The FAK Inhibitor VS-4718 Attenuates Breast Cancer Stem Cell Function and Inhibits Tumor Growth *in vivo*

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Abstract# 400

ABSTRACT

Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that mediates signal transduction by integrins as well as growth factor receptors. FAK has been implicated in different steps of tumor development including tumor initiation, growth, angiogenesis and metastasis. Amplification and overexpression of FAK have been observed in aggressive human cancers including breast and ovarian cancers. We report here that VS-4718, a selective FAK kinase inhibitor, exhibits preferential inhibitory effects on breast cancer stem cells both *in vitro* and *in vivo*.

VS-4718 is a potent and selective FAK kinase inhibitor that blocks FAK autophosphorylation at Tyr397 at low nanomolar concentrations. To determine if FAK plays a role in the biology of breast cancer stem cells, the effect of FAK inhibition on cancer stem cells was determined in a multitude of cellular assays using either VS-4718 or FAK shRNA. Treatment of SUM159 triple negative breast cancer cells *in vitro* with FAK shRNA inhibited tumorsphere formation. Similarly, pre-treatment of SUM159 cells with VS-4718 in matrigel attenuated tumorsphere formation. Furthermore, VS-4718 reduced the percentage of Aldefluor $^{+}$ cancer stem cells as well as the percentage of Hoechst dye-excluding side population (SP) of SUM159 cells *in vitro*. In direct contrast, the cytotoxic agent paclitaxel increased the percentage of cancer stem cells in these assays. Taken together, these data indicate a role of FAK in the maintenance of breast cancer stem cells and the activity of VS-4718 to attenuate cancer stem cell function.

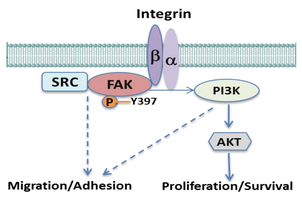
The effect of VS-4718 on cancer stem cells *in vivo* was examined in SUM159 and MDA-MB-231 human triple negative breast cancer xenograft models. Following systemic administration, VS-4718 caused significant reduction of cancer stem cells in tumors as assessed by a decrease in the percentage of Aldefluor + cells and a reduction in tumorsphere-forming efficiency relative to vehicle-treated tumors. The FAK inhibitor VS-4718 also induced significant dose-dependent tumor growth inhibition in the MDA-MB-231 xenograft model.

In summary, our results indicate the importance of FAK in maintaining breast cancer stem cells *in vitro* and *in vivo*, and support the clinical development of the selective FAK inhibitor VS-4718 to target cancer stem cells for the treatment of triple negative breast cancer.

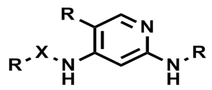
INTRODUCTION

FAK has been implicated in the self-renewal of cancer stem cells (CSC) and breast cancer development

- Inactivation of FAK or β1 integrin compromised mammary CSC self renewal (Taddei, Nature Cell Biol 2008)
- In the MMTV-PyMT model, targeted deletion of FAK in mouse mammary epithelium reduced the number & self renewal capability of cancer stem/progenitor cells & impaired tumor growth (Luo, Cancer Res 2009)
- FAK amplification correlates with poor survival of breast cancer patients (Pylayeva, JCI 2009)
- Integrin β1 – FAK signaling is critical for proliferation of micro-metastatic breast cancer cells in the lung (Shibue & Weinberg, PNAS 2009)



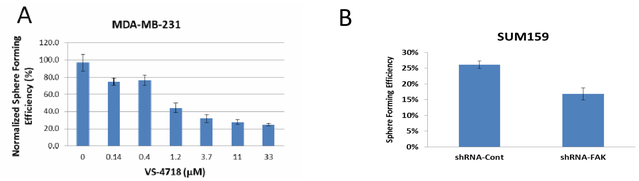
VS-4718 is a potent and selective FAK kinase inhibitor



Cellular pFAK EC_{50} = 4 nM

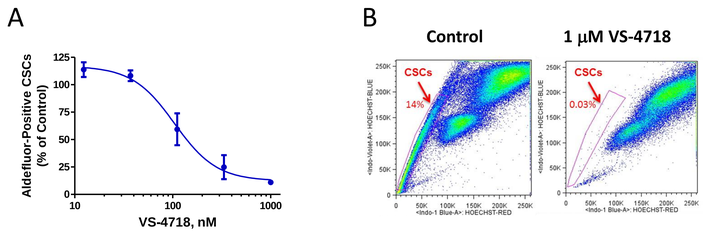
RESULTS

Fig 1: FAK is important for the self renewal of cancer stem cells *in vitro*



A, MDA-MB-231 cells were treated with compounds during tumorsphere formation (Experiment was performed by Epistem, Inc.). VS-4718 inhibited sphere forming efficiency of MDA-MB-231 breast cancer cells in a dose-dependent manner. *B*, SUM159 cells harboring FAK shRNA or control shRNA were cultured without compound in matrigel for 5 days . Cells were then dissociated and plated on low adhesion plates in serum free medium for tumorsphere assay.

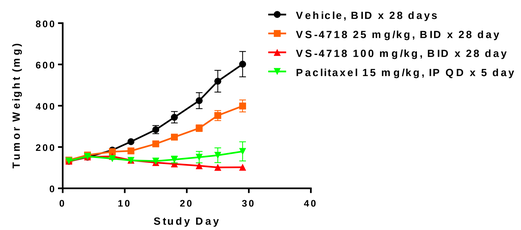
Fig 2: FAK inhibitor VS-4718 reduces the proportion of CSCs in Aldefluor and Hoechst dye exclusion assays



A, MDA-MB-231 cells were treated with VS-4718 for 4 days in 3D matrigel . Cells were then extracted from matrigel, plated on tissue culture plate and subjected to an Aldefluor assay. The percent of Aldefluor positive cells normalized to control is shown.

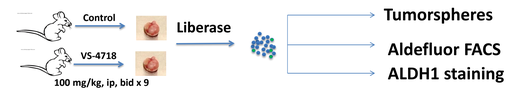
B, SUM159 cells were treated with VS-4718 for 4 days before a Hoechst dye exclusion assay was carried out.

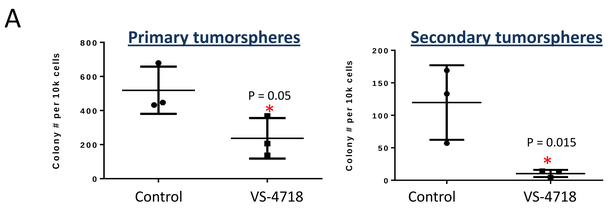
Fig 3: Potent *in vivo* antitumor activity of VS-4718

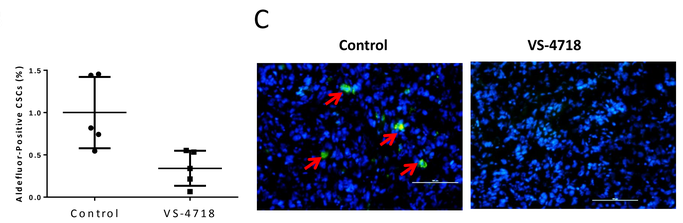


ICR-scid mice bearing MDA-MB-231 breast xenograft tumors were treated with VS-4718 and paclitaxel at the indicated doses and schedules (study was conducted by TGen).

Fig 4: VS-4718 reduces cancer stem cells in xenograft tumors *in vivo*







MDA-MB-231 tumor bearing mice were treated with VS-4718 at the indicated schedules. Cells were dissociated from harvested tumors and subject to tumorsphere assays (A) and Aldefluor FACS analysis (B). Frozen sections of harvested tumors were also prepared and subject to ALDH1 immunofluorescence analysis (C).

SUMMARY

1. VS-4718 is a potent and selective FAK kinase inhibitor
2. FAK inhibitor VS-4718 preferentially reduces cancer stem cells *in vitro* and *in vivo*
3. Single agent treatment with VS-4718 induces complete tumor growth inhibition
4. Our results demonstrate the importance of FAK in the self-renewal of cancer stem cells and support the clinical development of a FAK inhibitor to achieve more durable clinical responses for cancer patients

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