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 evasion1,2.
- 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 models3, 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.

Methods

- ZEN-3694 induces IFNγ secretion and Enhances T-cell killing activity in Anti-PD1 Resistant Melanoma
- Targeting BRD4 May Improve TIL Infiltration and Survival Rates in Melanoma and RCC
- ZEN-3694 reduces anti-PD1 resistance signatures 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 treatment2. 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.

Studies

- 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.

References

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4. Ahmed et al. (2017)” The BET bromodomain inhibitor ZEN-3694 modulates the expression of checkpoint receptors and immune suppressive markers in the blood of mCRPC patients. AACR-NCI-EORTC 2016
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Figure 1. BET inhibitors prevent the binding of BET proteins to acetylated lysines on histones, blocking the induction of superenhancer-driven gene expression

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 CDR T-cells are inhibited by ZEN-3694.

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 treatment2. 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 4. ZEN-3694 enhances IFNγ secretion and T-cell killing function. A) ZEN-3694 synergistically induces IFNγ secretion with pembrolizumab in an anti-PD1-resistant patient-derived tumor-TIL melanoma sample. B) ZEN-3694 enhances tumoridal activity of pome mouse CD8+ T-cells to kill B16F10 mouse melanoma cells in vitro.

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).

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.