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 domain (LBD) respectively. Patients initially responding to these drugs eventually relapse, highlighting the need for alternative therapies. Resistance is attributed to the emergence of constitutive active AR splice variants lacking C-terminal LBDs such as AR variant 7. Although AR variant 7 activity cannot be mitigated by current therapeutic approaches, it is known that AR mRNA is exported from the nucleus by eIF4E and export-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 mm³ before treatment with selinexor (10 mg/kg Qd) or KPT-8602 (15 mg/kg then reduced to 10 mg/kg Qd). Tumor growth and animal weights were monitored to determine tumor growth inhibition (TGI) and biolability to treatment. Tumors were analyzed using immunohistochemistry (IHC).

Results: We found that expression of AR, Arv7 and prostate specific antigen (PSA; transcribed by ARv7) by ARv7 proteins are reduced in androgen-independent PC xenografts 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 (IC50 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 options for androgen-independent mCRPC patients that fail first line therapies.

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 T-SP core-capped-dependent mRNA translation. Nuclear enrichment of eIF4E by SINE compound treatment results in decreased export (and translation) of target mRNAs such as Myc, Bcl2 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.

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 induced 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 compartment.

Figure 6: Immunohistochemistry 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 p53, 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 a 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 transcriptionally 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, Bcl2, cyclin D2, 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 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.