ZEN-3365 is a novel BET bromodomain inhibitor for the treatment of hematologic malignancies and solid tumors

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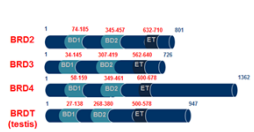
Abstract

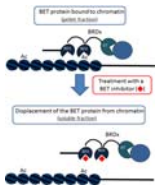
ZEN-3365 is an orally bioavailable small molecule discovered and developed from a BET bromodomain inhibitor platform. In vitro, ZEN-3365 binds BRD4(BD1) vs non-BET bromodomains with >20-fold selectivity, and binds both the first and second bromodomains of BRD2, BRD3, BRD4 and BRDT pan-selectively in a biochemical AlphaScreen assay, competing for binding to acetylated histone peptides with IC50 values of 8-36 nM. ZEN-3365 selectively displaces BRD4 protein from *MYC* and *BCL-2* promoters and from super-enhancers in cells, resulting in inhibition of *MYC* and *BCL-2* expression in acute myeloid leukemia (AML) and B-cell lymphoma cell lines with sub-uM IC50 values. In hematologic tumor types, including most lymphomas and many leukemia's, ZEN-3365 inhibits proliferation (IC50: 0.1 – 0.5 uM) and induces cell cycle arrest and apoptosis, consistent with inhibiting *MYC* and *BCL-2* expression. In AML xenograft tumor models, ZEN-3365, administered orally, dose-dependently inhibits *MYC* and *BCL-2* expression and can cause complete tumor regression with no regrowth for 6 months post cessation of dosing.

ZEN-3365 has also demonstrated strong activity against several solid tumor cell lines with sub-uM IC50 values, including breast, prostate, head and neck, and colorectal cell lines. Solid tumor xenograft studies conducted with ZEN-3365 have demonstrated that it is efficacious at well-tolerated doses. Robust PK/PD relationships have been established across a number of in vitro, in vivo and ex vivo systems for ZEN-3365 and will be explored further in the clinic. Promising target validation data, excellent pharmaceutical properties, clean off target selectivity profile, and robust activity of ZEN-3365 across a variety of hematologic malignancy and solid tumor settings support the clinical development of ZEN-3365 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 lymphoid/leukemia, CRC and breast cancer cells[1,2]. Inhibition of BET proteins results in their displacement from super-enhancers leading to down regulation of MYC, BCL-2 and other genes promoting cancer cell proliferation and survival[1]. BETi have been demonstrated to inhibit proliferation and suppress tumorigenicity in A549 lung cancer cells[3,4] There is clinical potential to combine an orally bioavailable BET inhibitor with the AML standard-of-care therapeutics cytarabine and AC220.





Results

ZEN-3365 selectively inhibits BET proteins, oncogenic gene expression & proliferation

Figure 1. ZEN-3365 binds BET bromodomains and displaces BET proteins from chromatin. **A) AlphaScreen assay:** Purified bromodomains were used to measure IC50 values competing BET bromodomains by Alpha-Screen. **B) ChIP Assay**: MM1s cells were treated with 2 uM ZEN-3365 for 3 hours. Abundance of BRD2, BRD3 and BRD4 was determined on the MYC promoter

A

BET Protein	IC50 (uM)
Brd2 (BD1/BD2)	0.02
Brd3 (BD1/BD2)	0.01
Brd4 (BD1/BD2)	0.04
BrdT (BD1/BD2)	0.04

B

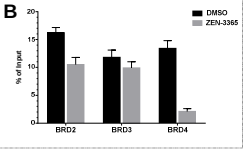


Figure 2. ZEN-3365 decreases BETi specific PD marker expression in multiple matrices. A) Whole blood (species indicated) and xenografts (cell lines indicated). **B)** Human whole blood treated ex vivo shows dose-dependent repression (Genes 1-5) or induction (Gene 6) upon treatment with ZEN-3365.

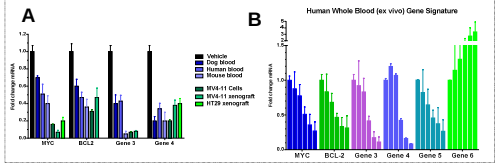
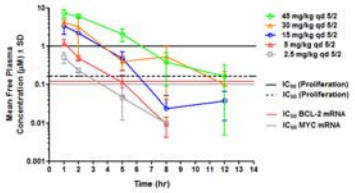


Figure 3. PK/PD for ZEN-3365. Dose dependent exposure and plasma concentration maintained for sufficient duration to inhibit cell proliferation, MYC and BCL2 expression in MV4-11 cells

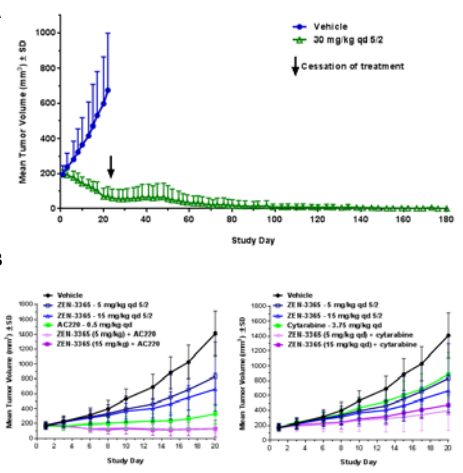


ZEN-3365 synergizes with AC220 and cytarabine to inhibit proliferation, induce apoptosis and reduce tumor growth

Table 1. Proliferation of MV4-11 cells treated with ZEN-3365 alone or in combination with cytarabine or AC220 (FLT3-ITD inhibitor). Chou-Talaly synergy indicated by values <0.7.

Treatment	Combination Index
ZEN-3365 + cytarabine	0.3
ZEN-3365 + AC220	0.6

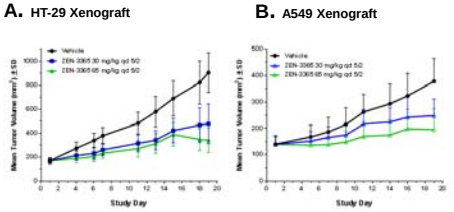
Figure 4. ZEN-3365 regresses MV4-11 tumors, has sustained effect and synergizes in combination: A. Mice bearing MV4-11 xenograft (200mm^3) were dosed orally with ZEN-3365 at 30 mg/kg qd daily for 21 days. Treatment was discontinued and mice observed for relapse of tumor for 160 days. **B.** ZEN-3365 dosed at 5 and 15 mg/kg in combination with AC220 or cytarabine.



ZEN-3365 inhibits CRC (HT-29) and lung carcinoma (A549) xenograft tumor growth

Figure 5. ZEN-3365 inhibits HT-29 and A549 xenograft tumor growth. Mice with tumors of approx 200mm^3 were orally dosed with ZEN-3365 at 30 & 65 mg/kg qd 5/2 for 19 days.

A. HT-29 Xenograft **B. A549 Xenograft**



Conclusions

1. **ZEN-3365 is novel and selective BET inhibitor that binds BET bromodomains in vitro and displaces BET proteins from chromatin in cells**
2. **ZEN-3365 inhibits BETi specific gene signature in multiple matrices including *MYC* and *BCL-2***
3. **ZEN-3365 synergizes with cytarabine and FLT3 inhibitor (AC220) to inhibit MV4-11 cell proliferation**
4. **ZEN-3365 is orally bioavailable, maintains plasma concentrations for sufficient duration and causes tumor regression in MV4-11 xenografts**
5. **ZEN-3365 inhibits tumor growth in CRC and lung carcinoma xenografts**

References

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