Voruciclib, an Oral, Selective CDK9 Inhibitor, Enhances Cell Death Induced by the Bcl-2 Selective Inhibitor Venetoclax in Acute Myeloid Leukemia

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Introduction

5-year survival rates for patients with acute myeloid leukemia (AML) remain frustratingly low (65% for children and 27% for adults). Resistance to frontline chemotherapy (cytarabine and an anthracycline-based) often develops; therefore, a new treatment modality is urgently needed.

Bcl-2 family proteins play an important role in balancing cell survival and apoptosis. The anti-apoptotic protein Bcl-2 is overexpressed in both bulk AML cells and leukemic stem cells (LSCs). Voruciclib (Venetoclax), a Bcl-2 inhibitor, was developed to selectively target Bcl-2. Even though ABL-199 has demonstrated promising anti-AML activity, another anti-apoptotic Bcl-2 family protein, Mcl-1, impairs its activity. Previous studies, including our own, have shown that direct targeting of both Bcl-2 and Mcl-1 with small molecule inhibitors in AML is effective. Alternatively, indirect targeting of Mcl-1 may preserve or enhance ABL-199 activity in AML cells, as well.

One approach to indirectly target Mcl-1 is to transcriptionally downregulate Mcl-1 through CDK9 inhibition. The CDK9 inhibitor flavopiridol (alvocidib) has progressed to phase II clinical trials in AML. However, cell target effects and toxicity remains a concern. A more selective CDK9 inhibitor, voruciclib, represses Mcl-1 and sensitizes high risk diffuse large B-cell lymphomas to Bcl-2 inhibition. Based on these data, we hypothesize that voruciclib will also downregulate Mcl-1 and therefore synergize with ABL-199 in AML cells.

Analogous to flavopiridol, voruciclib induced apoptosis in AML cell lines and primary patient samples at clinically achievable concentrations. Both voruciclib and flavopiridol were found to synergistically induce apoptosis in AML cells when combined with ABL-199. Voruciclib and flavopiridol were found to downregulate Mcl-1 transiently. The combination treatment was greatly enhanced when using a concentration of voruciclib or flavopiridol that downregulated Mcl-1.

Additional studies are underway to further elucidate the molecular mechanisms and to determine the in vivo anti-leukemia efficacy in NGS mouse AML models.

Conclusions and Future Work

Conclusions:

• Voruciclib and flavopiridol both synergize with ABL-199 to induce apoptosis in both venetoclax sensitive and resistant AML cells

• Voruciclib and flavopiridol both transiently downregulate Mcl-1

• Mcl-1 downregulation is likely responsible for the bulk of the synergy between voruciclib and ABL-199 as well as flavopiridol and ABL-199

Future studies:

• Further determination of the molecular mechanism underlying the synergy between voruciclib and ABL-199 or flavopiridol

• In vivo efficacy in MV4-11- and patient-derived xenograft models in NGS mice

• Alternative molecular mechanisms

Figure 1. Proposed mechanism. ABL-199 (VOR) (top right) treated releases p53 from Bcl-2 in sensitive cells, while in resistant cells, p53 is not transcriptionally activated (bottom right), resulting in the Bcl-2-Bcl-XL complex, acting as a supports to AML cells. Voruciclib (VOR) (bottom right) with and without BCL-XL (BCL) (top right) treatment releases p53 through AML cell death regardless of the lineages, resulting in Bcl-2-Bcl-XL complex, acting as a supports to AML cells.

Figure 2. Mcl-1 knockdown induces apoptosis in AML cell lines and primary samples upon Flavopiridol and Voruciclib treatment. A) Schematic for the knockdown of Mcl-1 by RNAi. (B) Mcl-1 knockdown of MV4-11 (24 h) cells were treated with Flavopiridol (FLV) at 200 nM and Voruciclib (VOR) at 2000 nM, alone or in combination, for 24 h. Whole cell lysates were subjected to Western blotting and probed with the indicated antibodies. Bcl-2, Bcl-XL, and Bax knockdown cells were treated with Flavopiridol for 24 h and then subjected to Annexin V staining and flow cytometry analysis. ** indicates p<0.01.