Selinexor, a Selective Inhibitor of Nuclear Export (SINE), shows enhanced activity in combination with PD-1/PD-L1 blockade in syngeneic murine models of colon cancer and melanoma

Matthew R. Farren[1], Reena Shakya[1], Rebecca Hennessey[1], Thomas Mace[1], Jennifer Yang[1], Omar Elnaggar[1], Gregory Young[1], Yosef Landesman[2], Robert Carlson[2], Sivan Elloul[2], Marsha Crochiere[2], Christin Burd[1], Gregory B. Lesinski[1]

[1]The Ohio State University, Columbus, OH
[2]Karyopharm Therapeutics, Newton, MA

The James
THE OHIO STATE UNIVERSITY
COMPREHENSIVE CANCER CENTER

Abstract

Exportin-1 (XPO1) is a nuclear export protein with >220 cargo proteins, including tumor suppressors and cell cycle modulators. Selinexor (KPT-330), an oral XPO1 inhibitor, has been administered to >1000 cancer patients in Phase I and II trials, with evidence of efficacy and tolerability. Selinexor blocks nuclear export of NFAT1c, STAT1 and STAT3, which have been implicated in regulation of the inhibitory T cell receptor PD-1 and its ligand, PD-L1. We hypothesized that selinexor would upregulate T cell checkpoint molecule expression, and that combination treatment with anti-PD-1 or anti-PD-L1 antibodies would thereby enhance the ability of selinexor to elicit anti-tumor activity.

Selinexor increased PD-1 gene expression by ~2--fold in normal lymphocytes and induced PD-L1 gene expression in tumor cell lines. Mice bearing syngeneic colon (C26) tumors and treated with selinexor ± anti-PD-1 antibodies for 2 weeks demonstrated a significant reduction in tumor growth rate ($p<0.05$), while monotherapy with either agent had no significant effect on tumor growth. Similar results were obtained in mice bearing syngeneic B16F10 tumors, whereby combined treatment with selinexor + anti-PD1 antibodies was superior to either single agent ($p<0.034$). Combined therapy of mice bearing B16F10 tumors with selinexor and anti-PD-L1 antibodies was similarly effective, with significantly smaller tumors at the study endpoint ($p<0.001$). Importantly, no weight loss or signs of toxicity were evident in any *in vivo* study.

Immunophenotypic analysis of splenocytes revealed that combined therapy with selinexor + anti-PD-1/anti-PD-L1 antibodies significantly increased the frequency of NK cells ($p\leq0.045$) and significantly increased the frequency of Th1 T cells ($p\leq0.016$) as compared to vehicle treated mice. Interestingly, combining selinexor with anti-PD-L1 antibodies significantly decreased the proportion of PD-L1$^+$ MDSC, dendritic cell, NK cell, and T cell ($p=0.029$, $p=0.004$, $p=0.019$, $p=0.005$, respectively). Anti-PD1 antibody treatment did not alter PD-1 or PD-L1 expression in the tumor or individual splenic cell populations. However, ~40% of splenic T cells expressed PD-1 in all treatment groups. These data indicate that the efficacy of selinexor may be enhanced by disrupting the pre-existing PD-1/PD-L1 signaling in effector cells (T and NK cells).

Altogether, these data suggest that the efficacy of selinexor in combination with anti-PD-1 or anti-PD-L1 in mouse syngeneic tumor models may be due to both disrupting immunosuppressive PD-1/PD-L1 signaling and increasing the frequency of potentially tumor reactive NK cells and Th1 T cells. This provides a rational basis for this treatment combination as a novel therapeutic approach for advanced cancer.

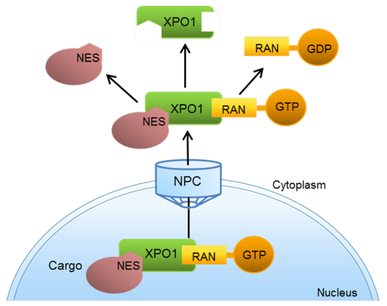
Combining nuclear export inhibition with immunotherapy in melanoma

Immunotherapy in melanoma

- **Immune checkpoint blockade (ipilimumab, nivolumab) has clinical efficacy against metastatic melanoma, having elicited significant and sustained responses in a proportion of patients.**
- **While promising, majority of patients still progress in < 2 years following immunotherapy.**

Nuclear export inhibition in melanoma

- **The nuclear export protein exportin-1 (XPO1) is upregulated during melanoma progression**
- **XPO1 transport >220 identified cargo proteins including tumor suppressors and cell cycle modulators**
- **Selinexor (KPT-330), a Selective Inhibitor of Nuclear Export (SINE) compound, inhibits XPO1**
- **In Yang *et al.* (*PlosONE,* 2014), we demonstrated this drug has direct anti-tumor activity in melanoma**
- **In addition to its antitumor activity, selinexor inhibits nuclear export of NFAT1c, STAT1, and STAT3 and may have immunomodulatory properties**



Hypothesis: Selinexor leads to direct anti-tumor activity but also upregulate T cell checkpoint molecule expression. Thus, combination treatment with anti-PD-1 or anti-PD-L1 will synergize with selinexor to control tumor growth

Experimental design

B16F10 Melanoma (5 x 10^5 cells)

Treatment
Selinexor (oral) 2/week
Antibody (IP) 2/week

Necropsy & immune assays

Figure 1. Experimental Design: 5.0 x 10^5 B16F10 melanoma cells were implanted subcutaneously into (immunocompetent) female C57BL/6 mice. When tumors became palpable, mice were treated with 15 mg/kg selinexor (orally) and 100-200 µg of the relevant antibody or isotype control (i.p.). All treatments were twice per week and continued until control tumors > 1500 mm^3. Mice were then euthanized and tumor tissue, splenocytes, and plasma analyzed.

Selinexor induces immune checkpoint molecule expression

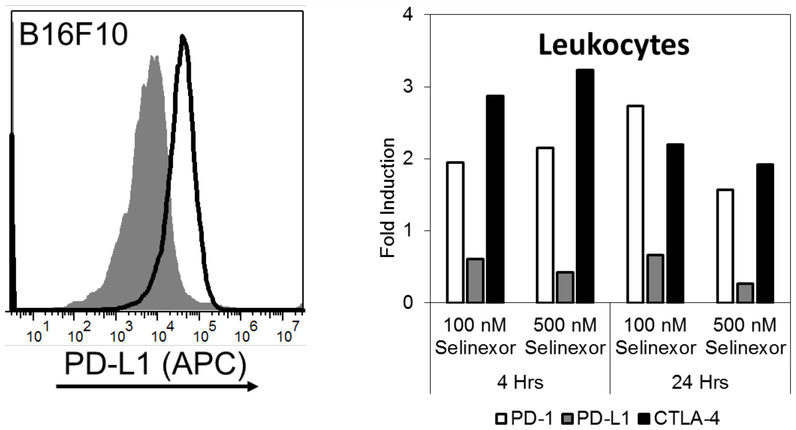


Figure 2. Melanoma cells express PD-L1 at baseline and selinexor induces increased immune cell expression of *PD-1,* and *CTLA-4*. (*left*) PD-L1 expression on B16F10 was assessed by flow cytometry. B16F10 expressed PD-L1 at baseline (*grey histogram*: isotype control, *black histogram*: anti-PD-L1 stained). Selinexor incubation (up to 1 µM for 72 hours) did not further induce PD-L1 expression (data not shown). (*right*) Human leukocytes from healthy donors were incubated with 100-500 nM selinexor or diluent control for 4-24 hours and T cell checkpoint molecule expression assessed by qRT-PCR. Selinexor strongly induced *PD-1* and *CTLA-4* expression, while marginally suppressing *PD-L1* expression in human leukocytes.

PD-1/PD-L1 blockade + XPO1 inhibition by selinexor slows melanoma tumor growth

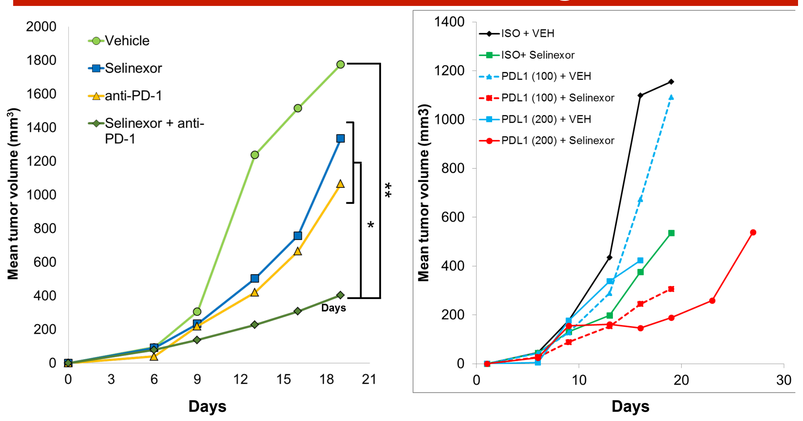


Figure 3. Combination therapy with PD-1/PD-L1 blockade + selinexor exerts superior anti-tumor activity to either therapy alone. Mice bearing B16F10 tumors were treated as described above with selinexor ± α-PD-1 or α-PD-L1 antibodies (PD-1/PD-L1 blockade), or diluent vehicle / isotype controls (VEH, ISO), twice weekly when tumors became palpable. Mice were euthanized when tumors grew to > 1500^3 or ulcerated. Lines are median tumor volume, n = 6 mice per group. * $p < 0.05$; ** $p < 0.01$.

Selinexor increases the frequency of NK cells in the spleen

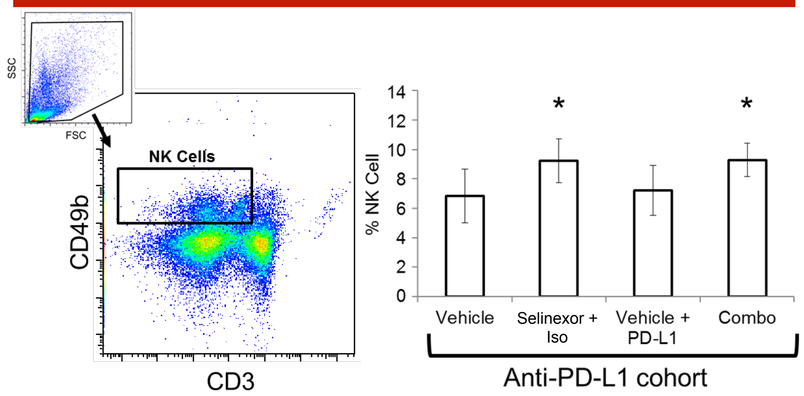


Figure 5. Therapy with selinexor ± PD-1/PD-L1 blockade induces increased NK cell frequency. (*left*) Gating strategy: NK cells were identified by gating $CD3^-$ $CD49b^+$ cells. (*right*) Selinexor induced an increased frequency of this potentially cytotoxic and anti-tumor cell type. n = 5-6 mice per group. * $p < 0.05$.

PD-L1 blockade decreases the frequency of PD-L1$^+$ splenocytes

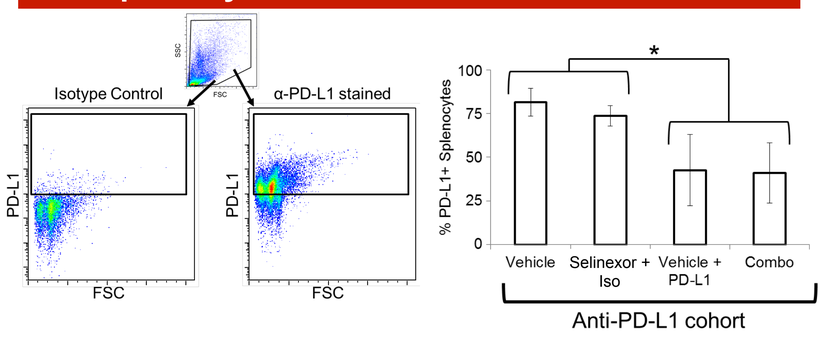


Figure 6. Therapy with PD-L1 blockade ± selinexor decreased surfaced expression of PD-L1 on bulk splenocytes. (*left*) Gating & validation strategy: PD-L1 expression on bulk splenocytes was assessed by flow cytometry. Expression was determined relative to isotype-control stained cells. To verify that decreased fluorescence was a result of decreased PD-L1 expression and not an artifact resulting from interference between the treatment α-PD-L1 antibody and the fluorescently labeled antibody, residual unlabeled (treatment) α-PD-L1 staining was assessed by staining "unlabeled" cells with a secondary antibody conjugated to FITC. (*right*) α-PD-L1 alone or in combination with selinexor significantly decreased PD-L1 expression on bulk splenocytes. n = 5-6 mice per group. * $p < 0.05$.

Selinexor + PD-L1 blockade induces splenic T_H1 differentiation

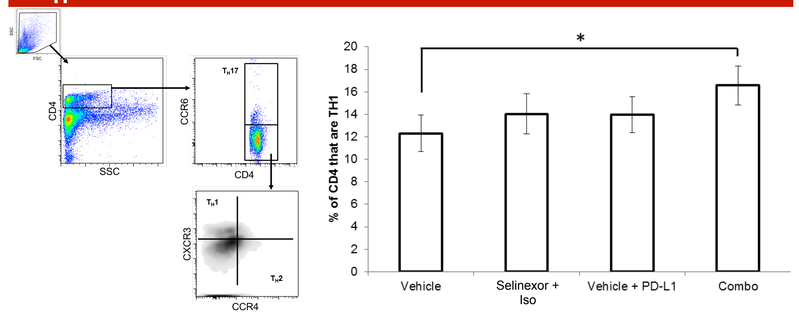


Figure 7. Combination therapy significantly increases T_H1 differentiation. (*left*) Gating strategy: Helper T cell phenotypes were determined based on $CD4^+$ T cell expression of $CXCR_3$ (T_H1), CCR4 (T_H2), and CCR6 (T_H17). (*right*) Combo therapy with selinexor + PD-L1 blockade significantly increased the frequency of cells with a T_H1 phenotype. n = 5-6 mice per group. * $p < 0.05$.

Selinexor increases *in vivo* splenic T cell activation

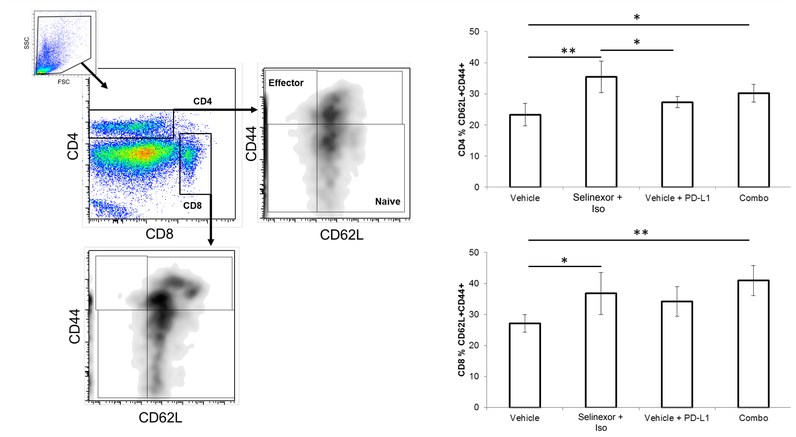


Figure 8. Combination therapy significantly increases $CD4^+$ and $CD8^+$ T cell activation *in vivo*. (*left*) Gating strategy: T cell activation status was assessed by first gating on $CD4^+$ or $CD8^+$ cells and then assessing CD44 and CD62L levels. $CD62L^+$ $CD44^-$ - Naïve phenotype; $CD62L^+$ $CD44^+$ - early activation and central memory phenotype; $CD62L^-$ $CD44^+$ - Effector phenotype Helper T cell phenotypes were determined based on $CD4^+$ T cell expression of $CXCR_3$ (T_H1), CCR4 (T_H2), and CCR6 (T_H17). Selinexor treatment significantly increased the proportion of T cells with early activated/central memory phenotype (*right*), as well as increasing effector phenotype and decreasing naïve phenotype (data not shown). n = 5-6 mice per group. * $p < 0.05$.

Conclusions

1. **Combination of selinexor + PD-1 or PD-L1 blockade exerts considerable anti-tumor activity in an aggressive murine melanoma model.**

2. **Combination of selinexor + PD-1 or PD-L1 blockade shows significant immunomodulatory activity, inducing changes in the frequency and phenotype of immune cell populations including:**

   - **Increased frequency of NK cells**
   - **Induced frequency of differentiated TH1 cells**
   - **Increased frequency of activated T-cells**

3. **These results support the future clinical evaluation of the combination of selinexor + PD-1/PD-L1 blockade in melanoma patients**

Acknowledgements

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