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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Targeting EYA1 Activity in MLL- Rearranged Leukemia

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The *Mixed Lineage Leukemia* gene (also known as *MLL* or *KMT2A*) is involved in chromosomal translocations in a subtype of acute myeloid (AML) and lymphoblastic leukemias (ALL). *MLL* translocations have been identified with more than 80 partner genes, and the most common AML translocation partner is *MLLT3* (AF9). MLL-rearranged (MLL-r) leukemia is associated with a sudden onset, aggressive progression, and poor prognosis in comparison to non-MLL-r (MLL-nr) leukemias, and there is a need for more effective therapies. Previous work determined that AF9 directly interacts with multiple different regulatory proteins, including the BCL6 corepressor (BCOR). An AF9 point mutation (E531R) was identified that selectively disrupts BCOR binding to AF9 and MLL-AF9. The loss of BCOR binding to MLL-AF9 abrogated its leukemogenic ability in a mouse transplant model. RNA-seq analysis determined that of the MLL-AF9 direct target genes, *EYA1* was the most down-regulated in the MLL-AF9 (E531R) point mutant-transformed cells. Addback of EYA1 into MLL-AF9 (E531R) cells partially rescued its leukemogenic ability, suggesting that EYA1 has an important role in MLL-AF9 leukemogenesis. EYA1 is a transcriptional coactivator and protein tyrosine phosphatase that is expressed during embryonic development and its re-expression has been implicated in several cancers, including MLL-r leukemia.

The objective of this study is to investigate the in vitroand in vivo effects of EYA1 inhibition in MLL-AF9 transformed murine bone marrow stem/progenitor cells and in human MLL-rearranged (MLL-r) and non-rearranged (MLL-nr) leukemia cell lines. Cells were treated with small molecule inhibitors at varied concentrations and time points. Effects of inhibition were assessed with viability, cell morphology, cell cycle, surface and intracellular marker expression analyses, and in vivo transplant studies. EYA1 inhibition reduced cell viability by 50% - 90% in MLL-AF9 transformed murine bone marrow (mBM) cells and multiple human AML cell lines. EYA1 inhibition did not affect some human B-ALL cell lines, which also express undetectable levels of EYA1, indicating a correlation between the response to EYA1 inhibition and EYA1 expression. In MLL-r and MLL-nr cells, cell cycle analysis indicated a reduction in cells entering S-phase, stalling in G0/G1, with EYA1 inhibition as well as an induction in cellular senescence. Inhibiting EYA1 activity induced cell morphology changes toward a more differentiated state in MLL-AF9 mBM and several MLL-r and -nr cell lines. To confirm these qualitative results, cells were stained for myeloid differentiation markers, and cell surface expression of CD11b, Gr1, and CD14 increased in a dose- and time-dependent manner in all cell types, while HSC markers CD117 (c-KIT) and CD34 were reduced in MLL-AF9 mBM and human AML cells, respectively.

In assessing the mechanism of EYA1 in MLL-r leukemia, MLL-AF9 cells treated with EYA1 inhibitors showed increased RNA pol II CTD Tyr1 phosphorylation levels (known EYA1 substrate) compared to vehicle-treated cells. Thus, unregulated EYA1 phosphatase activity targeting RNA Pol II CTD may contribute to leukemia development. Mice that received MLL-AF9 mBM cells treated with an EYA1 inhibitor had a significantly decreased rate of disease progression and increased survival compared to control. Bone marrow cells isolated from these mice showed increased expression of myeloid differentiation markers CD11b and Gr1 compared to vehicle-treated mice, confirming that EYA1 activity contributes significantly to leukemogenesis.

The inhibition of EYA1 induces leukemia cell differentiation, cell cycle arrest and senescence, and cell death. Ongoing experiments include additional in vivo murine leukemia, further mechanistic analyses, and combinatorial experiments to evaluate the efficacy of a multi-target approach with EYA1 inhibition and other targeted-therapies. This work may provide a promising, novel therapeutic approach to MLL-r and MLL-nr leukemias that will help improve patient outcomes.

Disclosures No relevant conflicts of interest to declare.

OffLabel Disclosure: Benzbromarone used for preclinical anti leukemia agent

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