Combination therapy of immune checkpoint and nuclear export inhibitors in a renal cell carcinoma mouse model

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Introduction

- Renal cell carcinoma (RCC) is frequently metastatic at diagnosis. Current treatments are inadequate and associated with resistance
- New RCC therapeutic targets are desperately needed
- Selective inhibitor of nuclear export (SINE) compounds represent a novel class of drugs
- SINE compounds have therapeutic equivalence to multiple kinase inhibitors in pre-clinical studies of RCC
- SINE compounds (eg Selinexor) are in phase I/II clinical trials for patients with advanced malignancies including RCC
- Selinexor (KPT-330) is being evaluated in multiple later stage clinical trials in patients with relapsed and/or refractory hematological and solid tumor malignancies (ClinicalTrials.gov). To date >1400 patients have been dosed with selinexor
- Using antibodies against PD-1, PD-L1 and/or CTLA-4 to block immune checkpoints shows promise in RCC phase I/II/III clinical trials, with objective tumor responses of 20-50%

HYPOTHESIS

The oral SINE compound, selinexor, will rapidly kill RCC tumor cells and prime the tumor microenvironment for a response to checkpoint inhibition with antibodies against PD-1 or CTLA-4.

Procedures

Materials: *InVivo*MAb anti-mouse CTLA-4 (9D9) and *InVivo*MAb anti-mouse PD-1 (RMP1-14) were purchased from BioXCell. Selinexor (KPT-330) was provided by Karyopharm Therapeutics.
Animals: Groups of 10, six-week old, male Balb/c mice were injected heterotopically (subcutaneous) with 500,000 syngeneic renal cell adenocarcinoma (RENCA) cells. After visible tumors appeared seven days later, mice were treated every three days with vehicle (controls), selinexor (15 mg/kg), anti-PD-1 (250 μg/mouse), or anti-CTLA4 alone (250 μg/mouse), or with selinexor in combination with either antibody. Mice were euthanized 10 days after the initiation of treatment when the control groups reached the tumor endpoint. Tumor volume, tumor size, and tumor growth rate were determined by physical measurements.
Flow Cytometry: Whole tumors were harvested at necropsy and preserved in complete media for analysis. A representative section of each tumor was preserved in 10% neutral buffered formalin for routine histological analysis to confirm the diagnosis. The remaining tumor was mechanically disrupted into a single cell suspension and stained to assess infiltration of stromal cells and immune cells by multi-parameter flow cytometry. To assess intracellular cytokine production, cells were stimulated with BD Leukocyte Activation Cocktail (PMA/ionomycin/brefeldin A) for 4 hours followed by fixation, permeabilization, and intracellular staining. Analysis was performed using a LSRII and population gating was performed in FlowJo.
Statistics: Figure 1 tumor volumes were analyzed using a Students t-test to compare treatments with control; *p<0.05. Data are mean±SE. In figures 2-5 box-and-whisker plots show the percent of the total number of cells collected in the stromal and immune gate from each sample. Box = 75% confidence intervals; whiskers = 95% confidence intervals for 7 mice (Selinexor group) and 8 mice (control, Selinexor + anti-CTLA-4, Selinexor + anti-PD-1 groups).

Results

Figure 1. Ten days of selinexor treatment with or without antibodies against PD-1or CTLA-4 inhibits growth of syngeneic RENCA tumors *in vivo*.

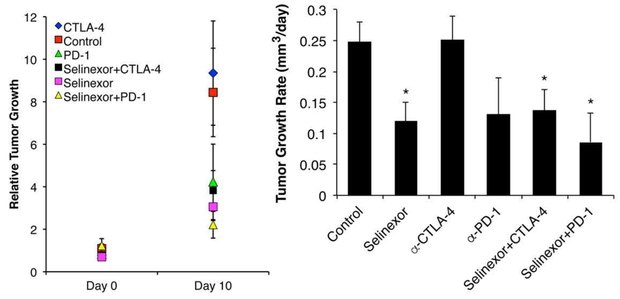
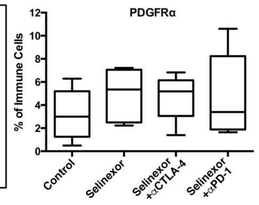


Figure 2. Selinexor increases stromal cell infiltration into syngeneic RENCA tumors. Representative 2D contour plots with outliers for stromal cells (PDGFRα⁺) from tumors. SSC, right angle side scatter.



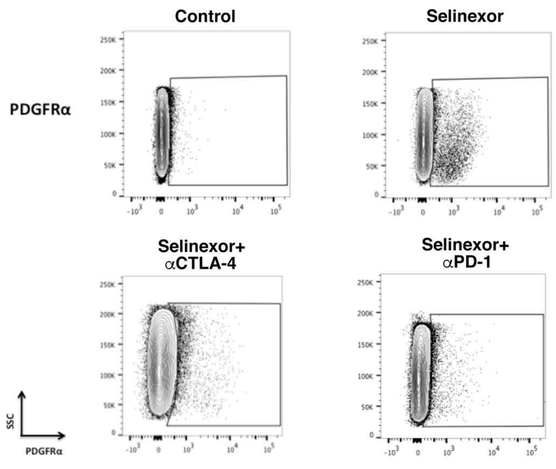


Figure 3. None of the treatments affected the proportion of natural killer (NK) cells in the total cells isolated from syngeneic RENCA tumors.

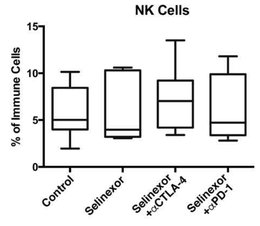
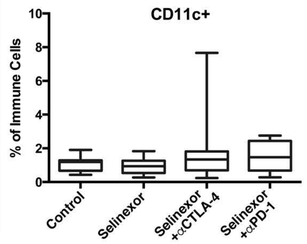


Figure 4. Selinexor increases the proportion of dendritic cells (DCs) in syngeneic RENCA tumors. Representative 2D contour plots with outliers for dendritic cells (CD11c⁺). SSC, right angle side scatter.



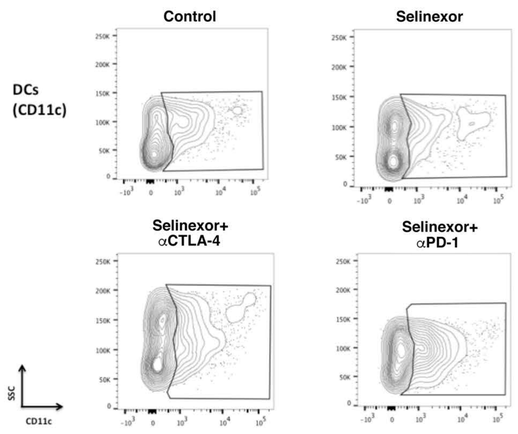
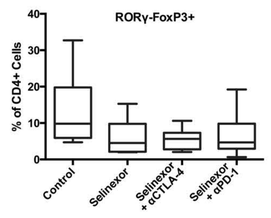


Figure 5. Selinexor decreases the proportion of regulatory T cells (Tregs) in syngeneic RENCA tumors. Representative 2-dimensional contour plots with outliers for Tregs (FoxP3⁺/RORγ-).



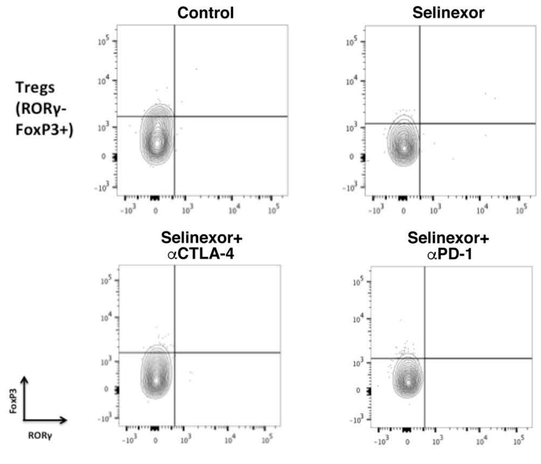


Table 1. Selinexor decreases the absolute number of stromal cells and leukocytes in syngeneic RENCA tumors

Group	Ave % of Cells in Gate	Fold Change (compared to control)	p Value (compared to control)
Control	35.30		
Selinexor	28.85	0.82	0.023
Selinexor+ αCTLA-4	27.52	0.78	0.037
Selinexor+ αPD-1	29.66	0.84	0.088

Summary

- All combinations of selinexor treatment inhibited tumor growth
- Selinexor reproducibly reduced the total number of cells in the gate of interest (leukocytes, host stroma) by about 20%
- Modest and consistent changes associated with selinexor treatment in the number of stromal fibroblasts (PDGFR-alpha+), dendritic cells (CD11c), and NK-T cells (CD3+/NK1.1+) in tumors, and an equally modest, but reproducible decrease in the number of Tregs defined by expression of FoxP3 and absence of ROR-γ.
- No changes in NK cells, B cells or myeloid derived suppressors (CD11b/Gr1)

Conclusions

The data suggest that treatment with selinexor promotes a rapid reduction in tumor burden, while priming the inflammatory and immune environment to potentially maximize the therapeutic effects of checkpoint inhibition. Additional pre-clinical assessments of dose and schedule for this combination can and will be feasibly done in the RENCA model of RCC.

Acknowledgements

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