The investigational drug ZEN-3694, a novel BET-bromodomain inhibitor, inhibits multiple tumor immune escape mechanisms and has the potential to combine with immunotherapies

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Abstract

ZEN-3694 is an orally bioavailable, potent inhibitor of both bromodomains of the Bromodomain and Extra-Terminal domain (BET) proteins. *In vitro*, ZEN-3694 has demonstrated strong activity against cell lines representing a broad range of solid tumor and hematological malignancies with submicromolar potency, and shows *in vivo* activity in several xenograft models, including prostate, breast, colon and AML. ZEN-3694 is currently in phase 1 clinical trials for metastatic castration resistant prostate cancer patients who have progressed on enzalutamide and/or abiraterone.

Immunotherapies targeting the PD1/PD-L1 axis have shown remarkable durable efficacy for many cancers, but still, the majority of patients do not respond to these therapies alone, and therefore there is a need to identify combination agents which will increase the response rate.

Here we show that ZEN-3694 targets many pathways which suppress the anti-tumor immune response. In solid tumor cell lines representing a variety of malignancies, ZEN-3694 downregulates the checkpoints B7H3 and PD-L1, and upregulates the MICA antigen. In activated CD8+ T cells, ZEN-3694 targets multiple checkpoint receptors known to be involved in tumor escape. ZEN-3694 also inhibits the differentiation and function of Regulatory T cells (Tregs), and strongly inhibits the suppressive cytokines/chemokines IL-10 and CCL2. ZEN-3694 also targets several recently identified markers of intrinsic PD-1 resistance, and inhibits the angiogenic factor VegF. Effects on these various markers of immunomodulation are being confirmed in our current phase I study.

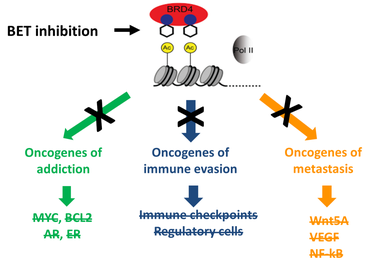
Immunomodulatory effects were also measured *in vivo.* In an MC-38 colon cancer syngeneic xenograft model, the addition of ZEN-3694 increases the efficacy of anti-PD1 in tumor growth inhibition. The ZEN-3694 treated mice showed a significant increase in IFNg+ CD8 T cells in the draining lymph nodes, as well as an increase in CD8+ tumor infiltrating lymphocytes (TILS) in the tumor. Analysis of the tumors showed a decrease in markers of myeloid suppressive cells.

Taken together these data suggest that ZEN-3694 targets several mechanisms of resistance to PD1 therapy, and has the potential to synergize with a variety of cancer immunotherapies.

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]. Additionally, BET inhibitors (BETi) target pathways involved in metastasis, such as the NF-kB and Wnt5a pathways[3,4]. BET inhibitors have been demonstrated to inhibit proliferation and suppress tumorigenicity in numerous solid and hematological malignancies.

Although PD1 antibodies have shown remarkable and durable efficacy in a portion of cancers, a variety of immune mechanisms contribute to both innate and acquired resistance in the majority of patients. These include upregulation of alternate tumor and T cell checkpoint receptors, recruitment of suppressive cells which dampen the T cell response, and tumor mechanisms to decrease immune recognition. Here we show that BET inhibitors have an additional unique mechanism of action: they inhibit multiple complementary mechanisms of tumor immune escape, which suggest that they could synergize with immunotherapies.



Results

ZEN-3694 targets a unique set of checkpoint receptors, cytokines, and tumor factors

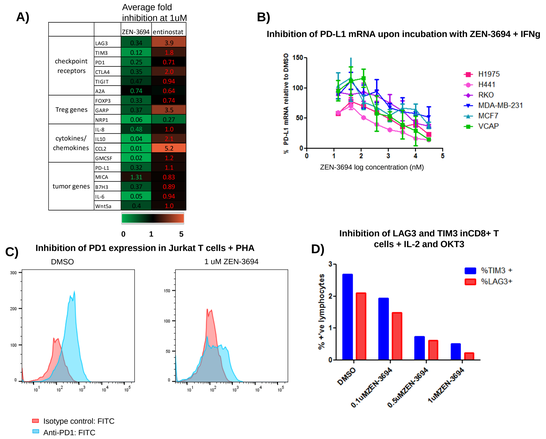


Figure 2. ZEN-3694 inhibits mRNA expression of checkpoint receptors, cytokines, and tumor antigens, and the cell surface expression of the checkpoints PD-1, LAG3, and TIM3. A) BET inhibitors are unique in their capacity to inhibit several important markers involved in tumor immune escape compared to entinostat (shown) and other epigenetic inhibitors (not shown). Gene expression was measured in PBMCs, or A549 lung cancer cells. B) ZEN-3694 inhibits PD-L1 mRNA expression in a variety of solid tumor cancer cell lines. C) Treatment of Jurkat T cells with ZEN-3694 reduces the levels of cell surface PD-1 D) Dose-dependent inhibition of cell surface levels of LAG3 and TIM3 checkpoints upon treatment of CD8+T cells with ZEN-3694

ZEN-3694 inhibits Treg differentiation and function

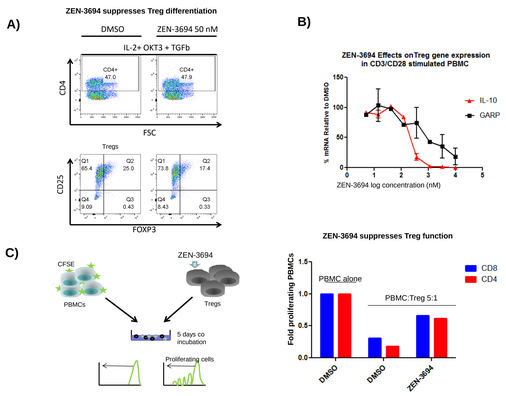


Figure 3. ZEN-3694 inhibits Treg differentiation, activation, and function. A) Tregs were differentiated from PBMCs. Incubation with ZEN-3694 inhibits the differentiation of Treg (BOTTOM) without affecting the number of CD4+ T cells (TOP) B) ZEN-3694 inhibits the mRNA of two markers of activated Treg cells, IL-10 and GARP. C) LEFT: schematic of experiment for Treg functional assay: Tregs were pre-treated with ZEN-3694, and then incubated with PBMCs stimulated to proliferate. Proliferating PBMCs gradually lose the CFSE label. RIGHT: Pre-incubation of Treg cells with ZEN-3694 decrease their inhibitory potential which results in increase proliferation of PBMCs.

ZEN-3694 increases markers of tumor immunogenicity

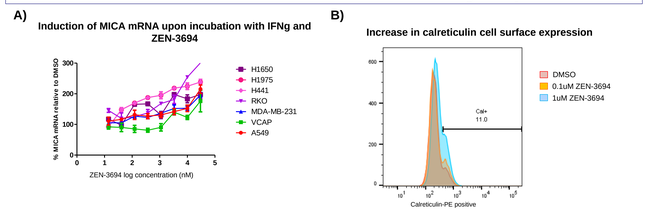


Figure 4. ZEN-3694 induces expression of the NK cell ligand MICA, and the cell surface expression of calreticulin, a phagocytosis marker for dendritic cells. A) ZEN-3694 increases mRNA levels of the major histocompatibility complex class I polypeptide-related sequence A (MICA) in a variety of solid tumor cell lines, without increasing shedding of MICA in the media (not shown). B) MV4-11 cells were incubated for 24 hours with ZEN-3694, which resulted in the increase of cell surface calreticulin, which is associated with increased immunogenicity of tumors, also known as immunogenic cell death.

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ZEN-3694 targets multiple suppressive factors, and influences T cell activation in MC-38 colon cancer syngeneic mouse models

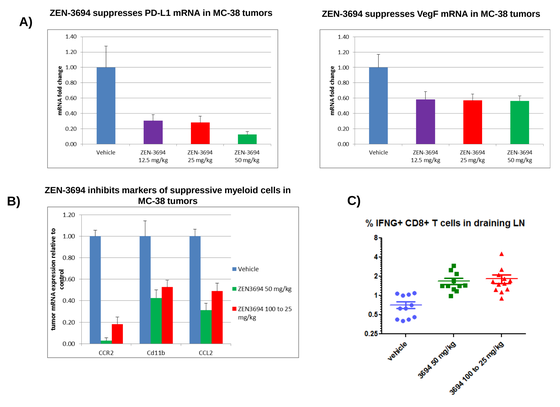
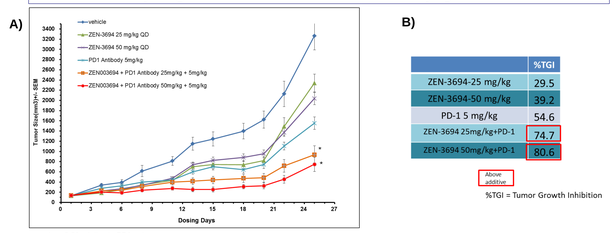


Figure 5. ZEN-3694 inhibits expression of PD-L1 mRNA and other immune suppressive markers in MC-38 tumors, and increases T cell activation in draining lymph nodes and tumors A) Treatment of mice with ZEN-3694 results in a decrease in PD-L1 and VEGFA tumor mRNA B) Decrease in CCR2, CD11b, and CCL2 , markers of the suppressive TAM and MDSC response. C) ZEN-3694 induces an increase in activated (IFNG+) CD8+ T cells in the draining lymph node D) ZEN-3694 increases TIL infiltration into the tumor, including CD8, CD4, and total activated (CD69+) T cells

ZEN-3694 is synergistic with anti-PD1 in the MC-38 syngeneic mouse model



*p<0.01 relative to PD1 only group

Figure 6. ZEN-3694 shows synergistic anti-tumor activity with anti-PD1 in the MC-38 model. A) MC-38 tumors were injected subcutaneously in C57Bl/6 mice. Once the tumors reached approximately 130 mm³, mice were dosed daily with ZEN-3694 given orally, or once every 3 or 4 days intraperitoneally with anti-PD1 (BioXcell). Mice were sacrificed for final tumor weights after 25 days of dosing. B) Final % TGI relative to vehicle.

ZEN-3694 has the potential to increase the anti-tumor immune response

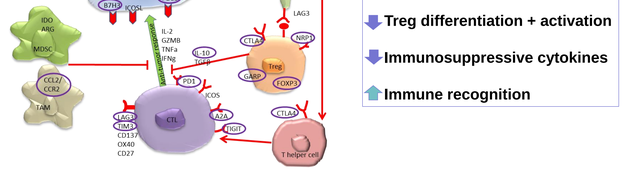


Figure 7. ZEN-3694 targets the expression of tumor and immune cell factors to increase the anti-tumor immune response. BETi have the potential to prime the tumor for increase immune recognition and killing, by inhibiting the checkpoints B7H3 and PD-L1, and increasing tumor antigen MICA expression. They also target suppressive cytokines such as IL-6, IL-10, and VegF. Furthermore, BETi target factors involved in the suppressive cell response, and ,most importantly, target multiple immune checkpoint receptors, suggesting that they may combine well with PD1 and other checkpoint antibodies

Summary

- **ZEN-3694 inhibits Treg cell differentiation and suppressive function.**
- **ZEN-3694 modulates a unique set of checkpoint receptors, cytokines and antigens on immune and tumor cells which were not targeted by the epigenetic modifier entinostat**
- **ZEN-3694 induces the tumor antigen MICA, and increases calreticulin cell surface expression, a marker of immunogenic cell death**
- **ZEN-3694 inhibits the mRNA expression of tumor PD-L1 and VegF, as well as the myeloid and macrophage suppressive cell markers CCR2, Cd11b, and CCL2, which could potentially stimulate the anti-tumor immune response**
- **ZEN-3694 increases the number of activated T cells in the tumor and draining lymph node**
- **ZEN-3694 synergizes with anti PD1 antibody to inhibit tumor growth in the MC-38 syngeneic tumor model**
- **ZEN-3694 has the potential to epigenetically prime the tumor to increase the frequency and duration of PD1 response, with a mechanism distinct from HDAC inhibitors**

References

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