The clinical candidate ZEN-3694, a BET bromodomain inhibitor, is efficacious in the treatment of a variety of solid tumor and hematological malignancies, alone or in combination with several standard of care therapies

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Abstract

ZEN-3694 is an orally bioavailable small molecule discovered and developed from a BET bromodomain inhibitor platform. In vitro, ZEN-3694 selectively binds to BET proteins with >20 fold selectivity over non-BET bromodomains inhibiting the interaction of acetylated histone peptide with IC50 values in low nM range. ZEN-3694 inhibits proliferation of MV4-11 AML cells with an IC50 of 0.2 uM, and inhibits MYC mRNA expression with an IC50 of 0.16 uM.

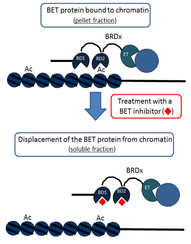
ZEN-3694 has also demonstrated strong activity against many solid tumor and hematological cell lines with sub-uM IC50 values. In vitro synergy with Standard of Care (SOC) agents has been shown in a wide variety of malignancies including Breast, Prostate, Lung, Melanoma, AML, and DLBCL. Xenograft studies conducted with ZEN-3694 in AML, prostate and breast cancer models have demonstrated that it is efficacious at well-tolerated doses, modulating target gene expression and halting tumor growth in a dose-dependent manner.

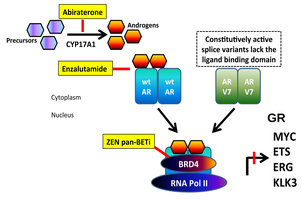
In the AR positive VCAP prostate cancer cell line, ZEN-3694 inhibits proliferation synergistically with the AR antagonists enzalutamide and ARN-509. In an in vitro enzalutamide resistance model, glucocorticoid receptor (GR) is upregulated and is sufficient to confer enzalutamide resistance, as reported by others. Here, we show that ZEN-3694 inhibits GR expression, and that enzalutamide resistant cells are sensitive to ZEN-3694.

Robust PD modulation has been observed across multiple matrices for ZEN-3694 and will be explored further in the clinic. Promising target validation data, excellent pharmacological properties, and robust activity of ZEN-3694 across a variety of hematological malignancy and solid tumor settings support the clinical development of ZEN-3694 in both of these therapeutic indications.

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 transcriptional programs involved in key oncogenic programs, including members of the MYC, BCL-2 families[1]. Inhibitors of the BET bromodomains (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, and BETi have the potential to target abiraterone and enzalutamide resistant patient populations.[4]





Results

ZEN-3694 is a novel BETi that inhibits proliferation of several cancer cell lines, and synergizes with various anti-cancer agents

Figure 1. ZEN-3694 has an excellent in vitro profile.

	ZEN-3694
FRET BRD4 (1) IC_{50}	<25 nM
C-Myc IC_{50}	<200 nM
MV4-11 proliferation IC_{50}	<250 nM

Figure 2. CRPC cell lines are more sensitive to ZEN-3694 than AR antagonists in the presence of androgen. Cells were treated for 3 or 7 days in a 96-well assay in the presence of R1881 and compounds, and proliferation was measured by Cell Titer-Fluor.

Cell line	Mutations	AR Splice variants	ZEN-3694 IC50 (uM)	Enzalutamide IC50 (uM)	Abiraterone IC50 (uM)
22RV1	• AR H874Y	AR-V3 and variants, AR-V7	0.19	~50	>100
VCAP	• AR AMP • TMPRSS2-ERG	AR-V1 to V3, AR-V7, AR-V8 to V11, ARv567es	0.9	>50	>50
LnCAP	• AR T877A • MIPOL1-ETV1	AR45	0.40	~6.4	62.6
LAPC4	• WT AR	ARV3	11.5	85	>50
PC3	• AR-, PTEN	No androgen receptor expressed	>30	N/A	N/A

Figure 3. ZEN-3694 inhibits proliferation of TNBC and breast ER+ cell lines in vitro Cells were treated for 3 or 7 with ZEN-3694, and proliferation was measured by Cell Titer-Fluor.

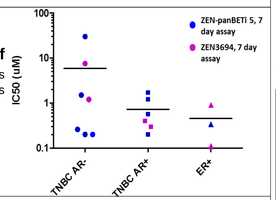
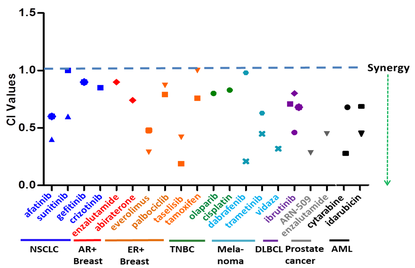


Figure 4. ZEN-3694 synergizes with several standard of care and targeted therapies in numerous types of cancer. Cells were treated in constant dose ratio combination for 3 days and proliferation was measured using Cell-Titer Fluor. Combination Index (CI) was calculated using the Chou-Talalay Index[5]



ZEN-3694 targets mechanisms of enzalutamide resistance in CRPC

Figure 5. ZEN-3694 can inhibit AR signaling in cells with high AR splice variant ratios. VCaP and 22RV1 cells were treated with ZEN-3694 or enzalutamide for 24h in the presence of androgen, and the AR target gene KLK2 was measured by realtime PCR

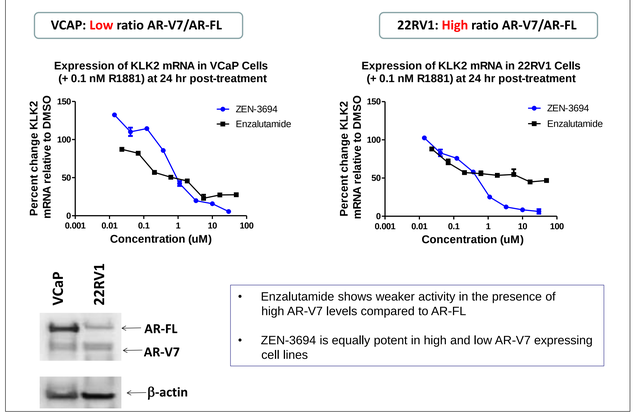
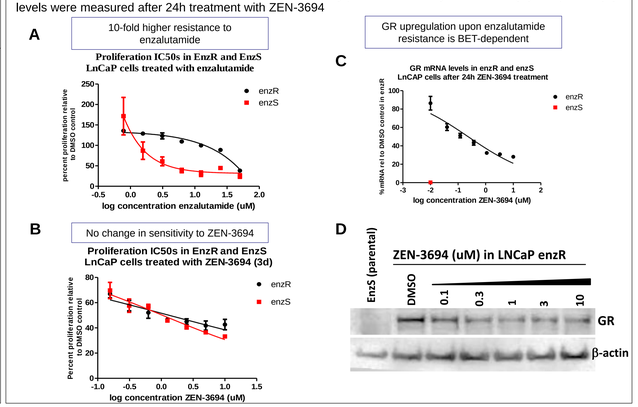
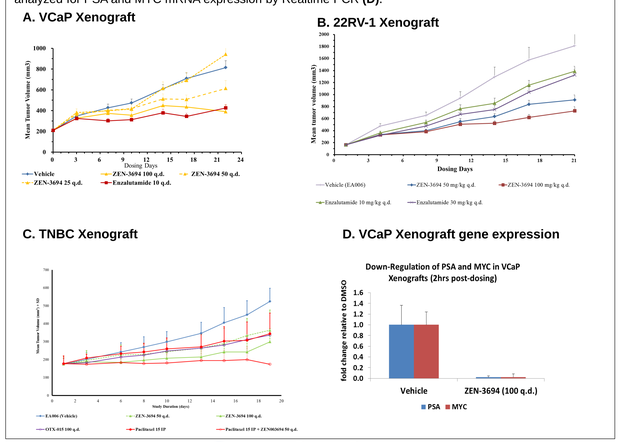


Figure 6. ZEN-3694 can inhibit GR expression in an in vitro model of enzalutamide resistance. LNCaP cells were cultured in 10uM enzalutamide for 60 d, and sensitivity to enzalutamide **(A)** and ZEN-3694 **(B)** was measured by 3 day proliferation assay. GR mRNA **(C)** and protein **(D)** levels were measured after 24h treatment with ZEN-3694



ZEN-3694 inhibits CRPC and TNBC xenograft tumor growth

Figure 7. ZEN-3694 inhibits VCaP and 22RV-1 xenograft tumor growth, and downregulates AR and MYC signaling in tumors, and inhibits TNBC xenograft tumor growth and synergizes with paclitaxel. Subcutaneous xenografts of VCaP **(A),** 22RV1 **(B)** and MDA-MB-231 **(C)** tumor cells in SCID or athymic mice were treated p.o. with ZEN003694 at doses ranging from 25 to 100 mg/kg q.d., the comparator OTX-015 at 100 mg/kg q.d., enzalutamide at 10 or 30 mg/kg q.d., or IP with Paclitaxel at 15 mg/kg. VCaP xenograft tumors were analyzed for PSA and MYC mRNA expression by Realtime PCR **(D)**.



Conclusions

1. **ZEN-3694 is a novel, selective, and potent BET inhibitor**
2. **ZEN-3694 is more active than AR antagonists in CRPC**
3. **ZEN-3694 targets mechanisms of enzalutamide resistance, including AR-V7 splice variants and GR upregulation**
4. **ZEN-3694 synergizes with many standard of care and targeted therapies in several types of cancers**
5. **ZEN-3694 is efficacious in CRPC and TNBC xenograft models**

References

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