Pre-clinical development and clinical validation of a whole blood pharmacodynamic marker assay for the BET bromodomain inhibitor ZEN-3694 in metastatic castration-resistant prostate cancer (mCRPC) patients

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Abstract

PD marker assay development and validation

Detection of drug activity in patients is essential to configure its mechanism of action, as well as to ensure proper target engagement of the selected drug to elicit optimal clinical activity. Pharmacodynamic (PD) markers are often developed to detect pharmacological responses and optimize drug dosing. Whole blood is an easily available and minimally invasive source of tissue material to measure critical aspects of drug exposure, engagement and pharmacodynamic effects in a clinical setting. Development and validation of pharmacodynamic markers are critical for assessing the effectiveness of the drug and can be used for clinical decision-making. Potential PD marker candidates were first identified using quantitative mRNA analysis using a qRT-PCR inhibitor (QI) and a stable (23S) rRNA (tRNA) level for normalization. Further microarray analysis of subcutaneous tissue samples and published data of BET bromodomain and extracellular (BET) domain family or protein currently in phase I clinical trials in mCRPC (NCI/CTG01000 and NCT02117168). Potential PD marker candidates were identified using Fold change mRNA of >2 and SD <0.73 (tRNA) in the CCR1, BCL2, MYCBCL2, CCR1, IL1R1, NGPR183, H2B, H2BE, hexim1, CNM, M4, ZMYND8, AML1, AML2, DLBCL1, DLBCL2, and DLBCL3 genes. Further testing was done by measuring the modulation of these PD markers by various BET inhibitors in multiple cell lines of different origins derived from solid and liquid tumors, as well as in agreement with human peripheral blood mononuclear cells (PBMCs). In vivo studies were also done where blood obtained from mice and human specimens that were dosed orally with ZEN-3694, as well as other BET inhibitors derived from normal control or patients diagnosed with various AML and acute large B cell lymphomas. There was also robust target engagement in tumors of mouse AML, myeloma cells, and normal control PBMCs. These results demonstrated the potential of using BET inhibition as a therapeutic strategy. The results of this study show that ZEN-3694 induces robust and substantial modulation in multiple BET bromodomain inhibitors in mCRPC patient’s whole blood, and that whole blood can be used as a surrogate tissue for measuring the target modulation of ZEN-3694 in the clinic, and can be a useful tool for guiding dose optimization in clinical studies.

Overview

A whole blood assay was developed to measure target engagement of ZEN-3694 in patients
- 6 PD markers were selected that showed modulation in multiple biological systems
- Detected robust dose-dependent target modulation for CCR1 and IL1RN in all patients tested
- CCR1 and IL1RN results were validated independently by Nanosting analysis

PDZ-3694 shows activity that is consistent with a BET bromodomain inhibitor

This assay could be used to measure activity of other BET inhibitors and may be a useful tool for guiding dose optimization in clinical studies.

Background

Overall objective:
- QCR assay to measure target modulation of ZEN-3694 in whole blood

Method:
- Identity genes that are modulated by ZEN-3694 in various cell lines and animal models

Requirements:
- Genes need to be robustly upregulated over a large concentration range of ZEN-3694

Goals:
- Confirm that whole blood can be used to easily measure target modulation by ZEN-3694

Development Scheme

PDZ-3694 gene comparison

Confirmed PD marker modulation across different expression

Whole blood assay development and validation

Whole blood can be used to measure the activity of ZEN-3694 activity

Figure 1: Development of whole blood PD marker assay for ZEN-3694. QPCR: Quantitative polymerase chain reaction. }

Target engagement in mCRPC patients treated with ZEN-3694 at 4hrs post-dose

Figure 2: PD marker modulation detected in whole blood and tumor xenografts

Figure 3: PD marker assay validation: reproducibility and repeatability parameters, intra- and inter-assay variability

Figure 4: PD marker modulation by ZEN-3694 in ex vivo treated human whole blood

Figure 5: PD marker modulation in different human cell types

Figure 6: Nanosting digital color-coded barcode dual probe hybridization assay validates qPCR assays. Whole blood samples from ZEN-3694 treated patients (left) and vehicle-treated patients (right) were analyzed on Nanostring NCounter arrays. A panel of 80 genes was analyzed for gene expression, and the ZEN-3694 treated patient samples showed a significant modulation of several genes (ex. IL1RN) using the HCQ050L-08 gene set.