The BET inhibitor ZEN-3694 Blocks Multiple Tumor Immune Suppressive Factors and Has the Potential to Increase the Efficacy of Anti-PD1 Treatment

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Background

- Bromodomain and Extra-Terminal domain (BET) family of proteins (BRD2, BRD3, BRD4, and BRDT) promote gene transcription through binding of tandem bromodomains to acetylated lysines on histones.
- BET bromodomain inhibitors (BETi) target 'super enhancers' and inhibit several oncogenic programs such as proliferation, metastasis, invasion, and immune evasion[1,2].
- ZEN-3694 has been previously shown to target checkpoint receptors, and act synergistically with anti-PD1 antibodies in both *in vitro* and *in vivo* pre-clinical models[3], as well as significantly modulate checkpoints and immune suppressive markers in the blood of mCRPC patients (NCT02705469)[4].
- Here, we assessed if ZEN-3694 could modulate markers of anti-PD1 resistance in immune-infiltrated, anti-PD1-approved cancers NSCLC, melanoma, and RCC.

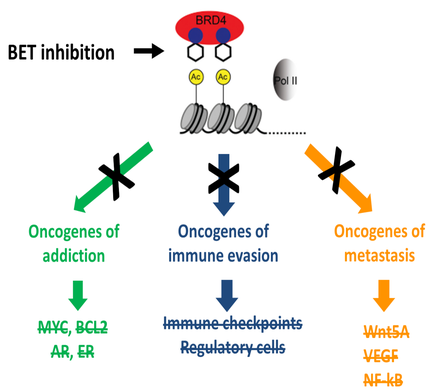

Figure 1. BET inhibitors prevent the binding of BET proteins to acetylated lysines on histones, blocking the induction of superenhancer-driven gene expression

Methods

NSCLC Blood

- Fresh blood from 6 non-small cell lung cancer patients was treated ex vivo for 4h with 1 uM ZEN-3694.
- RNA was extracted and gene expression changes were detected by Nanostring nCounter® PanCancer Immune Profiling Panel.
- GSEA analysis was performed on select genesets.

Melanoma Cell RNA-seq and GSEA analysis

- A375 melanoma cells were treated with 1uM ZEN-3694 for 24h, and total RNA was extracted and RNA-seq performed.
- GSEA analysis was performed on the 26 genesets of the Innate PD1 Resistance (IPRES) signature [5].

Tumor/T-cell co-incubation studies

- An anti-PD1-resistant primary human melanoma tumor sample was co-incubated with patient-matched tumor infiltrating lymphocytes (TILS) for 24h, and media was collected and IFN-γ secretion assessed by ELISA.
- Mouse melanoma B16F10 cells were co-incubated with CD8+ T-cells from pmel-1 mouse spleens (transgenic for a T-cell receptor that recognizes the melanocyte differentiation antigen gp100), for 3 days, in the presence of IFN-γ and ZEN-3694. T cells were then removed, and cell viability measured using cell titer fluor.

Population studies

- TCGA datasets for mRNA expression in melanoma and Renal Clear Cell Carcinoma (RCC) tumors were separated by BRD4 expression levels (high vs. low/medium), and survival was plotted. The same analysis was performed after sorting the populations by CD8 expression or PBRM1 mutational status. All data downloaded from The Cancer Genome Atlas, Genomic Data Commons.
- mRNA expression datasets from a melanoma anti-PD1-treated cohort[5] were plotted for CD8A and BRD4 expression.

References

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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*

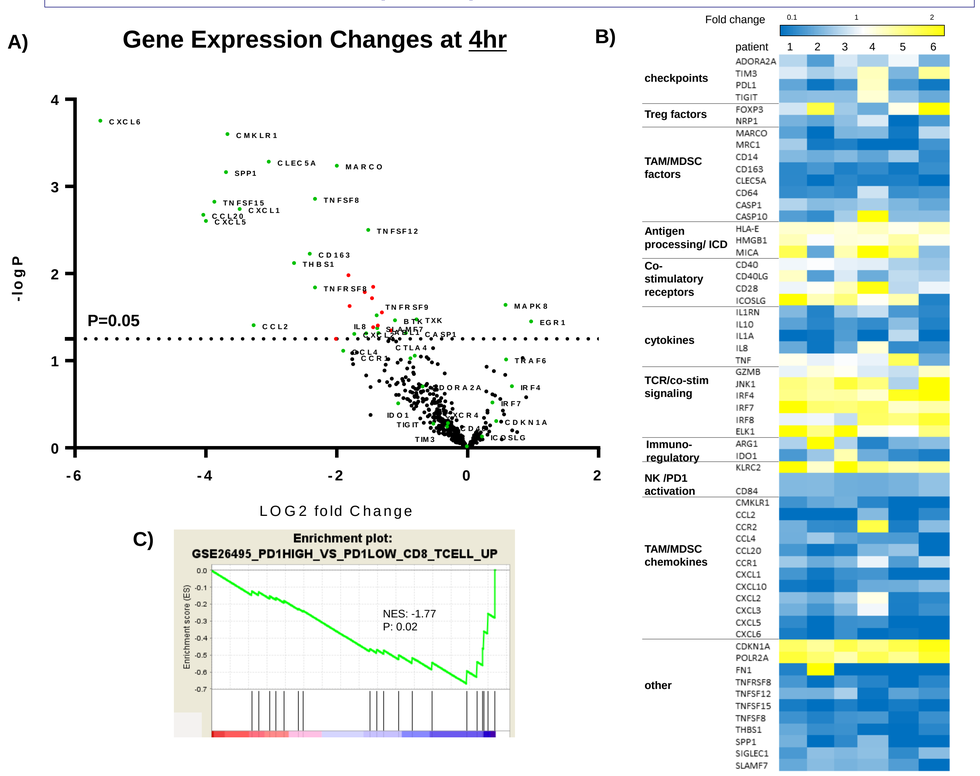
4. Attwell et al. (2017)* The BET bromodomain inhibitor ZEN-3694 modulates the expression of checkpoint receptors and immune suppressive factors in the blood of mCRPC patients. SITC 2017*

**poster presentations* available at http://www.zenithepigenetics.com/newsroom/presentations-&-publications

5. Hugo et al. (2016) Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma

Results

ZEN-3694 Targets Anti-PD1 Resistance Genes in Non-small Cell Lung Cancer (NSCLC) Patient Blood



ZEN-3694 Targets IPRES (Innate anti-PD1 RESistance) Signature in Melanoma Tumor Cells

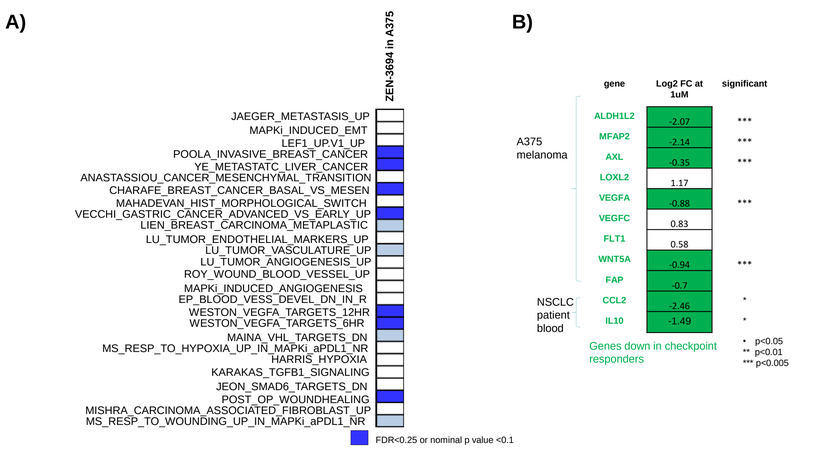


Figure 3. ZEN-3694 reverses an anti-PD1-resistance signature in melanoma cells. GSEA analysis was performed on RNA-seq data from A375 melanoma cells treated with 1uM ZEN-3694 for 24h. The IPRES signature consists of 26 pathways significantly downregulated in melanoma patients who responded to anti-PD1 treatment[5]. Of these 26 pathways, 7 were significantly inhibited by ZEN-3694, and none were significantly induced. **A**) GSEA pathway analysis **B**) Effect of ZEN-3694 on key genes in the IPRES signature.

Figure 2. ZEN-3694 inhibits anti-PD1 resistance genes in blood Gene modulation in the blood of NSCLC patients treated with 1uM ZEN-3694 for 4h ex vivo. Gene expression changes were detected by Nanostring nCounter® PanCancer Immune Profiling Panel. ZEN-3694 significantly inhibits expression of several genes involved in primary and adaptive anti-PD1 resistance, including checkpoint receptors and immune suppressive cell factors. **A**) Volcano plot of all changes **B**) Fold change of select genes. **C**) Genes associated with PD1-*high* vs PD1-*low* CD8 T-cells are inhibited by ZEN-3694.

ZEN-3694 Induces IFN-γ secretion and Enhances T-cell killing Activity in Anti-PD1 Resistant Melanoma

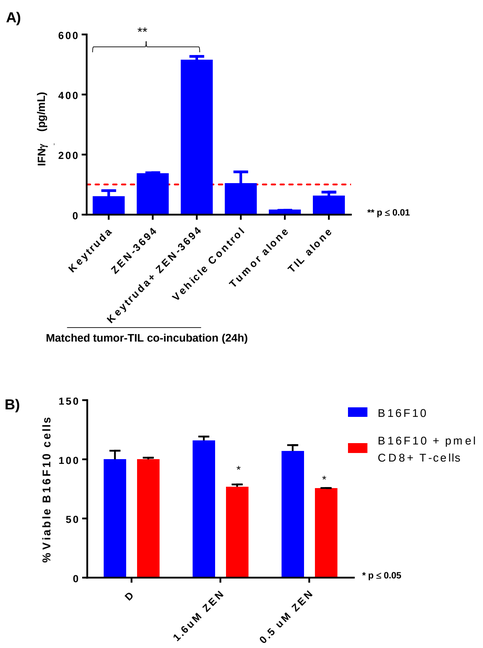


Figure 4. ZEN-3694 enhances IFN-γ secretion and T-cell killing function. A) ZEN-3694 synergistically induces IFN-γ secretion with pembrolizumab in an anti-PD1-resistant patient-derived tumor-TIL melanoma sample. **B)** ZEN-3694 enhances tumoricidal activity of pmel mouse CD8+ T-cells to kill B16F10 mouse melanoma cells in vitro.

Targeting BRD4 May Improve TIL Infiltration and Survival Rates in Melanoma and RCC

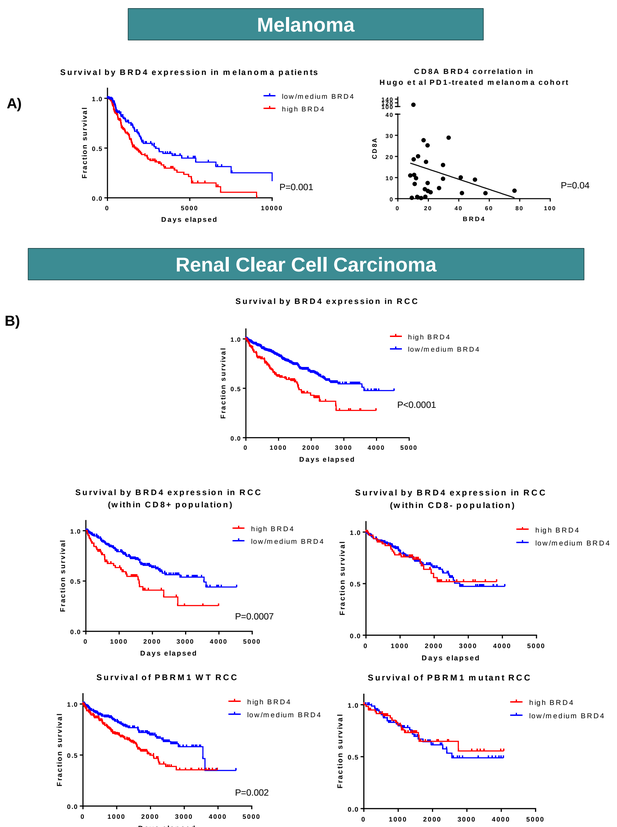


Figure 5. BRD4 is a poor prognosis factor in melanoma and Renal Clear Cell Carcinoma. A) BRD4 mRNA expression is a poor prognosis factor in a melanoma TCGA cohort (left). In an anti-PD1-treated melanoma patient cohort, tumor expression of BRD4 negatively correlates with CD8A expression (right) **B)** BRD4 expression is a poor prognosis factor in Renal Clear Cell Carcinoma (top), specifically in the CD8+ T-cell infiltrated population, and the PBRM1 wildtype population (middle and bottom).

Summary

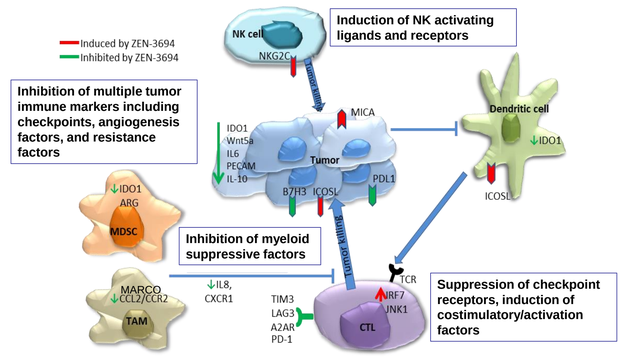


Figure 6. ZEN-3694 targets multiple mechanisms of adaptive resistance to immunotherapies. ZEN-3694 modulates multiple immune markers in blood and tumor cells that play a role in anti-PD1 resistance. We have previously shown that ZEN-3694 acts synergistically with anti-PD1 in a syngeneic mouse model, and modulates immune markers in the blood of mCRPC patients at tolerable doses. Taken together, these results suggest that ZEN-3694 could combine synergistically with anti-PD1 in the approved indications NSCLC, melanoma, and RCC, both to prevent or reverse resistance and increase response rates.

- **BET inhibition displays unique properties among epigenetic modulators for their potential to increase the effectiveness of anti-PD1 therapies.**
- **ZEN-3694 reverses anti-PD1-resistance signatures in both blood and tumor cells.**
- **ZEN-3694 increases T-cell activation and decreases tumor cell survival in tumor/T-cell co-incubation studies.**
- **Population studies suggest that targeting BRD4 in melanoma and RCC may be an effective treatment strategy leading to increased TIL infiltration and survival rates.**
- **Further functional studies are underway to measure long term effects of daily dosing of ZEN-3694 on immune activation and tumor recognition in patients.**
- **Potential identification of biomarkers of anti-PD1 resistance or ZEN-3694 response could aid in the design of a combination trial.**