VS-4718, a Potent FAK Kinase (FAK) Inhibitor, Exhibits Anticancer Activity in Leukemia Models *in Vitro* and *in Vivo*

Abstract# 982

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ABSTRACT

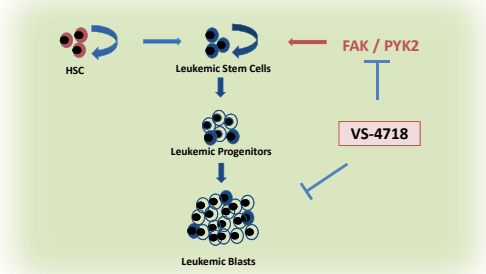
Current chemotherapy for leukemia is effective in killing leukemic blasts in the periphery, but not leukemic stem cells (LSCs) in the bone marrow, which are thought to be responsible for the high relapse rate in leukemia. Thus, new therapies that effectively target LSCs are urgently needed to prevent cancer relapse. Accumulating evidence supports an essential role for adhesion pathways: particularly integrin beta 3 and its downstream target focal adhesion kinase (FAK) in the maintenance of LSCs.[1,2] Previously, we have shown that FAK inhibitors preferentially target cancer stem cells in solid tumors.[3,4] We are extending our investigation of FAK inhibitors into hematological malignancies and report here that the FAK inhibitor VS-4718 displays anticancer activity in leukemia models both *in vitro* and *in vivo*.

VS-4718 is a potent and orally bioavailable small molecule that targets cancer stem cells through inhibition of FAK which is currently being evaluated in a Phase 1 clinical trial (NCT01849744). The anti-proliferative effect of VS-4718 was evaluated in a panel of 10 cell lines derived from patients with acute promyelocytic leukemia (APL), T-cell acute lymphocytic leukemia (T-ALL), B-cell acute lymphocytic leukemia (B-ALL), chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) using a CellTiter-Glo cell viability assay on tissue culture or collagen coated plates. VS-4718 displayed anti-proliferative effects against most of these cell lines, with MV4-11 AML being the most sensitive with an EC_{50} of 100 nM. We further investigated the *in vivo* efficacy of VS-4718 in both subcutaneous and disseminated xenograft models of AML using the MV4-11 cell line. Nude mice bearing subcutaneous MV4-11 tumors were treated orally twice daily (BID) with either vehicle control or VS-4718 for 14 days. The 75 mg/kg dosage of VS-4718 caused 50% tumor growth delay and significantly extended median survival of mice from 28 days to 48 days ($p < 0.05$). Moreover, tumor regression was observed in 4 out of 10 mice. We extended these observations to a disseminated MV4-11 AML model to incorporate bone marrow stromal biology. When compared with vehicle control, VS-4718 dosed at 25 or 75 mg/kg, on an oral BID dosing schedule for 14 days, resulted in a 40% and 76% increase in mouse life span, and significantly extended survival with p values < 0.05 and < 0.001 (log rank test), respectively. The effect of VS-4718 on leukemic stem cells and minimal residual disease (MRD) is currently under investigation.

Taken together, results of our preclinical studies suggest that VS-4718 may have activity against leukemia that warrants further investigation.

INTRODUCTION

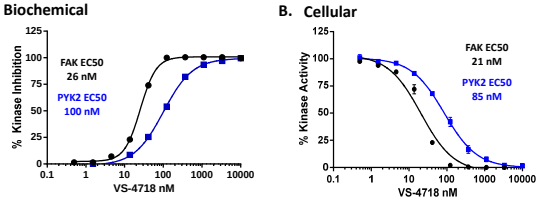
Hypothesis: VS-4718 effectively targets both leukemic blast and leukemic stem cells



- Focal Adhesion Kinase (FAK) is an established anti-cancer stem cell target.[3,5]
- FAK inhibitors have been demonstrated to induce apoptosis and decrease self-renewal capacity of AML leukemic stem cells.[1]
- PYK2, a related FAK family kinase, plays a role in hematopoietic cell responses[6].
- Depletion of integrin beta 3 and its downstream effector, PYK2, inhibits leukemic stem cell maintenance *in vivo*.[2]

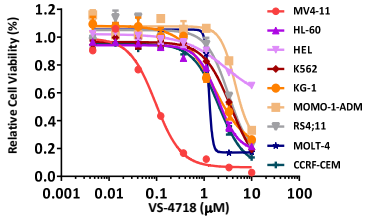
RESULTS

Fig 1: Biochemical and Cellular Activity of VS-4718



(A) VS-4718 inhibited FAK and PYK2 with biochemical IC50s of 26nM and 100nM, respectively.
(B) VS-4718 inhibited FAK and PYK2 with cellular IC50s of 21nM and 85nM, respectively.

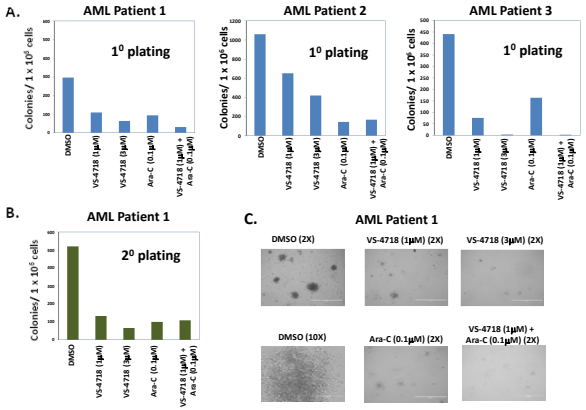
Fig 2: *In vitro* Activity of VS-4718 in Hematological Malignant Cells.



Cell Line Name	μM
MV4-11 (AML M5)	0.095
KG-1 (AML M1)	1.6
HL-60 (APL M3)	1.9
HEL (AML, M6)	2.9
MOMO-1-ADM (AML M5b)	4.5
CCRF-CEM (T-ALL)	2.1
RS4;11 (B-ALL)	3.2
K562 (BC-CML)	3.7
MOLT-4 (T-ALL)	>10

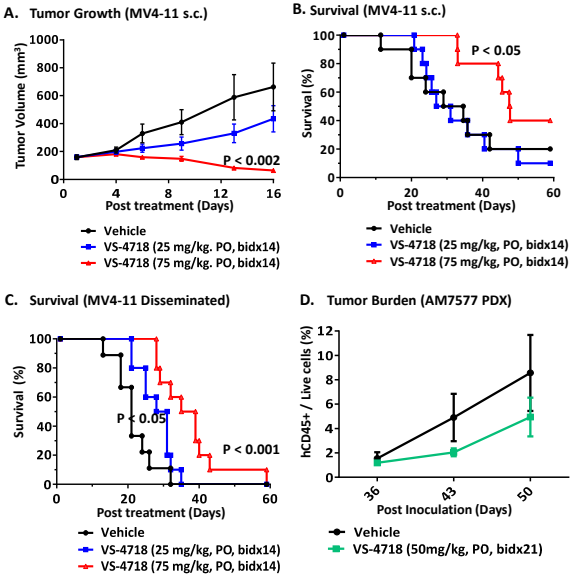
VS-4718 displayed a range of anti-proliferative activity against a panel of hematological malignant cell lines with EC50s of 0.1-10μM. Cell viability was assayed with CellTiterGlo (Promega) after 72 hours of treatment.

Fig 3: VS-4718 Inhibited Colony Forming Activity of Primary AML Cells



(A) Viably frozen AML bone marrow mononuclear cells from three patients were treated with VS-4718, Ara-C or in combination as indicated, in methylcellulose media. The number of colonies (>50 cells) were counted after 14 days of culture. (B) Cells were harvested from 1⁰ plate of patient 1 cells and 1 x 10^5 cells were replated on 2nd plate. Colonies were counted and (C) photographed after 14 days .

Fig 4: VS-4718 Inhibits Tumor Growth and Extends Survival in AML Models



VS-4718 is active in preclinical AML mouse models. (A) Mice with subcutaneous AML MV4-11 tumors showed tumor regression after 14 days of treatment with 75mg/kg VS-4718 and (B) had a significant increase in survival. (C) In a disseminated mouse model of AML using MV4-11 cells, mice treated with VS-4718 showed a dose dependent increase in survival. (D) In a PDX AML model (AM7577, CrownBio Inc.), mice treated with 50mg/kg VS-4718 had a decreased tumor burden in peripheral blood compared to the vehicle control.

SUMMARY

1. VS-4718 is a potent FAK and PYK2 inhibitor.
2. VS-4718 displayed anti-proliferative activity in primary AML and different subtypes of AML cell lines with EC_{50} values of 95 nM – 5μM.
3. Inhibition of FAK/Pyk2 activity by VS-4718 induced tumor regression and significantly increased survival in the *in vivo* models of AML.
4. A Phase I study in patients with relapsed or refractory acute leukemia is planned (NCT02215629).

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CONFLICT OF INTEREST DISCLOSURE

All the authors are employees of Verastem, Inc.

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