Advanced Image Analysis of H3K27 Trimethylation in Skin from Subjects Dosed with the EZH2 Inhibitor Tazemetostat

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Introduction

Tazemetostat (EPZ-6438 or E7438) is a selective small molecule inhibitor of the enhancer of zeste homolog 2 (EZH2) histone methyltransferase (HMT). EZH2 is the catalytic subunit of the multi-protein HMT complex known as polycomb repressive complex 2 (PRC2), which is responsible for mono-, di-, and trimethylation (me3) of histone H3 lysine 27 (H3K27). An exploratory objective for the phase 1 study of tazemetostat in subjects with advanced solid tumors or B-cell lymphomas was to explore the pharmacokinetic-pharmacodynamic (PK:PD) relationships such as correlation of H3K27me3 inhibition in patient tissue with systemic drug exposure. Pre-clinical xenograft models of EZH2 inhibition have previously shown tumor regression correlating with dose-dependent H3K27me3 decreases in both tumor tissue and skin epidermis (Knutson 2014). H3K27me3 reduction in the epidermis was calculated as a change in percentage of H3K27me3 positive cell employing an immunohistochemistry (IHC) assay with subsequent image analysis. Results from pre-clinical animal models and the low feasibility of obtaining matched pre- and post-treatment tumor biopsies, led to the selection of skin as a surrogate tissue for PD evaluation in the phase 1 study.

Methods

Phase 1 skin punch biopsies were successfully collected at screening and four weeks post dose from patients enrolled in the dose escalation and dose expansion cohorts n=32.

Examining the biopsies exhibited dose and exposure-dependent decreases in the percentage of H3K27me3 positive cells, however, the magnitude of reduction (60%) was less than observed in pre-clinical models (80%). Further investigation of the IHC images revealed a difference of H3K27me3 levels in distinct layers or strata of the skin. The stratum spinosum exhibited a distinct dose and exposure-dependent loss of H3K27me3 staining while the stratum basale showed a concomitant minimal loss of H3K27me3. These observations gave rise to the possibility of a stratum-specific PD response in skin to tazemetostat exposure. To fully evaluate this notion, a more sophisticated, unbiased, image analysis algorithm was employed to measure H3K27me3 levels across the strata of the epidermis. The algorithm was designed to identify three distinct regions (Figure 3): the full thickness epidermis, stratum basale to stratum corneum (a); the stratum basale alone (b); and the stratum spinosum (c). Iterative rounds of optimization were performed to identify the positive and negative thresholds in addition to the basale layer while excluding follicles and glands (Figure 5). Because skin has inherent challenges related to the chromogenic similarity between DAB and melanin, careful attention was paid to the negative control to ensure that endogenous melanin was not included in the analysis. PK/PD relationship between steady-state systemic and H3K27me3 mark in skin was performed using a logarithmic scale.

Results

Overview of Human Epidermal Layers

Figure 3: An H&E image from skin delineating the multiple layers/stratum: (a) full epidermal layer, (b) basale, the single cell layer at the base of the epidermis and (c) spinosum.

Human PK/PD Relationship Analysis

Figure 7: Fractional change from baseline when comparing both dosage at Day 28 (left) and AUC (right) exhibits an improved correlation of H3K27me3 in the stratum spinosum vs. the epidermis.

Conclusions

• Segregation of the stratum basale layer from the remainder of the epidermis led to a refined H3K27me3 analysis of tazemetostat exposure in skin as a surrogate tissue.
• Analysis of the different stratum resulted in an improved correlation and dynamic range of H3K27me3 inhibition with drug exposure.
• This analysis highlights that the zonal layers of the epidermis may require separate evaluation when analyzing skin as a surrogate tissue for proof of mechanism studies.
• Although more accessible for repeat biopsy, the use of skin as a surrogate tissue does not preclude the need to understand the PD effects in the target tumor tissue.
• Optional paired tumor biopsies are being collected in the Phase 2 tazemetostat trial and will be evaluated for H3K27me3 PD in addition to other biomarkers.

References


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