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Selinexor, a Selective Inhibitor of Nuclear Export (SINE) Compound, Shows Synergistic Anti-tumor Activity when Combined with PD-1 Blockade in a Mouse Model of Colon Cancer

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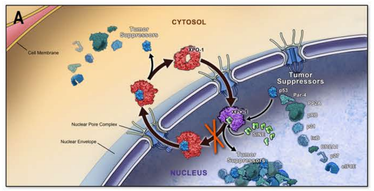
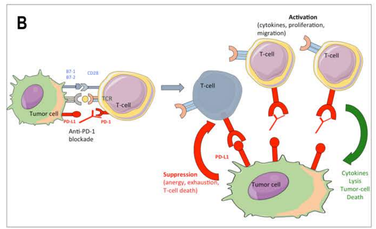
ABSTRACT

Introduction: Selinexor is an oral, first-in-class SINE compound that specifically binds to the primary nuclear exporter XPO1/CRM1. XPO1 exports over 200 cargos, including major tumor suppressor proteins (TSPs), leading to their functional inactivation. Inhibition of XPO1 results in nuclear retention of TSPs and restores their normal functions. Interestingly, XPO1 also mediates the export of NFAT1c, STAT1 and STAT3, which have been implicated in regulation of the inhibitory T-cell receptor PD-1 (NFAT1c, STAT3) and its ligand, PD-L1 (STAT1). Therefore, we hypothesized that selinexor treatment will result in up-regulation of PD-1 and PD-L1, making tumor cells more amenable to immunotherapy.

Figure 1. Mechanisms of action of selinexor (A) and anti-PD1 (B)



A). Selinexor is a SINE compound that increases the nuclear retention of major tumor suppressor proteins by inhibiting the nuclear export protein XPO1. Selinexor has been tested in >1400 patients to date in Phase 1 and 2/3 clinical trials with promising signs of efficacy, tolerability and safety.



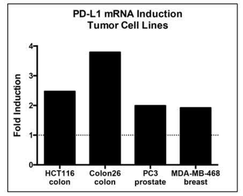
B). PD-1/PD-L1 immune checkpoint pathway impedes the CD8+ cytotoxic T-lymphocytes to recognize and destroy tumor cells and is believed to be exploited by tumors to promote survival and evade host's anti-tumor immune response.
Blocking PD-1-PD-L1 signaling by anti-PD-1 or anti-PD-L1 monoclonal antibodies allows for T-lymphocytes to mount a robust immune response against tumor cells.

Methods: The effect of selinexor on PD-1 and PD-L1 gene expression was tested in human normal donor leukocytes and in tumor cells [HCT-116(colon), PC-3 (prostate) MDA-MB-468 (breast) and Colon26 (colon)], respectively by quantitative PCR. A Colon26 syngeneic mouse model of CRC was generated and animals were assigned to the following treatment groups: (i) vehicle, (ii) selinexor at sub-therapeutic dose of 5 mg/kg (M/W/F), (iii) Anti-PD-1 (BioXCell), 100ug biwk and (iv) selinexor + anti-PD-1 combination. Tumor growth, weight loss and signs of toxicity were monitored for 45 days. Xenografts were harvested, RNA and DNA were collected and tumors were analyzed microscopically and by immunohistochemestry (IHC).

RESULTS

Selinexor Induces PD-1 and PD-L1 Gene Expression *in vitro* and *ex vivo*

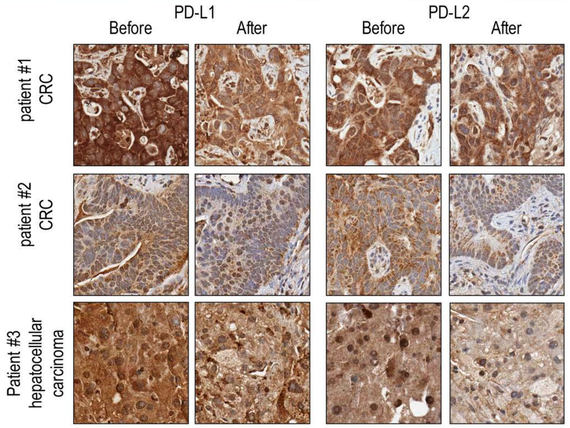
Leukocyte Source	Treatment	Dose	Time (Hours)	Fold Induction
Mouse	Ex vivo	100 nM	24	1.17
		1000 nM	24	4.07
	In vivo	15 mg/kg	8	2.39
			24	1.03
Normal Human Donor	Ex vivo	100 nM	4	1.95
			24	2.73
		500 nM	4	2.15
			24	1.57
Selinexor Patients	In vivo	Various	24	1.14
			48	1.87



Ex vivo: leukocytes from mice or normal human donors were incubated in culture with varying concentrations of selinexor for 4-24 hours. In vivo: leukocytes from mice or humans treated with selinexor were harvested at 8-48 hours post dose. mRNA from leukocyte samples was extracted and PD-1 levels relative to pre-treatment were quantitated by PCR.

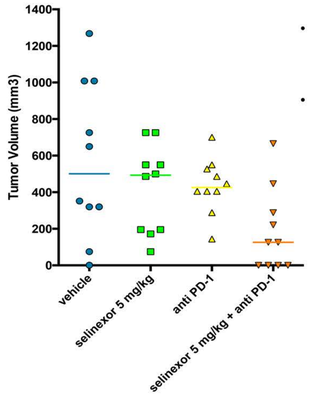
Human and mouse tumor cell lines were incubated for 24 hours with 1000 nM selinexor followed by mRNA extraction and quantitation of PD-L1 levels relative to pre-treatment by PCR.

Post-selinexor Treatment Reduction in PD-L1 & PD-L2 Staining Intensity in Patients Tumor Biopsies



The effect of selinexor on PD-L1 and PD-L2 expression in patient biopsies was determined by IHC. Note, despite the mRNA increase observed in tumor cell lines, a reduction in protein levels is observed 3-4 weeks post-selinexor treatment.

Selinexor Synergizes with anti-PD-1 to Increase Tumor Growth Inhibition

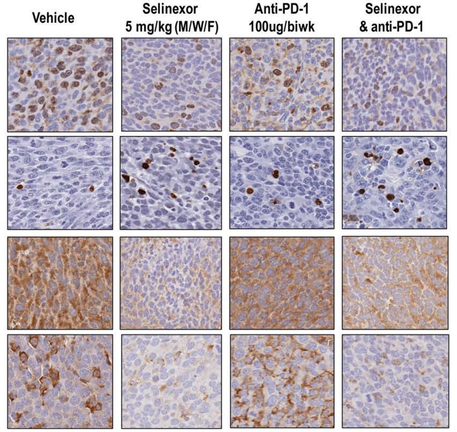


- 4/10 mice (female BALB/c) in the combination group did not have detectable tumors at day 22. These mice were also tumor free at the end of the study (day 45)
- No weight loss or signs of toxicity were evident in any *in vivo* study

Treatment	% TGI (day 22)
Selinexor 5 mg/kg (M/W/F)	27
Anti-PD-1* 100ug biwk	24
Selinexor + anti -PD-1	67

*Anti-PD-1: InVivoMAb anti-Mouse PD-1, BioXCell (#BE0146)

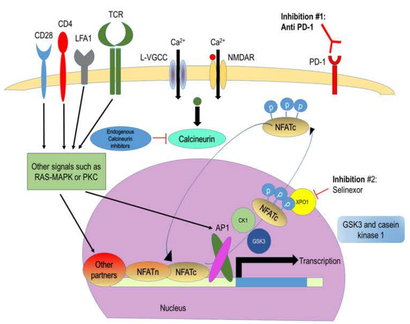
Inhibition of Proliferation, Induction of Apoptosis and Reduction in PD-L1 and PD-L2 Protein Levels In Selinxor-anti-PD1-Combination Treated Colon26-derived Xenografts



IHC analysis of Colon26-derived mouse xenografts treated with selinexor and anti-PD1 alone and in combination. Note decreased expression of PD-L1 & PD-L2, induction of apoptosis (Apoptag) and decreased cell proliferation (Ki67) in selinexor alone and in combination-treated xenografts.

Hypothesis and Suggested Model

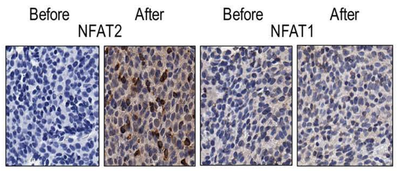
- Nuclear factor of activated T cells (NFAT) transcriptional complexes are composed of cytoplasmic (NFATc) and nuclear (NFATn) components respectively.
- Calcium influx through L-type calcium channels activates the calcium/calmodulin-dependent phosphatase calcineurin.
- Dephosphorylation of NFATc-family members by calcineurin unmasks nuclear localization signals, triggering the translocation of NFATc to the nucleus.
- In the nucleus, NFATc-family members can bind to NFATn and other transcriptional co-activators that are regulated in response to cell surface receptors activation, including proteins, AP-1 (activator protein 1), GATA and MEF2 (myocyte-enhancer factor 2).
- Within the nucleus, the kinases GSK3 (glycogen synthase 3) and CK1 (casein kinase 1) oppose calcineurin activity by phosphorylating NFATc, resulting in its nuclear export by XPO1 and the termination of NFATc activity.



Hypothesis:

- Inhibition of XPO1 activity results in nuclear retention of the XPO1 cargos NFAT and AP-1 thereby enhancing NFAT and AP-1 nuclear activity, downstream of TCR signaling.
- In addition, PD-L1 and PD-L2 protein levels are down regulated on the surface of tumor cells following selinexor treatment.
- In combination, anti-PD-1 antibodies and selinexor cooperatively block the tumor inhibitory effect on T-cells via blockade of PD-1 and downregulation of PD-L1 and PD-L2, respectively.
- The synergistic combination activity may be explained in part by the novel mechanism of enhanced TCR signaling via nuclear retention of NFAT and AP-1.

Enhanced Nuclear NFAT2 in Lymphocytes from a Selinexor Treated Thymoma Patient



Immunohistochemical analysis was performed on a thymoma patient paired tumor biopsies obtained at pre- and 3.5 weeks post-Selinexor treatment initiation. Note increase in nuclear staining intensity of NFAT2 and to a lesser extent of NFAT1 post-treatment.

SUMMARY

- Selinexor synergizes with anti-PD-1 to inhibit tumor proliferation and to induce apoptosis *in-vivo*.
- Selinexor treatment down regulates PD-L1 and PD-L2 proteins on tumor cells, which is hypothesized to cooperate with anti-PD-1 treatment in blocking tumor cells-induced T-cell inhibition.
- Selinexor may also enhance TCR signaling via nuclear retention of NFAT and AP-1.
- These data provide rational support for further investigation of selinexor/ anti-PD-1 combination in pre-clinical and clinical studies.