Abstract

Preclinical data have suggested that small molecule inhibitors for the histone methyltransferase EZH2 may represent potential new treatment modalities for Non-Hodgkin lymphomas (NHL) expressing EZH2 change of function mutations that merit further investigation in a preclinical or clinical setting, as appropriate. Our group has previously reported that selective inhibition of EZH2 results in specific killing of lymphoma cells bearing EZH2 mutations in vitro and in vivo, with minimal effects on non-mutant lymphoma cells [Knutson et al. Nature Chemical Biology 2012]; Killiches et al. Blood (ASH Annual Meeting Abstracts) 2012, 120. Abstract 7312]. Since genetic changes have been suggested to be involved in resistance of cancer cells to many anticancer agents, we studied EPZ-6438 (E7438), our clinical stage EZH2 inhibitor, in combination with standard of care agents for NHL second line therapies, or targeted therapies that are being explored in this indication. With continuous exposure to EPZ-6438, cell-based assays of two different EZH2 mutant cell lines demonstrated combination benefits with all components of the CHOP chemotherapy regime, second line therapies, but also with several targeted therapies (for instance other epigenetic drugs, PI3K pathway or other inhibitors). These effects were not observed in an EZH2 wild type lymphoma cell line of the activated B cell type. Strong combination benefit with CHOP was only observed in two different EZH2 mutant xenograft models. For instance, in the SUDHL4 Y646N xenograft model neither EPZ-6438 nor CHOP chemotherapy alone induced a significant antitumor effect, yet their combination produced durable tumor regressions even after cessation of dosing. Importantly, this effect was preserved when down-regulation was consented from the CHOP chemotherapy regime in a third study with another EZH2 mutant xenograft model. Subsequently we showed that glucocorticoid receptor agonism may be a key mechanism of the combination benefit observed with CHOP, as the antiproliferative effect of EPZ-6438 was enhanced by either prednisolone or dexamethasone alone, in several EZH2 mutant lymphoma cell lines (in vitro). Taken together these data suggest that the single agent activity of EPZ-6438 in EZH2 mutant NHL may be further enhanced and expanded through rational combination strategies.

Methods

Design of In vitro Combination Assays

Pre-treatment Model

Pre-treatment model: Lymphoma cells were pre-treated in flasks with 7 doses of EPZ-6438 and DMSO for 4 days. Cell densities were normalized with a DMSO control and each dose of drug was normalized with columns which received 7 doses of EPZ-6438 (A) or 7 doses of DMSO (C). Cell viability was determined with Guava ViaCount® Reagent.

Co-treatment Model

Lymphoma cells were treated with 7 doses of EPZ-6438 and 1 dose of compound of interest for either 4 or 7 days. Viability was determined with Guava ViaCount® Reagent.

Data Analysis-Chou-Talalay: Pre-treatment Model A

Combination Index Equation

Fractional Effect of treatment = Viability of treatment / Viability of vehicle

Combination Index Equation

Combination Index (CI) = [Fractional Effect of treatment] / [Mean Fractional Effect of drugs]

Summary Table of Combinations with EPZ-6438

Combination benefit with EPZ-6438 is achieved with all drugs tested in this in vitro assay. Fractional Effect of treatment model A and data analysis with Calcusyn software (see Methods section).

Results

Combination benefit was observed in SUDHL4 Y646N xenograft with CHOP at both 2:1 and 1:2 doses treatment with prednisolone (A) or dexamethasone (B), according to pre-treatment model B (see Methods section). In contrast, no combination benefit was observed in Toledo cells, an EZH2 wild type ABC lymphoma line. Doses ranged from 10µM-1000nM for prednisolone and from 0.156-10µM for EPZ-6438 in SUDHL4 Y646N xenografts and from 15nM-1000nM for prednisolone and 1.56-100nM for EPZ-6438 in Toledo xenografts. Cell viability was measured via ATP content using CellTiter-Glo® (EZH2 mutant lymphoma lines), or fraction of cell viability (WT EZH2 ABC lymphoma cell line).

Conclusions

• EPZ-6438 and glucocorticoid receptor agonists (GRag) cooperate to dramatically enhance antiproliferative activity in NHL of the germinal center subtype.
• The in vitro cell killing activity of EPZ-6438 is enhanced by GRag in EZH2 mutant bearing cells.
• EZH2 enhances the GRag antiproliferative activity in WT EZH2 GCB cell lines.
• The combination of EZH2i and GRag reverses intrinsic WT EZH2 mutant resistant cell lines.
• In two different EZH2 mutant xenograft models, strong combination benefit was demonstrated with EPZ-6438 and CHOP, and this effect was preserved in a study in a third EZH2 mutant xenograft model in which doxorubicin was omitted from the chemotherapy regime.
• These results suggest that:
  - Glucocorticoid receptor agonism may play a key role in the amplified antitumor activity observed with combinations of EPZ-6438 and CHOP in EZH2 mutant lymphoma xenografts.
  - EPZ-6438 may have a broader role in GCB lymphoma beyond the subset with an EZH2 mutation.

References


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