Selinexor (KPT-330), a Novel Selective Inhibitor of Nuclear Export (SINE), Shows Single Agent Efficacy Against Alveolar Soft Part Sarcoma (ASPS) *In Vivo*

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ABSTRACT

Chromosomal Region Maintenance Protein 1/Exportin 1 (CRM1/XPO1) is a key nuclear export protein whose inhibition leads to the nuclear accumulation of Tumor Suppressor Proteins (TSPs) and renders cancer cells susceptible to apoptosis. Selinexor is orally bioavailable and represents a novel class of small molecule compounds with activity against a wide variety of cancers. Selinexor is currently in Phase 1 clinical studies in hematological and solid cancer patients (Clinicaltrials.gov NCT01607892 and NCT01607905). We tested the activity of selinexor in a soft tissue sarcoma -- ASPS that is resistant to traditional chemotherapy and irradiation treatment. Here we report *in vitro* activity of selinexor against ASPS and *in vivo* efficacy results in xenograft models.

Methods

We used MTT, FACS, qPCR, immunofluorescence, immunostaining and immunoblots to measure the *in vitro* and *in vivo* effects of KPT-330 on the ASPS-KY cell line and in xenograft models.

Results

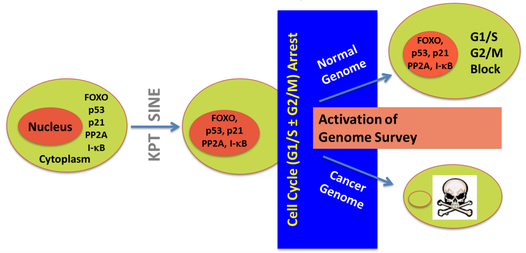
The IC_{50} of the ASPS-KY cell line treated with selinexor for 72 hours was 10 μM. This concentration induced nuclear accumulation of p53, p21, IκB and FOXO3A within 4 hours of treatment, and by 24 hours cells stopped DNA synthesis and were arrested at G1 phase of the cell cycle. By 72 hours, 25% of the cells died. Prior to cell death, the drug reduced the survival protein BCL2 as well as other pro-proliferative proteins such as CDK4, Cyclin D and E2F. In addition, selinexor induced dephosphorylation of pRb activating its tumor suppressor activity and also induced Caspase 3/7 and PARP cleavage. To assess the *in vivo* activity of selinexor in a xenograft model of ASPS, we treated mice with 10 or 20 mg/kg of KPT-330 using a 3 times weekly oral dosing schedule. Following a week of treatment, tumors showed accumulation of TSPs as well as significant reduction of the proliferation marker Ki-67. Following treatment with selinexor for 40 days, tumor growth was inhibited by 70% at 10 mg/kg and by 80% at 20 mg/kg compared with vehicle treated animals. Analysis of immunoblots from these tumors showed induction of p21, with corresponding reduction of the pro-survival and proliferation markers c-Jun, c-Met, Survivin, ERK and HSP70. Histological analysis revealed apoptosis and large areas of fibrosis in treated tumors. These results indicated that selinexor not only inhibited tumor growth, but also induced ASPS cell death *in vivo*.

Conclusions

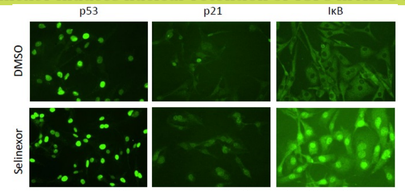
Our results demonstrated that selinexor is effective against ASPS *in vivo* as a single agent and further support the development of SINE-based therapies for alveolar soft part sarcoma that has currently no cure. We will perform further studies to test selinexor in drug combination studies to identify synergism with other therapies.

INTRODUCTION

The evolution of human neoplasms requires loss or inactivation of multiple tumor suppressor proteins (TSPs) and/or their associated pathways. XPO1 (Exportin 1, CRM1) is an essential nuclear exporter of many proteins and RNAs, including nearly all major TSPs (p53, p21, FOXO1a, FOXO3a, APC, IκB, p27, and PP2Aα). XPO1 inhibition can restore endogenous tumor suppressing activities by enhancing the nuclear localization and reducing the proteasome-mediated degradation of multiple TSPs and growth factors. In malignant cells, the genome is disrupted such that forced nuclear localization of TSPs leads to cell death, whereas normal cells undergo reversible cell cycle arrest and are therefore spared. Selinexor, a Selective Inhibitor of Nuclear Export (SINE), has been shown to have broad activity in a variety of cancers both *in vitro* and *in vivo*. SINE are small molecule, drug-like compounds that form slowly reversible covalent bonds with Cys528 of XPO1, inducing nuclear retention of the TSPs, cell cycle arrest and apoptosis in tumor cells. Recently, selinexor has been shown to be effective at inducing apoptosis and inhibiting proliferation in a broad panel of human bone and soft tissue sarcoma cell lines as well as at reducing tumor volume in xenograft models representing each type of these sarcomas (Nair et al., AACR 2013, poster #6086). Selinexor was evaluated for a stage 1 preclinical pediatric testing program and was found to have tumor regressing activity against selected solid tumor xenografts including a panel of Ewing sarcomas (Houghton et al., AACR 2013, poster #LB-354). The first signs for clinical efficacy in heavily pre-treated patients was reported to be observed in a number of patients, including a patient with metastatic endometrial sarcoma who had been receiving selinexor treatment for ≥ 24 weeks at 6 mg/m^2 and resulted in stable disease (Razak et al., ASCO 2013, oral presentation). Here, we describe the use of the SINE XPO1 inhibitor selinexor on ASPS. Orally bioavailable SINE compounds showed 80% inhibition of ASPS xenografts *in vivo*, with tumor tissue showing forced nuclear retention of TSPs, reduction in S phase and apoptosis. To date >240 patients with advanced solid and hematological cancers have been treated with oral selinexor in Phase 1 studies. Thus far, selinexor has shown broad spectrum and durable, single-agent anti-cancer activity, with lack of significant organ toxicity, minimal myelosuppression, and manageable anorexia/nausea/fatigue. These early results provide a promising outlook for successful development of selinexor for treatment of a wide variety cancers.

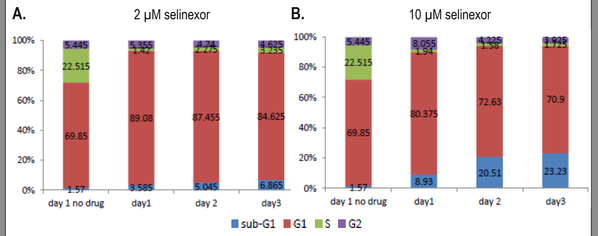


Selinexor induces nuclear retention of TSPs in ASPS cells



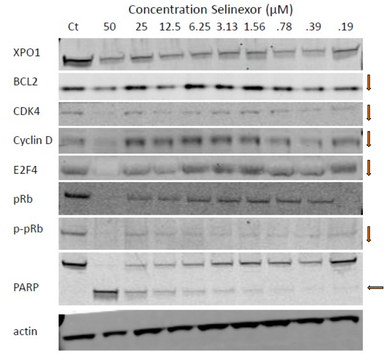
ASPS-KY cells were treated with 10 μM selinexor for 4 h (p53 and IκB) or 24 h (p21) prior to staining.

Selinexor inhibits DNA synthesis and induces growth arrest and cell death in ASPS cells



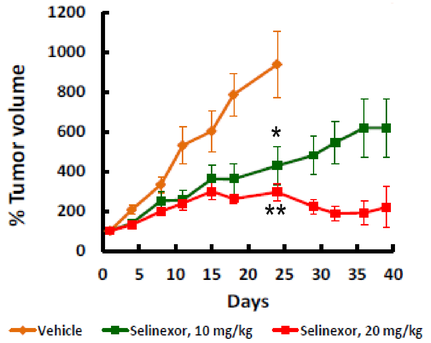
Cells were treated with vehicle (no drug) or with A) 2 μM selinexor or B) 10 μM selinexor and incubated with BrdU for 2 hours prior to collection and fixation after 1, 2 or 3 days in culture. Fixed cells were stained for both BrdU and 7-AAD incorporation and evaluated by flow cytometry. With 2 or 10 μM selinexor treatment, S phase was reduced and G1 arrest induced within 24 hours. However, treatment with 10 μM selinexor (the IC_{50} concentration), induced a more pronounce time-dependent increase in sub-G1 (cell death) than with 2 μM treatment.

Selinexor reduces pro-proliferative and survival protein expression and induces cell death via apoptosis *in vitro*



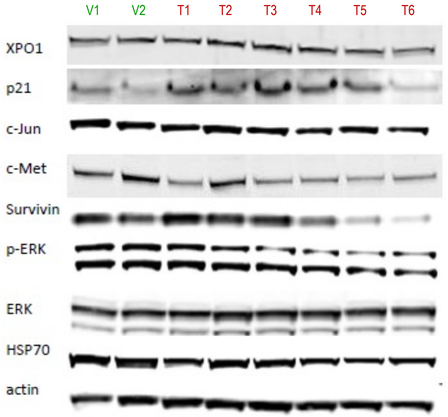
Immunoblots of whole cell lysates from ASPS-KY cells treated with 0, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, and 0.19 μM selinexor for 24 hours. Treatment with selinexor reduced the survival protein BCL2 and the pro-proliferative proteins CDK4, Cyclin D, and E2F4. Selinexor also induced dephosphorylation of pRb and cleavage of PARP.

Treatment with selinexor reduces mean tumor volume in an ASPS xenograft model



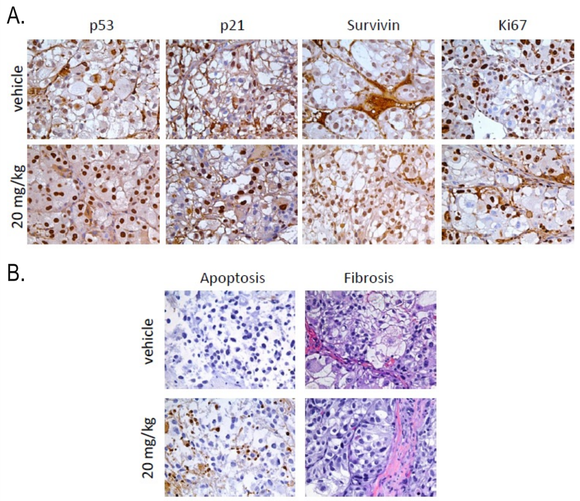
Treatment with selinexor for 40 days inhibited tumor growth by 70% at 10 mg/kg and by 80% at 20 mg/kg compared with vehicle treated animals. Selinexor treatment had very little effect on body weight at either dose compared to control vehicle treatment (data not shown). * Day 24 vehicle to 10 mg/kg selinexor, p = 0.016. **Day 24 vehicle to 20 mg/kg selinexor, p = 0.001.

Selinexor effects expression of TSPs, pro-survival, and proliferation proteins in ASPS-KY xenograft tumors



Tumors from two mice treated with vehicle (V1, V2) and from 6 mice treated with 20 mg/kg selinexor (T1-T6) three times weekly (MWF) for 40 days were isolated and evaluated for changes in protein expression levels by immunoblot analysis. Immunoblots of tumors lysates after 40 days selinexor treatment. Treatment with selinexor induces p21 protein expression and reduces the levels of the pro-survival and proliferation proteins c-Jun, c-Met, Survivin, ERK, and HSP70 compared to vehicle controls.

Selinexor causes nuclear accumulation of several TSPs, reduction in proliferation, and induction of apoptosis and fibrosis in ASPS-KY xenografts



In human ASPS-KY xenograft model, selinexor caused nuclear accumulation of several TSPs as well as reduction in proliferation after 1 week of treatment, and induced both apoptosis and fibrosis after 40 days of treatment. A) Mice were treated with either vehicle or 20 mg/kg selinexor 3 times for one week (MWF) and then tumors were harvested for evaluation by immunohistochemistry. Selinexor induced nuclear accumulation of p53, p21, and Survivin. Selinexor induced reduced proliferation, as indicated by loss of Ki67 staining. B) Tumors from mice treated with either vehicle or with 20 mg/kg selinexor three times weekly (MWF) for 40 days were harvested and evaluated by histological analysis for apoptosis and fibrosis by staining with TUNEL and Masson's trichrome, respectively. Selinexor treatment induced both apoptosis and fibrosis compared to vehicle-treated tumors.

Summary and Conclusions

- Selinexor is currently in Phase 1 testing with >240 hematological and solid tumor patients treated and is showing evidence of broad spectrum anti-cancer activity and good tolerability.
- In ASPS cells *in vitro* and *in vivo*, selinexor acts through XPO1 inhibition to force nuclear retention of TSPs and other critical regulatory proteins, leading to reduction in pro-survival and pro-proliferation proteins and subsequent cell cycle arrest and death by apoptosis of ASPS cells.
- The results from the phase I clinical studies as well as the data presented here suggest that selinexor holds promise as an effective therapy for ASPS.

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