Preclinical Characterization of ZEN-3694, a Novel BET Bromodomain Inhibitor in Phase I Studies for Metastatic Castration-Resistant Prostate Cancer (mCRPC)

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Abstract

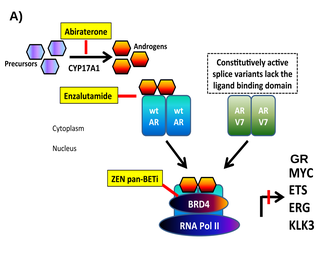
Metastatic Castration Resistant Prostate Cancer (mCRPC) is a major unmet medical need due to its widespread occurrence and incurable status. Current standard of care for advanced prostate cancer is androgen-deprivation therapy (ADT), and upon failure, patients are administered secondary ADT with androgen receptor (AR) antagonists such as enzalutamide and abiraterone. While most patients display an initial response to these agents, eventually all become resistant via various mechanisms that often result in constitutive AR signaling including mutations of the AR, and the generation of AR splice variants that bypass the ligand binding domain. Other mechanisms of resistance to AR antagonists include up-regulation of the glucocorticoid receptor (GR), and partial to complete loss of AR signaling through neuroendocrine differentiation. Recent evidence suggests that BET bromodomain inhibitors (BETi) could be efficacious in AR-signaling positive or negative mCRPC that are resistant to current therapies.

ZEN-3694 is an orally bioavailable, potent BETi that selectively binds to both bromodomains of the BET proteins. *In vitro*, ZEN-3694 has demonstrated strong activity against several prostate cancer cell lines with submicromolar potency, including AR-positive and AR-negative, neuroendocrine, and enzalutamide-resistant cell lines. In VCaP AR-positive prostate cancer cells, ZEN-3694 inhibited proliferation synergistically with enzalutamide, resulting in potent up-regulation of the *CDKN1C/KIP2* tumor suppressor gene. In 22Rv1 cells displaying constitutive AR signaling through the AR-V7 splice variant, ZEN-3694 inhibited AR signaling, and in an *in vitro* LNCaP model of acquired resistance to enzalutamide characterized by GR up-regulation, ZEN-3694 decreased levels of GR in a dose-dependent manner. Furthermore, in the PC3 AR-null cell line, the expression of a subset of NF-KB-dependent genes reported to be involved in mCRPC bone metastasis was found to be inhibited by ZEN-3694. *In vivo*, using multiple prostate cancer cell line xenografts such as 22Rv1, and VCaP, ZEN-3694 showed efficacy in inhibiting tumor progression at well-tolerated doses, and modulating target gene expression. ZEN-3694 also inhibited progression of a patient-derived xenograft (PDX) LuCaP 35CR that is resistant to enzalutamide.

In summary, our results indicate that ZEN-3694 demonstrates potent activity in advanced metastatic prostate cancer targeting multiple mechanisms of enzalutamide resistance, including AR-V7 signaling and GR up-regulation in different preclinical models. This together supports the clinical development of ZEN-3694 as a single agent, and in combination with enzalutamide in mCRPC patients that have failed first line ADT. We are implementing a robust translational medicine program in the phase 1 study to measure target engagement and explore mechanisms of enzalutamide resistance and sensitivity to ZEN-3694 in patients.

Background

The Bromodomain and Extra-Terminal domain (BET) family of proteins BRD2, BRD3, BRD4, and BRDT are epigenetic readers that bind via their tandem bromodomains (BD1 & BD2) to acetylated lysines in histones and promote gene transcription. Tumor type specific super-enhancers associated with key oncogenes involved in tumor pathogenesis have been identified in hematological as well as solid tumor malignancies[1,2]. Inhibition of BET proteins results in their displacement from super-enhancers leading to down regulation of key oncogenic programs, including members of the MYC, and BCL-2 families[1]. Inhibitors of the BET proteins (BETi), have been demonstrated to inhibit proliferation and suppress tumorigenicity in numerous solid and hematological malignancies. In castration-resistant prostate cancer (CRPC), BET proteins act downstream of the androgen receptor (AR) to regulate AR target gene expression (**Fig. 1A**), and BETi have the potential to target abiraterone and enzalutamide resistant patient populations.[4] ZEN-3694 shows potent inhibition of various prostate cancer cell lines, including AR-positive (AR+) and AR-negative (AR-) cell lines (**Fig. 1B**).



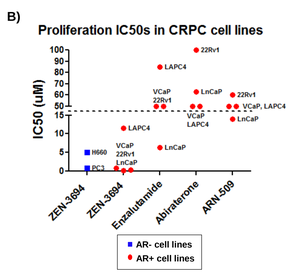
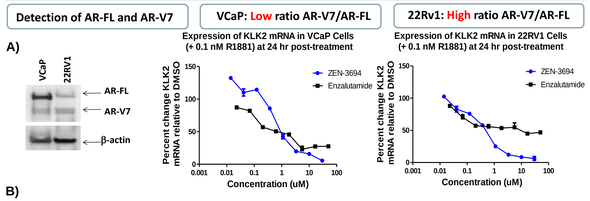


Figure 1. A) The BET proteins interact with AR to promote downstream signaling. **B)** ZEN-3694 inhibits proliferation of various prostate cancer cell lines, including AR+ and AR- cell lines that have low sensitivity to the AR antagonists enzalutamide and ARN-509, as well as the CYP17A1 inhibitor abiraterone.

Activity of ZEN-3694 in various CRPC resistance models

ZEN-3694 can inhibit AR signaling regardless of AR-V7 splice variant

Detection of AR-FL and AR-V7 | VCaP: Low ratio AR-V7/AR-FL | 22Rv1: High ratio AR-V7/AR-FL



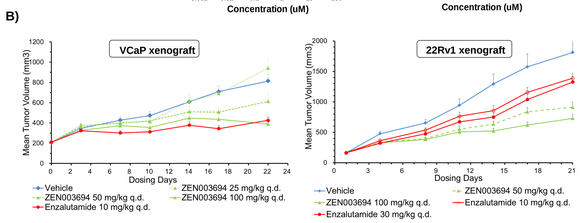


Figure 2. ZEN-3694 can inhibit AR signaling regardless of AR splice variant ratios. AR-V7 splice variant results in constitutive AR signaling and resistance to AR antagonists. **A)** VCaP cells have a low ratio of AR-V7 to full length (FL) AR, whereas 22Rv1 cells have a high ratio. ZEN-3694 can inhibit AR signaling in both VCaP and 22Rv1 cells as measured by KLK2 mRNA levels. **B)** ZEN-3694 also displays potent *in vivo* activity in subcutaneous xenografts of VCaP (enzalutamide sensitive) and 22Rv1 (enzalutamide resistant) tumors at well tolerated doses (data not shown).

GR up-regulation upon enzalutamide resistance is BET-dependent

Cell line	Enzalutamide IC50 (uM)	ZEN-3694 IC50 (uM)
LnCaP-EnzS (parental cell line)	8	1
LnCaP-EnzR (resistant cell line)	44	1

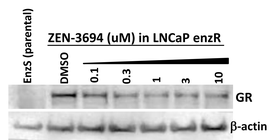


Figure 3. ZEN-3694 inhibits GR expression in an *in vitro* model of enzalutamide resistance. LEFT: Enzalutamide-resistance LnCaP cells have 5-10 fold increased resistance to enzalutamide, whereas sensitivity to ZEN-3694 is unaltered. RIGHT: Resistance to enzalutamide is associated with GR up-regulation in LnCaP[6] and ZEN-3694 reduces GR mRNA and protein levels.

Down-regulation of NEPC markers and induction of apoptosis in H660 (AR-)

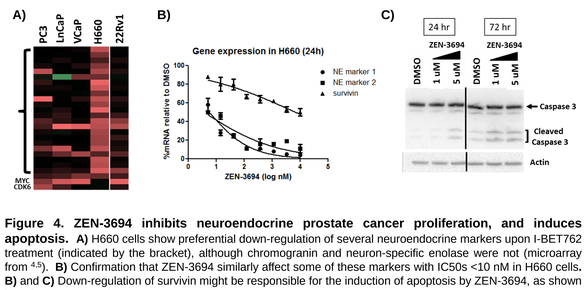


Figure 4. ZEN-3694 inhibits neuroendocrine prostate cancer proliferation, and induces apoptosis. A) H660 cells show preferential down-regulation of several neuroendocrine markers upon I-BET762 treatment (indicated by the bracket), although chromogranin and neuron-specific enolase were not (microarray from [4,5]). **B)** Confirmation that ZEN-3694 similarly affect some of these markers with IC50s <10 nM in H660 cells. **B)** and **C)** Down-regulation of survivin might be responsible for the induction of apoptosis by ZEN-3694, as shown here by caspase 3 cleavage.

A candidate BET-dependent NF-kB bone metastasis signature in PC3 cells

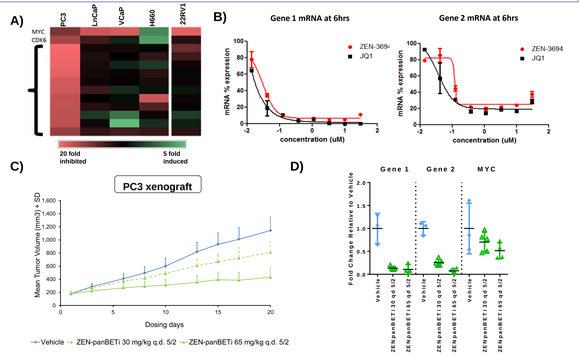


Figure 5. ZEN-3694 targets NF-kB signaling in AR null PC3 cells. A) NF-kB target genes are selectively down-regulated by I-BET762 in PC3 cells, (indicated by the bracket, microarrays adapted from [4,5]). **B)** Confirmation that ZEN-3694 similarly affects some of these markers in PC3 cells. **C)** Subcutaneous xenografts of PC3 cells treated with ZEN-panBETi show potent tumor growth inhibition at well tolerated doses (data not shown). **D)** Confirmation that NF-kB dependent genes are also down-regulated *in vivo* by ZEN-panBETi.

Activity of ZEN-3694 in the LuCaP 35CR enzalutamide-resistant PDX model

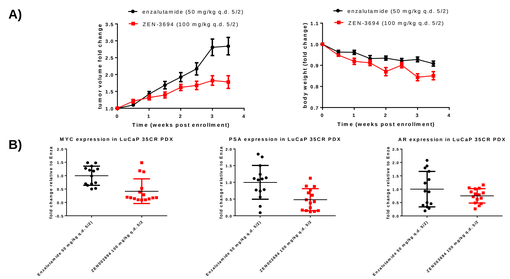


Figure 6. ZEN-3694 is active in the enzalutamide-resistant, AR-amplified, LuCaP 35CR patient-derived xenograft. Subcutaneous xenografts of the LuCaP 35CR tumors treated with ZEN-3694 or enzalutamide. **A)** tumor size and body weight. **B)** MYC, PSA, AR mRNA expression was analyzed by qPCR.

ZEN-3694 synergizes with enzalutamide and induces p57/KIP2 in VCaP cells

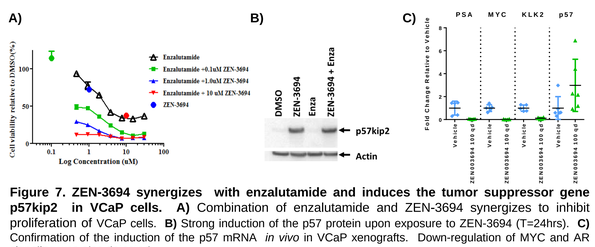


Figure 7. ZEN-3694 synergizes with enzalutamide and induces the tumor suppressor gene p57kip2 in VCaP cells. A) Combination of enzalutamide and ZEN-3694 synergizes to inhibit proliferation of VCaP cells. **B)** Strong induction of the p57 protein upon exposure to ZEN-3694 (T=24hrs). **C)** Confirmation of the induction of the p57 mRNA *in vivo* in VCaP xenografts. Down-regulation of MYC and AR signaling was also detected.

ZEN-3694 Phase I Trial in mCRPC

PD marker modulation in *ex-vivo* treated human whole blood

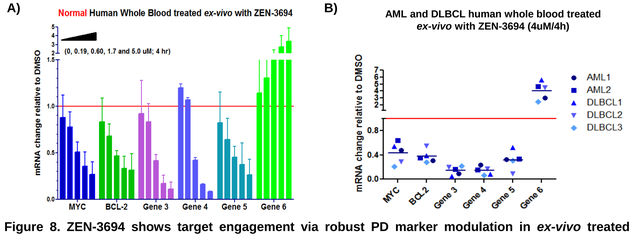


Figure 8. ZEN-3694 shows target engagement via robust PD marker modulation in *ex-vivo* treated normal and patient whole blood. A) Human whole blood from seven normal donors shows dose-dependent modulation of PD marker mRNA when treated *ex-vivo* with ZEN-3694 for 4hrs. **B)** PD marker modulation in whole blood from AML and DLBCL patients showing good inter-individual correlation

ZEN-3694 has entered phase I clinical trial in mCRPC

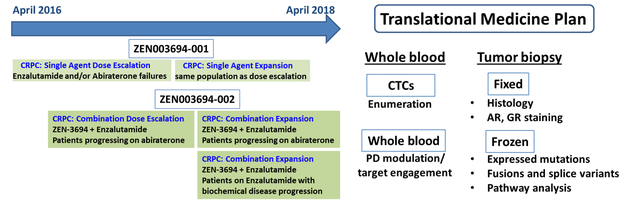


Figure 9. Design of the phase I clinical trial of ZEN-3694 in mCRPC. In the first protocols (ZEN003694-001, NCT02705469), a dose escalation phase with ZEN-3694 as single agent will start in Q1 2016, followed by a dose expansion phase planned for Q1 2017. In the second protocol (ZEN003694-002, NCT02711956), a combination with enzalutamide dose escalation phase and expansion phase are planned for Q2 2016, and Q1 2017, respectively.

Summary

- **ZEN-3694 shows potent activity in several models of CRPC enzalutamide resistance including AR amplification, AR-V7 splice variant, GR up-regulation, NEPC.**
- **A candidate BET-dependent NF-kB bone metastasis signature was found in the PC3 cell line model.**
- **ZEN-3694 synergizes with enzalutamide to inhibit proliferation of VCaP cells, and robustly induces the p57/KIP2 tumor suppressor gene.**
- **ZEN-3694 has entered clinical trials in mCRPC as a single agent, and in combination with enzalutamide.**

References

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