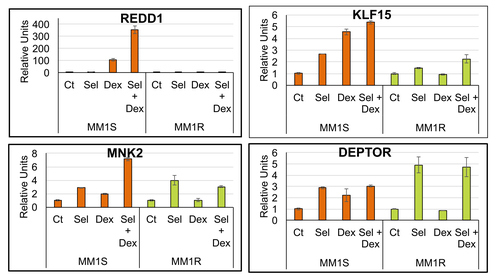


Figure 5: To understand inhibition of the mTOR pathway, the expression of known GR regulated targets of mTOR activity were evaluated. DEX treatment alone induced the expression of all target genes to varying degrees in MM.1S cells, while having no effect on MM.1R cells. Interestingly, the combination with selinexor dramatically enhanced the expression of REDD1 in MM.1S cells.

Selinexor Plus Dexamethasone Upregulates REDD1 and BCAT2 in GR^{wt} cells But Only BCAT2 in GR^{null} cells

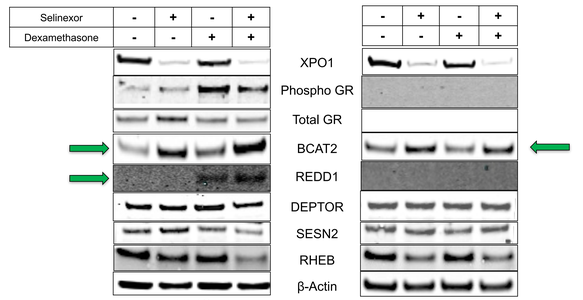


Figure 6: MM.1S and MM.1R cells were treated with 200 nM selinexor and 100 nM DEX for 24 hours. In MM.1S, BCAT2 and REDD1, both downstream of KLF15 (a GR target) exhibited combinatorial increases. In MM.1R, BCAT2 was significantly induced by selinexor treatment, although induction was not enhanced after adding DEX. DEX treatment induces the expression of REDD1 in MM.1S only, which is enhanced in combination. RHEB expression was significantly reduced by SINE-DEX combination.

Selinexor Reduces BCAA Levels in GR^{wt} and GR^{null} Cells

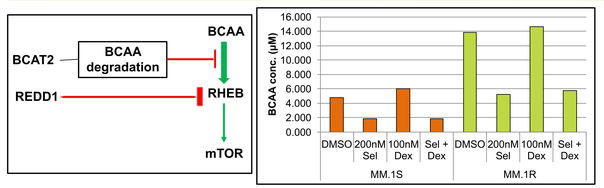
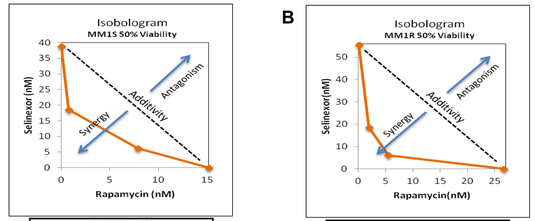


Figure 7: MM.1S and MM.1R cells were treated with 200 nM selinexor and 100 nM DEX for 24 hours. BCAT2 inhibits the mTOR pathway by initiating the degradation of branched chain amino acids (BCAA) blocking RHEB activation of mTOR. Selinexor, not DEX, decreases levels of BCAA in cells irrespective of GR status. Interestingly, MM.1R (GR^{null}) cells have higher BCAA levels than MM.1S (GR^{wt}) cells.

Selinexor Synergizes with mTOR Inhibitors in GR^{wt} and GR^{null} cells



MM1S Viability (%)	Selinexor (nM)					
Rapamycin (nM)	500	167	56	19	6	0
1000	14	15	22	32	35	42
333.3	14	16	22	39	46	43
111.1	14	16	26	38	45	50
37.0	17	18	29	38	43	45
12.3	14	16	29	42	46	51
4.1	18	18	31	45	56	58
1.4	16	20	31	51	60	65
0.5	16	21	36	50	57	68
0	16	21	43	68	88	100

MM1R Viability (%)	Selinexor (nM)					
Rapamycin (nM)	500	167	56	19	6	0
100	20	23	34	39	46	48.4
33.3	21	24	33	48	52	47.7
11.1	24	26	39	50	56	54.4
3.7	24	31	37	45	49	56.7
1.2	23	32	37	52	57	63
0.4	24	34	39	55	66	73.3
0.1	31	31	48	66	87	91.9
0.0	35	39	54	76	95	101
0	24	32	49	82	102	100

Figure 8: The effects of selinexor alone or in combination with rapamycin for 72 hours on cell viability of **(A)** MM.1S and **(B)** MM.1R cell lines using the MTT assay. Drug combination analysis using "isobologram" confirmed synergistic interaction between selinexor and rapamycin in both the cell lines.

SUMMARY

- SINE compounds induce mRNA and protein expression levels of Glucocorticoid Receptor (GR) in MM cells.
- Selinexor plus dexamethasone shows marked cytotoxic synergy in cells expressing GR by positively regulating the levels of key members of the intrinsic apoptosis pathway.
- Combination of selinexor and dexamethasone increases transcriptional activity of GR.
- The beneficial combinatory effect is mediated through mTOR inhibition as confirmed by Reversed Phase Protein Array (RPPA) and microarray.
- Among the known GR regulated modulators of mTOR; up-regulation of REDD1 (GR target gene) and BCAT2 by the combination lead to mTOR inhibiton.
- Selinexor-induced BCAT2 protein lead to a reduction in cellular BCAA concentration.
- In GR^{null} MM.1R cells, selinexor as a single agent can negatively regulate the mTOR pathway by inducing BCAT2 expression.
- Higher concentration of BCAA might make MM.1R cells more susceptible to mTOR inhibtion upon increase of BCAT2 levels.
- Selinexor synergizes with the mTOR inhibitor rapamycin, in both GR^{wt} and GR^{null} cells.
- Selinexor is currently being investigated in combination with DEX in a clinical study in refractory MM previously treated with/refractory to iMIDs, proteasome inhibitors and anti-CD38 monoclonal antibody (NCT02336815).

CONCLUSION

The current finding suggests that GR pathway status could be used as predictive marker to guide drug combination selection for MM patients. While selinexor combined with dexamethasone might be beneficial for GR^{wt} patients, selinexor with mTOR inhibitors could prove advantageous for patients with GR^{null} or GR^{mut} tumors.

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