Inhibition of FAK exerts anti-leukemic activity and potentiates ABT-199-induced apoptosis in AML

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Abstract

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that regulates cell adhesion, proliferation, stem cell functions, and cell-microenvironment communications. It is activated and/or overexpressed in many malignant cells and promotes tumor progression and metastasis. Several small molecule FAK inhibitors have been developed and some of them have reached clinical trials in solid tumors. High FAK expression was found to be associated with enhanced blast migration, increased cellularity, and poor prognosis in AML, indicating that FAK could be a potential therapeutic target in AML.

We showed previously that VS-4718, a potent and selective FAK inhibitor, effectively decreased viable cell number, and also induced cell death in leukemia cell lines with variable potencies in vitro, even in AML cells co-cultured with mesenchymal stromal cells (MSCs) (ASH 2015)

To further examine the effect of VS-4718 in vivo, we transplanted Molm14-GFP/Luc cells into NSGS (NOD-SCID IL2Rγnull-3/GM/SF, NSG-SGM3) mice, and treated the mice with VS-4718 (75 mg/kg) twice a day via oral gavage. We found that VS-4718 as a single agent exerted anti-leukemia activity as assessed by in vivo imaging for leukemia burden, human CD45 positivity in mouse peripheral blood, and histological staining of mouse tissues. VS-4718 treated mice survived significantly longer than the untreated controls (medium survival 27 vs 20 days, P = 0.0003).

FAK activates multiple signaling pathways and supports tumor cell survival. We found that inhibition of FAK with VS-4718 in Molm14 cells reduced the expression of MCL-1. The BCL-2 antagonist ABT-199 is being tested clinically for the treatment of hematological malignancies. However, as a single agent, ABT-199-treated cells can acquire drug resistance by upregulating MCL-1 and BCL-XL after treatment. We therefore hypothesized that combination of VS-4718 and ABT-199 would be more effective in inducing cell death and reversing the resistance of AML cells exposed to ABT-199 alone. In vitro studies showed that VS-4718 significantly improved the potency of ABT-199 in AML cell lines (ABT-199 EC50 at 24 h: 880.3 nM and 14.5 nM in the presence of 0.4 μM VS-4718, respectively, in Molm14 cells), and the combination of VS-4718 and ABT-199 also synergistically killed primary AML cells even when co-cultured with MSCs in the majority of samples examined, while largely sparing normal BM CD34+ cells.

Furthermore, the upregulation of MCL-1 in ABT-199-treated AML cells was antagonized by combining ABT-199 with VS-4718. BCL-XL is known to be regulated by STAT5. The activation of STAT5, which can be regulated by FAK, is considered to be significant in maintaining MCL-1 expression in FLT3-ITD AML cells. We observed that treatment with VS-4718 decreased the level of p-STAT5 as well as MCL-1 and BCL-XL in Molm14 cells harboring FLT3-ITD mutation.

These results suggest a novel therapeutic strategy for targeting FAK and BCL-2 family proteins for the treatment of AML.

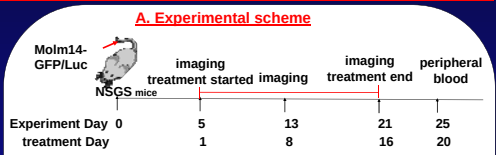
Background

- FAK, activated by integrins and cytokines promotes cell growth, survival, migration, and metastasis through regulating multiple cell signaling pathways
- FAK is overexpressed in various malignant cells in solid tumors, but is not well studied in myeloid leukemia
- We previously showed that VS-4718, a FAK inhibitor, effectively decreased viable cell numbers, and also induced cell death in leukemia cell lines with variable potencies *in vitro*, even in AML cells co-cultured with mesenchymal stromal cells (MSCs)
- BCL-2 antagonist ABT-199 is being tested clinically for the treatment of hematological malignancies; however, its activity is hampered by upregulation of MCL-1 and BCL-XL

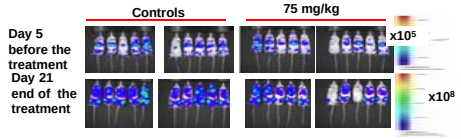
Results

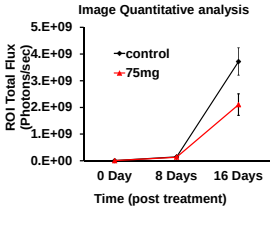
Inhibition of FAK by VS-4718 exerts antileukemia activity *in vivo* in human AML xenografted NSGS mice

A. Experimental scheme

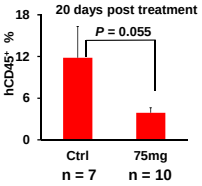


B. Image analysis

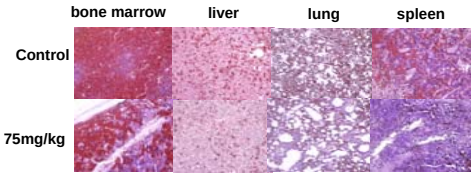




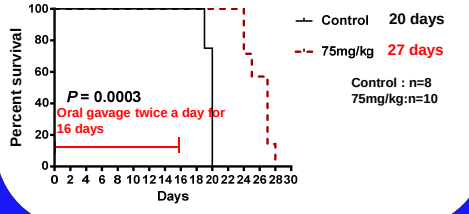
C. PB leukemia burden



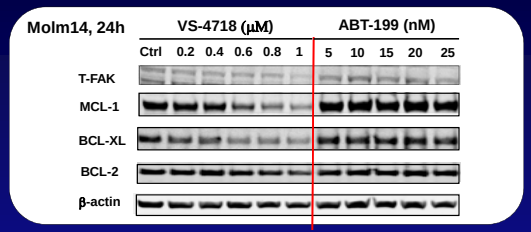
D. Leukemia burden in various tissues



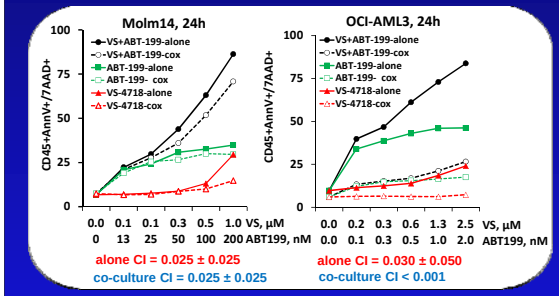
E. Survival curve



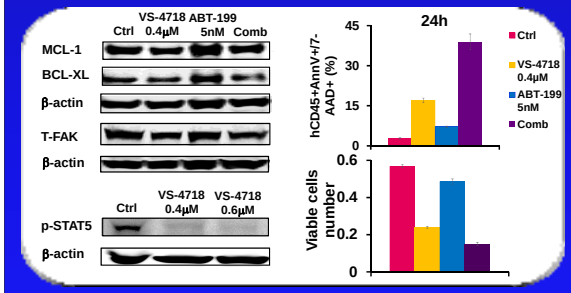
Inhibition of FAK by VS-4718 decreases, while ABT-199 increases MCL-1 and BCL-XL in AML cells



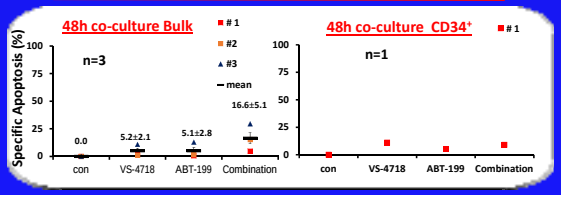
Combined inhibition of FAK and BCL-2 synergistically induces cell death in AML cell lines even when co-cultured with MSCs



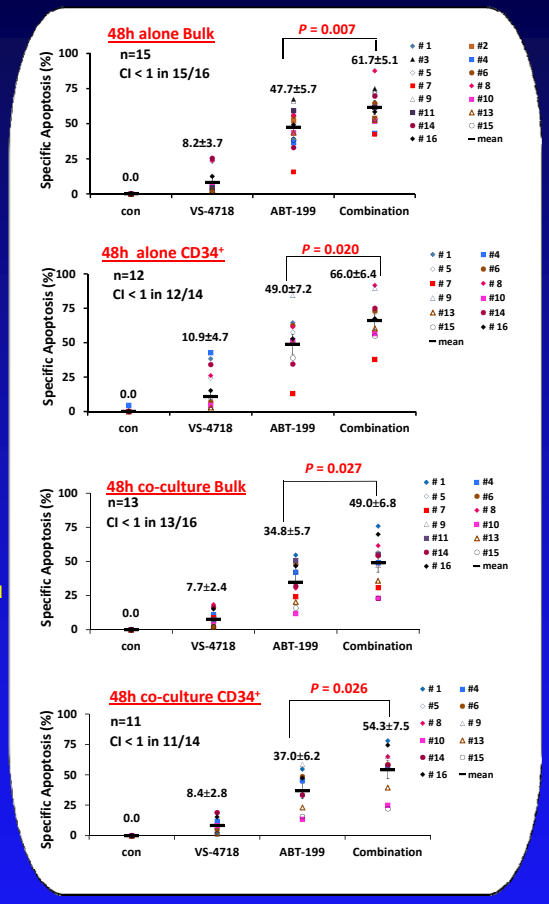
Combined FAK and BCL-2 inhibition antagonizes ABT-199-mediated induction of MCL-1 and BCL-XL and VS-4718 decrease p-STAT-5



Combined inhibition of FAK and BCL-2 has minimal toxicity to normal bone marrow cells



Inhibition of FAK enhances the efficacy of ABT-199 in primary AML cells even co-cultured with MSCs



Conclusion

- Inhibition of FAK has antileukemia activity both *in vitro* and *in vivo*.
- Combined inhibition of FAK and BCL-2 more efficiently induces apoptosis in AML cell lines and primary patient samples, while has minimal toxicity to normal BM cells.
- Inhibition of FAK decreases MCL1, BCL-XL, and p-STAT5 and antagonizes ABT-199-mediated increases of MCL-1 and BCL-XL.
- Combined targeting of FAK and BCL-2 may represent a novel strategy for the treatment of AML.

VS-4718 was kindly provided by Verastem Inc.