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DNA methylation therapy joins forces in *IDH2*-mutant AML

Isocitrate dehydrogenases 1 and 2 (*IDH1*/2) are frequently mutated in Acute Myeloid Leukemia (AML), with nearly 20% of patients carrying gain-of-function point mutations in these genes (Ley et al., 2013). *IDH2* is a metabolic enzyme that catalyzes the conversion of isocitrate to 2-oxoglutarate during the Krebs cycle. Patients carrying *IDH2* gain-of-function mutations produce instead high levels of the oncometabolite 2-hydroxyglutarate (2-HG), which inhibits oxoglutarate-dependent enzymes such as the TET family of methylcytosine dioxygenases, responsible for active DNA demethylation (Xu et al., 2011). As a consequence, *IDH2* mutations in AML patients induce DNA hypermethylation and inhibit hematopoietic differentiation (Figueroa et al., 2010).

Azacytidine (AZA) and enasidenib (ENA) are commonly used AML therapies which induce DNA hypomethylation, albeit through different mechanisms. AZA is a nucleoside analog that inhibits DNA methyltransferase enzymes (DNMTs) and ENA impairs *IDH2* mutant catalytic activity (Wang et al., 2013). In a study recently published in *Experimental Hematology* (MacBeth et al., 2021), Macbeth and colleagues hypothesized that combining both drugs in *IDH2*-mutant leukemia will produce a synergistic effect to drive hypomethylation and restore AML differentiation.

To test this hypothesis, the authors first performed combination or single agent treatments on a leukemia

cell line model overexpressing *IDH2* mutation (TF1 IDH2^{R140Q} cells) and evaluated dose-dependent erythroid differentiation via a hemoglobinization assay. Compared to single agent treatments, combination treatment with high AZA and ENA doses showed the highest rate of hemoglobinization.

Their findings were further strengthen using primary cells from AML patients carrying *IDH2*^{R172} and *IDH2*^{R140} mutations. Differentiation was assessed by flow cytometry using CD34^{+} (marking stem and progenitor cells) and CD15^{+} (marking granulomonocytic cells). Combination treatment showed a reduction in CD34 and increased CD15 intensity, indicative of myeloid differentiation, an effect that was not detected in *IDH2*-WT AML samples.

Next, they assessed genome-wide methylation profiles of AZA+ENA treatment on TF1 *IDH2*^{R140Q} cells. 5hmC levels were evaluated using hydroxymethylated DNA immunoprecipitation (hMeDIP) sequencing, showing an increase in 5hmC levels in ENA treated and ENA+AZA treated cells, with no measurable effects in AZA-only treated cells. Subsequently, 5mC was measured using EERBS (Enhanced Representation Bisulfite Sequencing), showing a decrease in both single-agent AZA and combination treatment, but no difference in single-agent ENA, concordant with the known mechanism of action of this *IDH2* inhibitor. Overall, ENA+AZA combination showed a greater degree of DNA hypomethylation than both single-agent treatments alone. These findings support a model in which restoration of TET activity cooperates with the inhibition of DNMTs to cooperatively induce DNA hypomethylation in leukemic cells.

Further mechanistic studies would be needed to elucidate the precise molecular mechanisms of AZA+ENA synergistic effect, such the identification of methylation patterns at specific genomic regions and functional regulatory elements responsible for the increased myeloid differentiation. DNA hypomethylation agents are widely used in AML therapy, although only a modest proportion of patients respond. Therefore, resolving the underlying mechanism of action of DNA methylation therapies and designing new rational combinations to enhance response rates continues to be a promising avenue for AML treatment.

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- *November 14, 2024*

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