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Abstract

Enhanced In Vivo Synergistic Efficacy of Pan-Histone Deacetylase and Cyclin-Dependent Kinase Inhibitors in Targeting TP53-Mutant Acute Myeloid Leukemia Cells

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Background: Acute myeloid leukemia (AML) is characterized by genetic and epigenetic alterations. Ten percent of AML patients display mutations in the tumor suppressor gene TP53, resulting in therapy resistance and reduced overall survival. TP53 alterations inhibit the P53/P21/Cyclin-CDK/RB-E2F pathway, activating the cell cycle. Recent clinical data showed that TP53-mutated patients unfit for cytotoxic chemotherapy do not benefit from venetoclax-azacytidine combinations. Accordingly, innovative treatments are warranted.

Aim: Validate the ability of experimental or clinically-approved pan-histone deacetylase inhibitors (HDACi) to inhibit AML cells with TP53 mutations/deletions, alone or in combination with the cyclin-dependent kinase inhibitor (CDKi) Dinaciclib.

Methods: Differential gene expression analysis was conducted on two independent cohorts of Acute Myeloid Leukemia (AML) patients, stratified by TP53 mutation status. Cell viability and proliferation assays were performed on TP53-mutated AML cell lines (HL60, U937, KG1 and HEL) treated with the pan-Histone Deacetylase inhibitors (HDACis) MC2726 and Vorinostat, either alone or in combination with Dinaciclib. RNA sequencing (RNAseq) analysis was carried out on HL60 and U937 cells treated with HDACis, and protein expression studies were conducted via western blotting in HL60 and U937 cells. Additionally, a U937-luciferase xenograft murine model was treated with MC2726 or Dinaciclib alone or in combination, compared to Vorinostat.

Results: Bioinformatic analysis revealed upregulation of genes predominantly involved in cell cycle processes (MYC, E2F, cyclins, CDK1/2/4, HDAC1, RB1) in TP53-mutated AML patients, while CDK-inhibitors (P21, P15) were downregulated.

Furthermore, HDACis exhibited cytotoxic effects on TP53-mutated AML cells. RNAseq analysis demonstrated the downregulation of genes encoding MYC, cyclins A/B/D, and CDK 4/6 in HL60 and U937 cells. Furthermore, our data showed an upregulation of P21 and P15 gene expression, which are repressed by MYC but activated by P53, in HDACi-treated cells. Additionally, E2F1 expression was inhibited. Combination treatments involving MC2726 and Dinaciclib synergistically reduced cell viability in U937 cells. Mice treated with MC2726 alone or in combination with Dinaciclib exhibited reduced tumor signals compared to control and Vorinostat-treated groups, in absence of adverse

effects. Overall survival was increased by MC2726 and Dinaciclib treatments alone, and in combination, compared to controls, with the combinatory treatment demonstrating superior efficacy.

Conclusion: Considering the dismal overall survival of TP53-mutated AML patients, we suggest pan-HDACis as a preclinical research avenue. Pan-HDAC-mediated inhibition of TP53-mutated AML cell viability and proliferation was accompanied by a reprogrammed expression of cell cycle genes and synergized with Dinaciclib. In vivo, luminescence decreased significantly in mice engrafted with U937-luciferase cells treated with MC2726 and Dinaciclib and survival was prolonged.