#2319

Selinexor, a Selective Inhibitor of Nuclear Export (SINE), Enhances the In Vivo Efficacy of Checkpoint Blockade with Antibodies Targeting CTLA4 or PD-1/PD-L1 in Melanoma

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Abstract

Selinexor (KPT-330) is a SINE (Selective Inhibitor of Nuclear Export) compound being evaluated in multiple later stage clinical trials in patients with relapsed and/or refractory hematological and solid tumor malignancies. (ClinicalTrials.gov). Selinexor has been administered to >1400 cancer patients in Phase I and II trials to date, with evidence of efficacy and tolerability. This small molecule targets exportin-1 (XPO1), a key nuclear export protein with >200 cargo proteins which include both tumor suppressors and cell cycle modulators. As a result, selinexor blocks nuclear export of proteins including IκB, NFAT1c, STAT1 and STAT3, which regulate expression of the inhibitory T cell receptors CTLA4, PD1 and its ligand, PD-L1. We hypothesized that selinexor would upregulate T cell checkpoint molecule expression, and thereby enhance the anti-tumor activity of antibodies targeting PD-1/PD-L1 or CTLA4. Human (A375, CHL-1) and murine (B16F10) melanoma cell lines expressed high levels of PD-L1 protein at baseline, and PD-L1 expression was induced following selinexor treatment in numerous other tumor cell lines (including HCT-116, MDA-MB-468, MV-4-11, OVCAR-8, and PC-3). Examination of lymphocytes revealed that selinexor also increased expression of PD-1 and CTLA4 by ~2-fold. Mice bearing syngeneic B16F10 melanoma tumors treated with selinexor (15 mg/kg 2 x weekly) and anti-CTLA4 (250 µg, 2 x weekly) demonstrated a significant reduction in tumor growth rate ($p = 0.0065$) while monotherapy had no significant effect on tumor growth. Similar results were obtained in mice bearing B16F10 melanoma treated with the combination of selinexor + anti-PD-1 (200 µg, 2 x weekly, $p < 0.034$) or selinexor + anti-PD-L1 (100-200 µg, 2 x weekly, $p < 0.001$). Importantly, no weight loss or signs of toxicity were evident in any *in vivo* study. Further immunophenotypic analyses have been completed in animals receiving selinexor alone or in combination with anti-PD-L1. In combination treated mice, we observed a significantly increased percentage of splenic NK cells ($p \leq 0.050$), and a significantly increased percentage of splenic Th1 T cells ($p \leq 0.011$), all compared to vehicle treated mice. Interestingly, combining selinexor with anti-PD-L1 significantly decreased the percentage of splenocytes that expressed PD-L1 ($p<0.001$). These changes are indicative of increased anti-tumor immune activity; however, they were accompanied by significantly increased percentages of myeloid cell subsets in combination treated mice ($p \leq 0.050$). The immunologic significance of this myeloid cell expansion is currently under investigation. These data indicate that the efficacy of selinexor may be enhanced by disrupting immune checkpoints in effector cells (T and NK cells). This provides data in support of novel, evidenced-based combinations involving immunotherapy with XPO1 inhibition that deserve further investigation for advanced cancer.

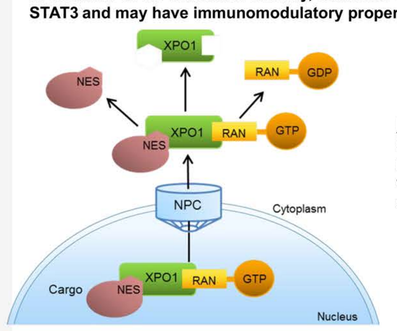
Combining nuclear export inhibition with immunotherapy in melanoma

Immunotherapy in melanoma

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

Nuclear export inhibition in melanoma

- **The nuclear export protein exportin-1 (XPO1) is upregulated during melanoma progression**
- **XPO1 >220 identified cargos include tumor suppressors, cell cycle modulators**
- **Selinexor, a selective inhibitor of nuclear export, 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 lead 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
KPT330 (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

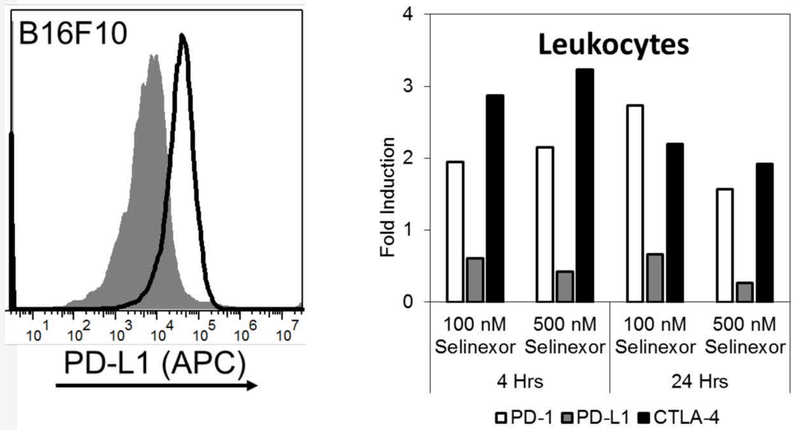


Figure 2. Melanoma cells express PD-L1 at baseline and selinexor induces increased immune cell expression of *PD-1,* and *CTLA-4*. (*left*) PD-L1 expression on B16F10 was assessed by flow cytometry. B16F10 expressed PD-L1 at baseline (*grey histogram*: isotype control, *black histogram*: anti-PD-L1 stained). 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 + XPO1 inhibition slows melanoma tumor growth

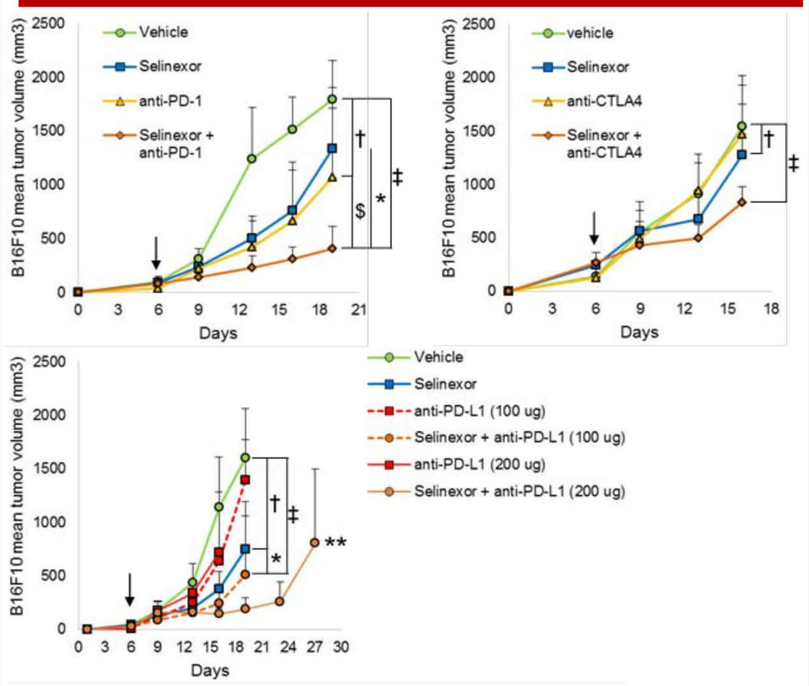


Figure 3. Combination therapy with PD-1/PD-L1/CTLA-4 blockade + selinexor exerts superior anti-tumor activity to either therapy alone. Mice bearing B16F10 tumors were treated as described above with selinexor (15 mg/kg) ± α-PD-1 or α-PD-L1 or α-CTLA-4(or diluent/isotype controls) twice weekly (Mondays and Thursdays) when tumors became palpable. Mice were euthanized when tumors grew to > 1500^3 or ulcerated. Lines are mean tumor volume + S.D., n = 6 mice per group. *, $p<0.05$ between combination treatment (combo) and selinexor alone; $, $p<0.05$ between combo and vehicle/checkpoint blockade; †, $p<0.05$ between selinexor and vehicle/isotype control; ‡, $p<0.05$ between combo and vehicle/isotype control; **, $p<0.05$ between selinexor + anti-PD-L1 (200 µg) and vehicle/isotype control. Arrows indicate when treatment was initiated.

Selinexor increases the frequency of NK cells in the spleen

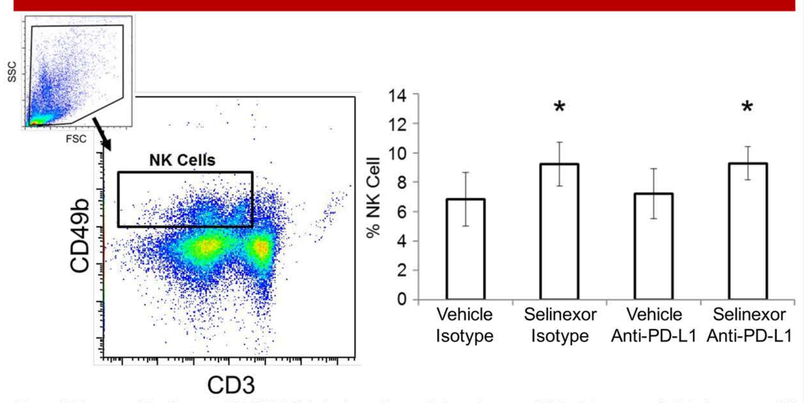


Figure 4. Therapy with selinexor ± PD-1/PD-L1 blockade + selinexor induces increased NK cell frequency. (*left*) Gating strategy: NK cells were identified by gating $CD3^-CD49b^+$ cells. (*right*) Selinexor induced an increased frequency of this potentially cytotoxic and anti-tumor cell type. n = 5-6 mice per group. * $p < 0.05$.

Selinexor + α-PD-L1 treatment induces T_H1 differentiation

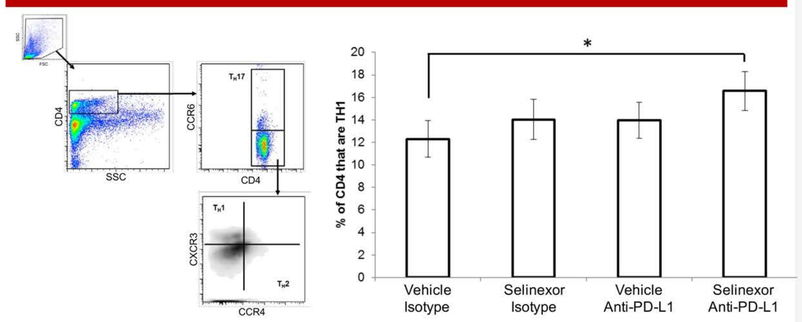


Figure 5. Combo therapy significantly increases T_H1 differentiation. (*left*) Gating strategy: Helper T cell phenotypes were determined based on $CD4^+$ T cell expression of $CXCR_3$ (T_H1), CCR4 (T_H2), and CCR6 (T_H17). (*right*) Combo therapy with selinexor + α-PD-L1 significantly increased the frequency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

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Selinexor increases *in vivo* T cell activation

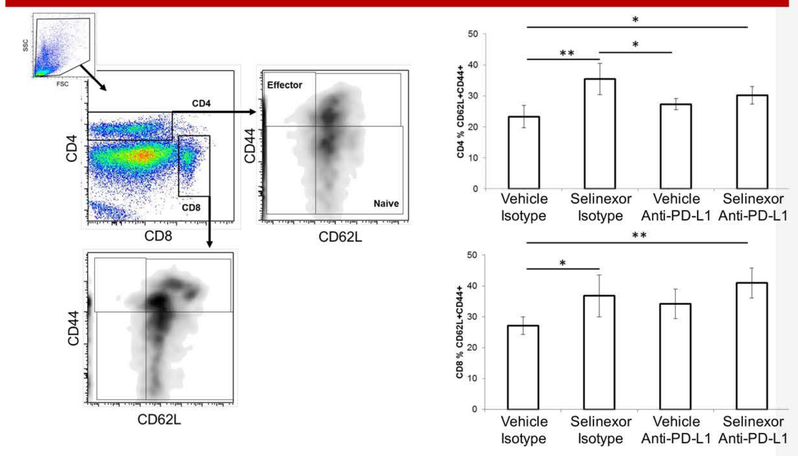

Figure 6. Combo therapy significantly increases $CD4^+$ and $CD8^+$T cell activation *in vivo*. (*left*) Gating strategy: T cell activation status was assessed by first gating on $CD4^+$ or $CD8^+$ cells and then assessing CD44 and CD62L levels. $CD62L^+$ $CD44^-$ - Naïve phenotype; $CD62L^+$ $CD44^+$ - 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. * $p < 0.05$.

Selinexor + checkpoint blockade remains effective across certain dosing schedules

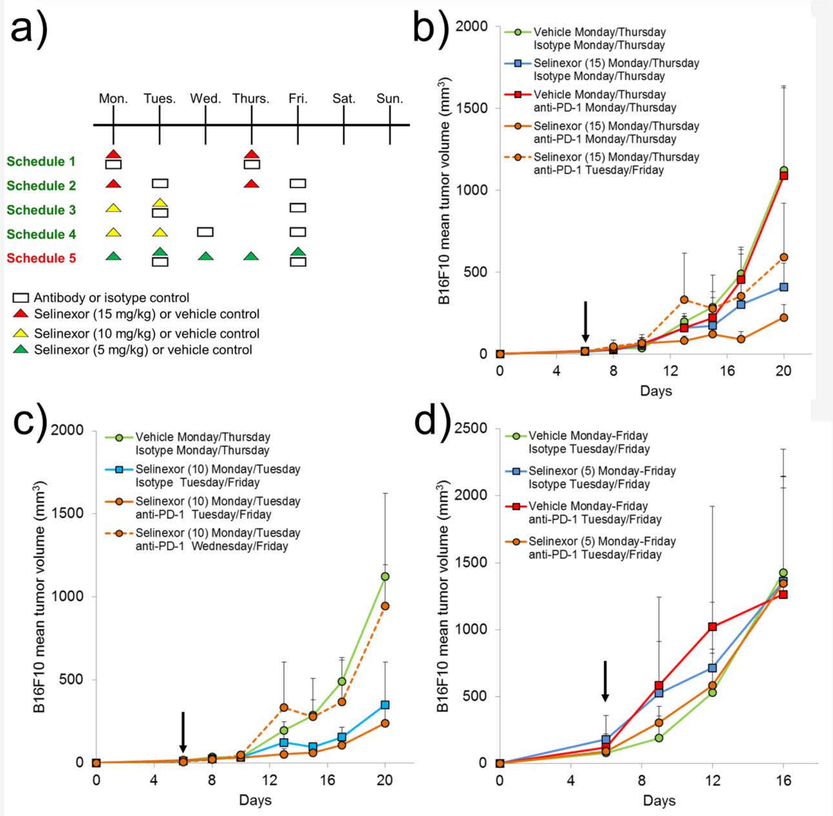

Figure 6: Evaluation of alternative dosing schedules for selinexor and anti-PD-1. Animals were injected subcutaneously with B16F10 on day 0 and were treated twice per week with selinexor and immune checkpoint blockade (or appropriate vehicle/isotype control) beginning when tumors became palpable. n=5 mice per group. **(a)** 5 alternative time/dose schedules for selinexor and anti-PD-1 antibody were evaluated for efficacy. A total of 5 different time and dose schedules were evaluated for efficacy. In these alternative schedules, selinexor was administered at 15 mg/kg (**schedules 1-2**, **b**), 10 mg/kg (**schedules 3-4**, **c**), or 5 mg/kg (**schedule 5**, **d**), while anti-PD-1 was always administered at 250 µg/animal. **Schedules 1-4 (b, c)** showed efficacy and benefit of the combination treatment, while **schedule 5** did not. Interestingly, these were also the treatment schedules wherein single agent selinexor (10 or 15 mg/kg, twice/week) exhibited some degree of tumor growth inhibition.

Conclusions

1. **Combination of selinexor + α-PD-1 or α-PD-L1 exerts considerable anti-tumor activity in an aggressive murine melanoma model.**
2. **This treatment combination had significant immunomodulatory activity, inducing changes in the frequency and phenotype of immune populations systemically.**
3. **In contrast to human leukocytes treated *in vitro*, selinexor did not induce PD-1 or PD-L1 expression in tumor bearing mice. There are two important differences in these models, however:**
   a) **The *in vitro* data was generated using human cells, while the *in vivo* data was generated in a murine setting. Immune cells from humans vs. mice are known to differentially regulate PD-1 and CTLA-4, and there may also be subtle differences in how they regulate these molecules in response to selinexor**
   b) **The human *in vitro* data was generated using healthy donor (i.e. tumor free) leukocytes, while the murine *in vivo* data only examined cells from tumor bearing mice. Thus, it is likely that immune checkpoint molecules were already upregulated on the murine cells, masking potential effects of selinexor on the regulation of these molecules**