The BET Bromodomain Inhibitor ZEN-3694 Modulates the Expression of Checkpoint Receptors and Immune Suppressive Factors in the Blood of mCRPC Patients

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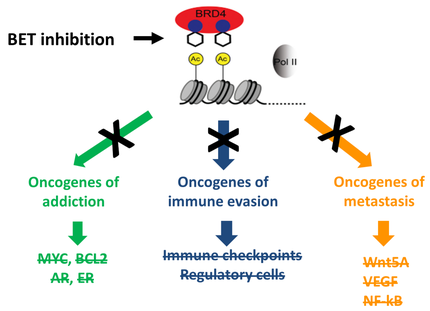
Background

- Bromodomain and Extra-Terminal domain (BET) family of proteins (BRD2, BRD3, BRD4, and BRDT) can bind acetylated lysines through their tandem bromodomains to promote gene transcription.
- BET bromodomain inhibitors (BETi) target super enhancers and inhibit several programs involved in tumorigenesis such as proliferation, metastasis, invasion, and immune evasion.[1,2]
- ZEN-3694 has been previously shown to target checkpoint receptors, and act synergistically with PD1 antibodies in both *in vitro* and *in vivo* pre-clinical models.[3]
- Here, we studied the immune profile of ZEN-3694 given at well-tolerated doses during the dose escalation and expansion clinical trial in metastatic castration-resistant prostate cancer (mCRPC) patients.



Patients and Methods

Patients dosed

- mCRPC patients enrolled in the single agent phase 1 dose escalation and dose expansion study of ZEN-3694 (NCT02705469) received a once daily dose of ZEN-3694.
- Whole blood was collected on C1D1 at 0, 4 and 24 hours post first dose.

Nanostring + RT-PCR

- Total RNA was isolated and the Nanostring nCounter® PanCancer Immune Profiling Panel was used to measure gene expression.
- Advanced analysis was performed on patients that were dosed below MTD (maximum tolerated dose).
- Real-time PCR analysis was performed on selected genes, either expressed at low levels, or to confirm the Nanostring results.

Pathway analysis

- 20 patients were selected and ranked by plasma drug concentration at 4h.
- Ingenuity® Pathway Analysis was performed to predict activated and repressed immune pathways.

References

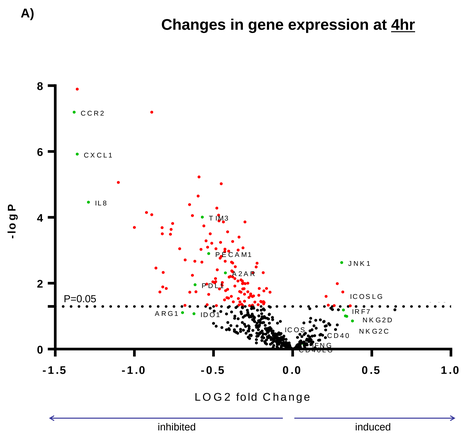
1. Loven et al. (2013) Selective Inhibition of Tumor Oncogenes by Disruption of Super-Enhancers. *Cell* 153, 320–334

2. Hnisz et al. (2013) Super-Enhancers in the Control of Cell Identity and Disease. *Cell* 155, 1–14

3. Attwell et al. (2016) The investigational drug ZEN-3694, a novel BET-bromodomain inhibitor, inhibits multiple tumor immune escape mechanisms and has the potential to combine with immunotherapies. AACR-NCI-EORTC 2016, *poster presentation* available at http://www.zenithepigenetics.com/newsroom/presentations-&-publications

Results

A single dose of ZEN-3694 below MTD modifies the expression of multiple tumor immune response genes



Gene name	Fold change relative to pre-dose	P value (16 patients)
CXCL1	0.37	3.13E-08
IL8	0.4	1.96E-05
CCR2	0.38	5.07E-08
PD-L1	0.66	0.020
TIM3	0.66	0.0001
A2AR	0.72	0.003
ARG1	0.59	0.067
IDO1	0.63	0.080
PECAM	0.68	0.001
IRF7	1.25	0.135
ICOSLG	1.26	0.069
NKG2C	1.38	0.110
NKG2D	1.27	0.12
JNK1	1.24	0.004

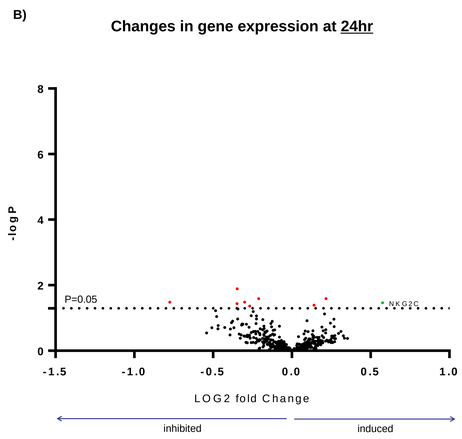


Figure 2. ZEN-3694 significantly inhibits checkpoint receptors and immune suppressive factors A) 131 significant changes (shown in red) were detected after the first 4 hours of dosing across 16 patients enrolled in dose escalation and expansion. Several checkpoint receptors and myeloid suppressive factors were inhibited across patients, while multiple co-stimulatory factors and NK cell activating receptors were either unchanged or slightly induced. ZEN-3694 significantly inhibited the checkpoint receptors TIM3, PD-L1, and A2AR, while leaving co-stimulatory receptors unchanged or slightly induced. Suppressive myeloid chemokine/chemokine receptors axes were also significantly inhibited: CCR2/CCL2, IL8/CXCL1. **B)** The majority of significant changes returned to baseline at 24h. Note: significant changes shown in red, genes of interest shown in green.

Analysis of gene expression of selected genes by real time PCR

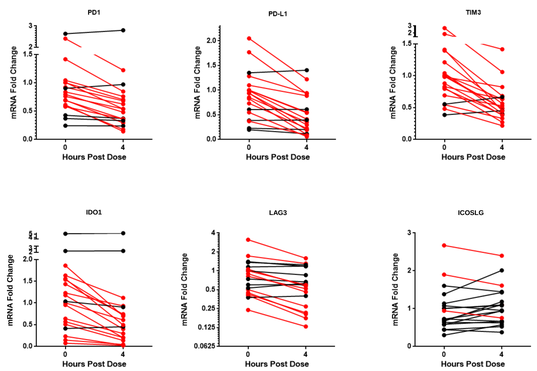


Figure 3. ZEN-3694 inhibits expression of multiple checkpoint receptors, but not the co-stimulatory receptor ICOSLG. Several genes were selected for confirmation by real time PCR. Additionally, PD1, and LAG3 were expressed at a level too low to be detected by the Nanostring platform, and thus were analyzed by real time PCR. Each line represents one patient, and inhibition of ≥10% is shown in red.

Differing patterns of exposure-response to ZEN-3694

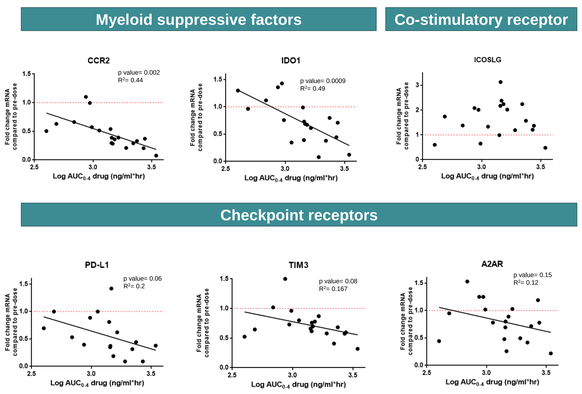


Figure 4. Several targets display a significant drug exposure-response correlation. Nanostring values were plotted against patient $AUC_{0\text{-}4h}$. Myeloid suppressive factors CCR2 and IDO1 show a significant drug exposure response correlation, whereas ICOSLG is induced in most patients regardless of drug concentration. Several checkpoint receptors showed a trend to be more inhibited with increasing exposure, although results were more variable across patients.

Low doses of ZEN-3694 may be superior to high doses for T cell signaling activation

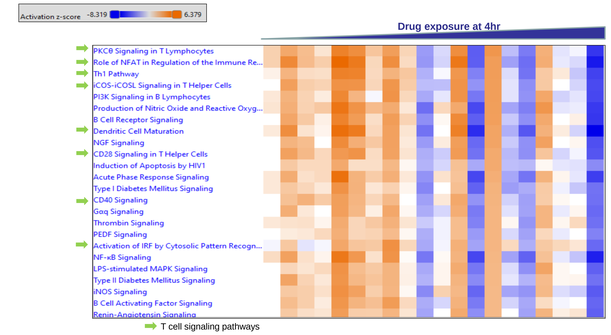


Figure 5. Low exposures of ZEN-3694 show superior activation of T cell signaling pathways over high exposures. Twenty patients were ranked according to drug exposure and Ingenuity® Pathway Analysis was performed. Orange= pathway activation and Blue= Pathway Inhibition

Summary

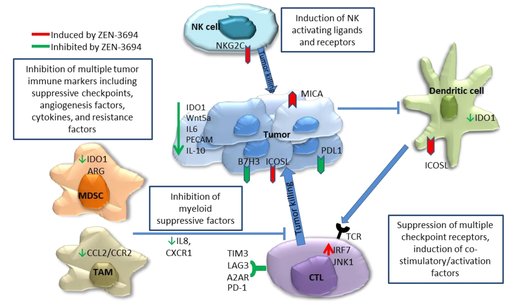


Figure 6. ZEN-3694 targets multiple mechanisms of adaptive resistance to immunotherapies. Here, we show that ZEN-3694 modulates multiple immune markers in patient's blood which play a role in adaptive resistance. We have previously shown that ZEN-3694 also targets multiple adaptive resistance mechanisms in the tumor[3]. Taken together, these results suggest that ZEN-3694 could combine synergistically with immunotherapies in the clinic, both to prevent or reverse resistance and increase response rates.

- **BETi display unique properties among epigenetic modulators for their potential to increase the effectiveness of immunotherapies, e.g. through checkpoint receptor downregulation.**
- **A single dose of ZEN-3694 significantly modulates tumor immune response genes at well tolerated doses in mCRPC patients.**
- **ZEN-3694 significantly inhibits several checkpoint receptors and suppressive myeloid chemokine/chemokine receptors axes, while co-stimulatory receptors are either unchanged or slightly induced.**
- **Patients with low drug exposure are predicted to show superior T cell pathway activation compared to higher drug exposures.**
- **Further immune function studies are underway to measure long term effects of daily dosing of ZEN-3694 on immune activation and tumor recognition in patients.**