DOT1L INHIBITOR EPZ-5676 SYNERGIZES WITH CYTARABINE AND AZACITIDINE IN PRECLINICAL MODELS OF MLLeukemia

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Abstract

EPZ-5676 is a small molecule inhibitor of the histone methyltransferase DOT1L currently in clinical development and represents a first in class novel therapeutic for the treatment of MLL-leukemia. In preclinical studies, EPZ-5676 selectively inhibited intracellular histone H3K79 methylation, downstream target gene expression and demonstrated complete tumor regression in a MLL-leukemia xenograft model. We previously reported synergistic and durable antiproliferative activity when EPZ-5676 was combined with current AML standard of care drugs, Cytarabine and Daunorubicin in MLL-leukaemia models MOLM-13 (MLL-ARF) and MV4-11 (MLL-4F4). Combination benefit was also observed when MLL+ cells were treated with Cytarabine, prior to co-treatment with EPZ-5676. Additionally, both Cytarabine and the DNA methyltransferase inhibitor Azacitidine, displayed synergistic anti-leukemic activity in MLL+ leukaemia xenograft model. We recently demonstrated synergistic and antiproliferative activity in MLL-rearranged cell lines and in a 7 day co-treatment model (see Klaus et al., JPET, 2014). In this report we discuss results of investigating additional treatment schedules using EPZ-5676 in combination with Cytarabine in MLL+ cells. Cells were pretreated with Azacitidine at nanomolar concentrations known to reverse promoter DNA-hypermethylation and alter the chromatin state (Tsai et al., Cancer Cell, 2012). We found treating MV4-11 and NOMO-13 cells daily for three consecutive days followed by sequential treatment with EPZ-5676 elicited a synergistic antiproliferative effect using the Chou-Talalay method (Chou, Pharmacol Rev., 2006). Results of studies to investigate the mechanism of this synergistic cell killing, including evaluation of differentiation markers and Annexin V staining will be reported. To determine if combinations of EPZ-5676 with Cytarabine or Azacitidine were tolerable and efficacious in vivo, nude rats implanted subcutaneously with MV4-11 tumors were treated using a range of doses and schedules. Azacitidine and Cytarabine were delivered by intraperitoneal injection once daily for 14 days at their respective maximum tolerated doses of 2 and 200 mg/kg. Dosing at the established MTD, these agents inhibited the subcutaneous MV4-11 tumor growth by 50% compared to vehicle controls. Efficacy results from the EPZ-5676 combination studies with Cytarabine or Azacitidine will be presented. In summary, these studies demonstrated that EPZ-5676 in combination with Cytarabine or Azacitidine revealed a synergistic effect, regardless of the treatment schedule used in preclinical models of MLL-leukemia. Tolerable in vivo rat combination doses for EPZ-5676 with both Cytarabine and Azacitidine have been determined in support of potential future assessment of these combinations in MLL-leukemia patients.

Results

EPZ-5676 Demonstrates Combination Benefit upon Priming with Cytarabine and Azacitidine in MLL-rearranged Cell Lines

EPZ-5676 In combination with Cytarabine Synergistically Decreases Time and Dose Sensitive Cell Death by Apoptosis in MLL-rearranged Cell Lines

EPZ-5676 Reduces ML1 Fusion Target Gene Expression as Single Agent and in Combination with Azacitidine

Conclusions

- EPZ-5676 acts synergistically with the AML standard of care drug Cytarabine and the DNMT inhibitor, Azacitidine, to induce a strong antiproliferative response in MLL-rearranged cell lines.
- In vitro synergistic cell killing with EPZ-5676 and either Cytarabine or Azacitidine is restricted to cell lines sensitive to single agents.
- Initial investigations indicate induction of apoptosis is likely the mechanism for synergies observed.
- Additional mechanisms of combination benefit observed in NOMO-1 cell line treated with EPZ-5676 and Azacitidine through induction of differentiation.
- Tolerable combination doses of EPZ-5676 and Cytarabine or Azacitidine achieved in tumor bearing rats.
- Greatest tumor growth inhibitory effects observed in combination arm in MV4-11 model.
- These studies indicate the potential for combining EPZ-5676 with Cytarabine or Azacitidine for treatment of MLL-leukemia.


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