Disruption of Nuclear Export with Selinexor or KPT-8602 Reduces Androgen Receptor Expression and Leads to Potent Anti-Tumor Activity in Preclinical Models of Androgen-Independent Prostate Cancer

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Abstract

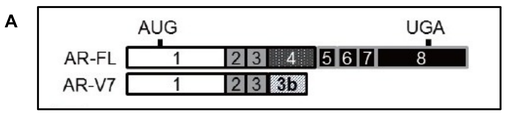
Background: Metastatic castration resistant prostate cancer (mCRPC) is an advanced form of prostate cancer (PC) associated with poor prognosis. Approximately 20 – 40% of mCRPCs are androgen-independent and do not respond to treatment with abiraterone or enzalutamide; drugs that suppress androgen synthesis or directly target androgen ligand binding domains (LBD) respectively. Patients initially responding to these drugs eventually relapse, highlighting the need for alternative therapies. Resistance is attributed to the emergence of constitutively active AR splice variants lacking C-terminal LBDs such as AR variant 7. Although ARv7 activity cannot be mitigated by current therapeutic approaches, it is known that AR mRNA is exported from the nucleus by eIF4E and exportin-1 (XPO1). Selinexor and KPT-8602 are orally bioavailable SINE (Selective Inhibitor of Nuclear Export) compounds that specifically target XPO1. The purpose of this study is to provide mechanistic evidence for using SINE compounds as novel therapies for androgen-independent mCRPC.

Methods: *In vitro,* selinexor or KPT-8602 were tested on androgen-independent PC cell line 22Rv1. RNA and protein were analyzed by qPCR and immunoblot. Cell viability was examined using the Celltiter-Glo assay. *In vivo*, nude mice were injected subcutaneously with 22Rv1 cells. Tumors were grown to ~150 mm^3 before treatment with selinexor (10 mg/kg QoDx3) or KPT-8602 (15 mg/kg then reduced to 10 mg/kg QDx5). Tumor growth and animal weights were monitored to determine tumor growth inhibition (TGI) and tolerability to treatment. Tumors were analyzed using immunohistochemistry (IHC).

Results: We found that expression of AR, ARv7 and prostate specific antigen (PSA; transcriptionally regulated by AR) proteins are reduced following 24-hour treatment with SINE compounds. Nuclear vs. cytoplasmic fractionation of RNA revealed that ARv7 and PSA mRNA localization was increased in the nucleus (4-fold and 3-fold, respectively) and reduced in the cytoplasm (5-fold and 3-fold, respectively). Moreover, KPT-8602 potently inhibited cell viability (IC_{50}: 100 nM), while enzalutamide and abiraterone had no effect. Finally, mice bearing 22Rv1 xenografts, treated with selinexor or KPT-8602 exhibited a complete reduction in tumor volume (95% and 94% TGI, respectively), which coincided with prolonged overall survival. IHC analysis showed a reduction of proliferation markers and a concomitant increase in cell death makers in selinexor and KPT-8602 treated tumors.

Conclusion: SINE compounds show strong anti-tumor activity in androgen-independent prostate cancer models *in vitro* and *in vivo* by reducing AR, ARv7, and PSA expression. These findings highlight the promise of SINE compounds as treatment options for androgen-independent mCRPCs patients that fail first line therapies.

Functional AR Variant 7 in mCRPC Patients



B

Compound	IC_{50} (μM)
	22Rv1
Selinexor	0.09
KPT-8602	0.11
Enzalutamide	>10
Abiraterone	>10

Figure 1: **(A) AR-V7** is a constitutively active androgen receptor splice variant lacking the ligand-binding domain and is no longer regulated by androgens or antiandrogens. AR-V7 is the only known variant encoding a functional protein product that is detectable in metastatic prostate cancer patient specimens. AR-V7 levels correlate with increased risk of biochemical relapse and shorter survival time of mCRPC patients. **(B)** The prostate cancer cell line, 22Rv1, expressing high levels of AR-V7, is resistant to conventional drugs used for mCRPC patients such as **enzalutamide** (binds to ligand binding domain of AR) and **abiraterone** (depletes adrenal and intratumoral androgens). However, 22Rv1 cells remain sensitive to SINE compounds **(i.e. selinexor and KPT-8602)**.

SINE Compound – Mechanism of Action

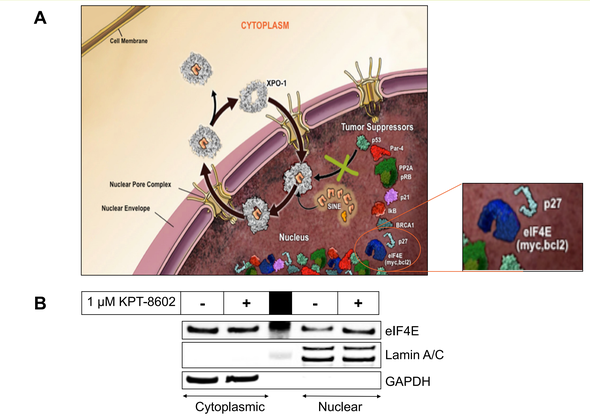


Figure 2: (A) Selinexor and KPT-8602 are novel, oral **Selective Inhibitor of Nuclear Export (SINE™)** compounds that inhibit XPO1-mediated nuclear transport by reversibly binding to the XPO1 cargo binding site. This inhibition leads to nuclear retention of TSPs including the translational control protein, eIF4E. eIF4E controls 7-methyl capped-dependent mRNA translation. Nuclear entrapment of eIF4E by SINE compound treatment results in decreased export (and translation) of target mRNAs such as Myc, Bcl-2 and AR. **(B)** In the CRPC cell line PC3, treatment with 1 μM KPT-8602 for 24 hours led to nuclear enrichment of eIF4E protein.

SINE Compounds Increase Nuclear Levels of AR-V7 mRNA

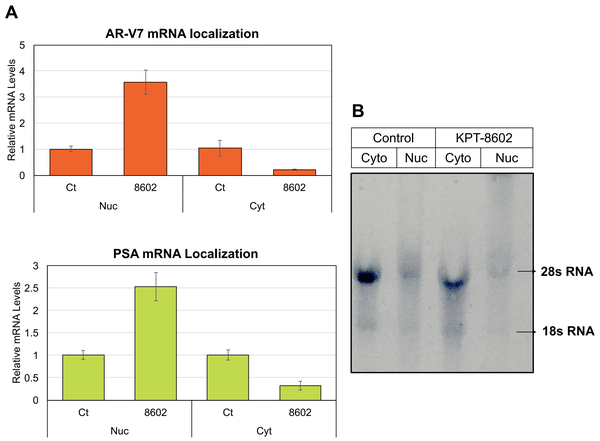


Figure 3: **(A)** 22Rv1 cells were treated with 100 nM KPT-8602 for 24 hrs. RNA was isolated as nuclear (nuc) and cytoplasmic (cyto) fractions using the RNA Subcellular Isolation Kit. Real time PCR for AR-V7 and prostate specific antigen (PSA) genes shows induction of mRNA in the nuclear fraction after KPT-8602 treatment. **(B)** 0.5 μg of each RNA fraction from the different treatment groups were loaded onto a 1.5% agarose gel for analysis.

SINE Compounds Reduce Levels of AR-V7 Protein

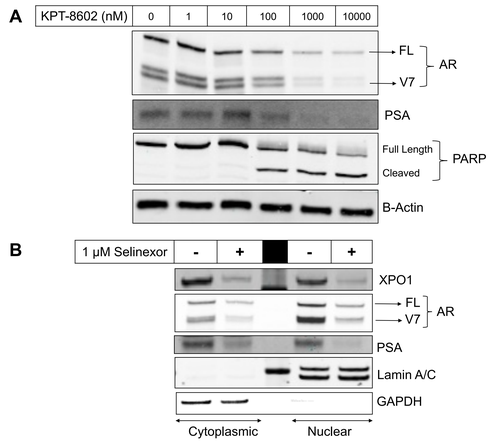


Figure 4: **(A)** 22Rv1 cells were treated with increasing concentrations of KPT-8602. KPT-8602 reduced levels of both full length (FL) and variant 7 (V7) AR in a dose dependent manner. Interestingly, KPT-8602 also decreased the levels of PSA. PARP cleavage confirms apoptosis by KPT-8602 treatment. **(B)** Cellular fractionation of 22Rv1 cells after treatment with 1 μM selinexor. The results show that the reduction of AR and PSA by SINE compounds is not limited to a particular cellular compartments.

SINE Compounds Lead to Tumor Reduction and Prolonged Survival in a 22Rv1 Xenograft Model

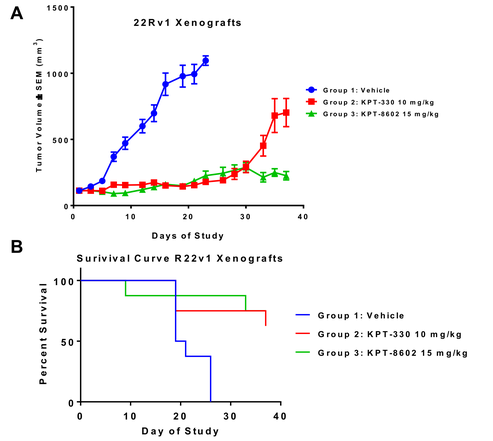


Figure 5: The study was designed to evaluate the efficacy of selinexor (10 mg/kg, QoDx3/week) and KPT-8602 (15 mg/kg, QDx5/week) in a 22Rv1 xenograft model in male CB.17 SCID mice. The treatment regimen of KPT-8602 was modified on Day 8 to 10 mg/kg QDx5/week based on tolerability. **(A)** %TGI on Day 16 was 84% and 87% by selinexor and KPT-8602, respectively, when compared to the vehicle. **(B)** Kaplan-Meier plot shows that vehicle treated mice have a median OS of 20 days while both SINE treatment groups have an undefined median OS at end of the study (Day 37).

SINE Compounds Decrease Proliferation, Increase Apoptosis and Nuclear Tumor Suppressors in 22Rv1 Xenografts

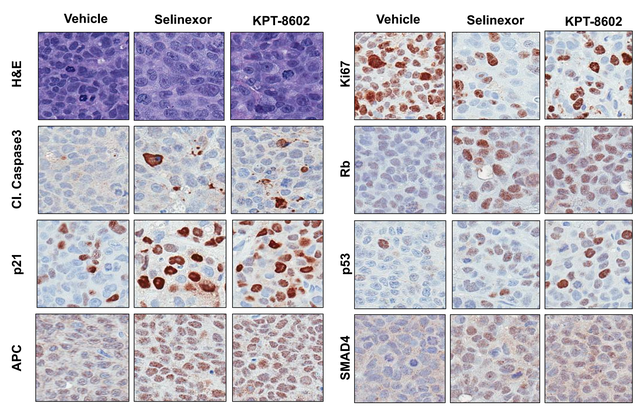


Figure 6: Immunohistochemistry analysis of xenograft samples derived from 22Rv1 cells treated with vehicle control, selinexor or KPT-8602 (**Figure 5**). Decreased cell proliferation (Ki67) and increased apoptosis (Cleaved Caspase 3) was observed in samples treated with SINE compounds. Increased nuclear staining of tumor suppressor proteins Rb, p21, p53, APC and SMAD4 were also observed in samples treated with SINE compounds.

SUMMARY

- Enzalutamide (synthetic non-steroidal antiandrogen) and abiraterone (androgen synthesis inhibitor) are therapies currently being used for treatment of metastatic castration-resistant prostate cancer (mCRPC).
- Alternative splicing of the androgen receptor (AR) mRNA is one potential mechanism for development of resistance to enzalutamide and abiraterone in mCRPC patients.
- AR-V7, a major AR splice variant lacking the ligand binding domain, is constitutively active and is not transcriptional regulated by androgens or anti-androgens.
- Cell lines carrying both full length AR or AR-V7 variant remain sensitive to SINE compounds.
- eIF4E is responsible for translation initiation of capped-dependent mRNA such as Myc, Bcl-2, cyclin D, and AR.
- eIF4E facilitates the nuclear export of capped mRNAs in an XPO1-dependent manner.
- SINE compounds inhibit XPO1 driven nuclear export and lead to nuclear retention of TSPs and growth regulatory proteins including the translation initiation factor eIF4E.
- SINE compound treatment traps eIF4E in the nucleus leading to nuclear retention of AR and AR-V7 mRNA and subsequent reduction in the protein levels of AR and AR-V7.
- *In vivo*, SINE compounds reduce proliferation, induce apoptosis and increased nuclear TSPs, leading to tumor reduction and prolonged survival in 22Rv1 xenografts.

FUTURE DIRECTIONS

Based on these findings, the ongoing First-in-Human clinical trial of KPT-8602 (NCT02649790) will be expanded to include patients with mCRPC.

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