Combinatorial Inhibition of Focal Adhesion Kinase and BCL-2 in AML

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Abstract
Focal adhesion kinase (FAK), activated by integrins and cytokines, promotes cell growth, migration and metastasis. We previously reported that a selective FAK inhibitor, VS-4718, inhibited cell growth and induced cell death in leukemia cell lines in vitro, effectively antagonized cell invasion and/ or scattering of leukemic cells, including bone marrow, spleen and liver, and significantly prolonged survival times in a human xenograft mouse model (Carter B K, et al., NCI, 6/07, 106/1103).

Although BCL-2 inhibition by the BCL-2 inhibitor ABT-199 (Venetoclax) has demonstrated remarkable activity in tumors, an expected drug resistance is currently noticed. When combined with ABT-199, VS-4718 significantly potentiated ABT-199-induced apoptosis in AML cell lines and in a PDX model of AML. ABT-199 treatment of Molm-14 AML cells decreased levels of p-STAT3, p-BCL-2, and p-IRa (Wang et al., ASH 2016) that play important roles in the acquired resistance to ABT-199.

To further examine the effect of VS-4718 as a single agent and in combination with ABT-199 on primary AML cells in vivo, we generated patient-derived xenografts (PDx) from AML patient bone marrow-derived cells expanded in NSGS (NOD-SCID IL2Rg−/−, 3G8SF, NSG-3G222) mice. The xenograft used AML cells from a patient who had failed chemotherapy and had NPM1, FLT3-ITD, TET2, DNMT3A, and TET1 gene mutations and a complex karyotype. The combination of VS-4718 and ABT-199 synergistically killed the PDx cells in vitro even under MDR conditions. Once PDx cells were engrafted into NSGS mice, animals were randomized into 4 groups (VS+ABT-199) and treated with vehicle, VS-4718 (73 mg/kg, twice daily), ABT-199 (100 mg/kg, once per day), or the combination via oral gavage for 29 days. We observed that ABT-199 had a stronger anti-tumorigenic effect on preclinical blood blast on tissue-resident cells, while VS-4718 was more effective in tissues. At the end of the treatments, VS-4718 significantly reduced circulating human CD45+ cells (P=0.0000), and ABT-199 alone (P=0.0000-0.07) and the ABT-199-VS combination (P=0.001-0.07) were more effective. VS-4718 alone or in combination with ABT-199 reduced leukemic burden in spleen and liver and effectively reduced splenic and hepatic metastases, while ABT-199 alone did not. VS-4718-treated mice survived significantly longer than the untreated controls (median survival 72 vs. 36 days, P=0.0000), while ABT-199 did not prolong survival (median survival 35 days). Also, the combination of VS-4718 with ABT-199 significantly prolonged survival (median survival 65 days, P=0.01 vs. control and P=0.0072 vs. ABT-199). No statistical difference in survival was found between VS-4718 and the combination treatment groups (P=0.03-0.1). All the mice had large spleens at death except the combination group; the first dead mouse had a small spleen, and the second through the fourth had slightly enlarged spleens, and only the last two had large spleens as others suggesting that additional reasons such as toxicity at the dosage used may contributed to the death of the mice in the combinatorial group, hence shortening the median survival in this group.

We determined expression of FAK and BCL-2 family proteins in cells from mouse spleens at the end of treatment and found that the combination of VS-4718 and ABT-199 decreased protein levels of FAK. Consistent with our in vitro results, the increased level of MCL-1 by ABT-199 was antagonised by its combination with VS-4718 in vivo, suggesting the latter was potentially be more effective.

Conclusions: These results suggest a potential clinical benefits of combining a FAK inhibitor with Venetoclax for patients with AML.

Results
Characteristics of the patient whose BM cells was used to generate PDX cells in NSGS mice

Combinational inhibition of FAK and BCL-2 synergistically induced apoptosis in PDX cells in vitro

Background

FAK, activated by integrins and cytokines is overexpressed in various malignant cells.

- FAK promotes cell growth, survival, migration, and metastasis through regulating multiple cell survival signaling pathways in solid tumors, but its role in AML is not well studied.

- We previously showed that VS-4718, a FAK inhibitor, effectively decreased viable cell numbers and induced cell death in leukemia cell lines with variable profiles both in vitro and in vivo, also induced apoptosis in primary AML cells in vitro, even in AML cells co-cultures with endothelial stromal cells (DSCA).

- We demonstrated that VS-4718 significantly potentiated BCL-2 inhibitor ABT-199 (Venetoclax)-induced apoptosis in AML cell lines and primary AML cells, in part by antagonizing ABT-199-induced MCL-1 upregulation, a resistance factor to ABT-199.

- Here we investigate the effect of FAK inhibitor and its combination with BCL-2 inhibitor in vivo using a PDX xenograft model in NSGS mice.

Conclusions
Inhibition of FAK, or combined inhibition of FAK and BCL-2, has anti-leukemia activity in a PDX NSGS mice model.

- Premature death of several mice in the combination treatment group, compared to VS-4718 treatment alone, was likely caused by drug toxicity.

- Consistent with our in vitro results, the combination partially reversed the ABT-199-induced MCL-1 increase and decreased FAK levels.

- Results suggest a potential benefit of FAK inhibition, alone or in combination with Venetoclax, in AML.

(VS-4718 was kindly provided by Verastem Inc.)