FAK Inhibitor VS-4718 Attenuates Breast Cancer Stem Cell Function *in vitro* and *in vivo*

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ABSTRACT

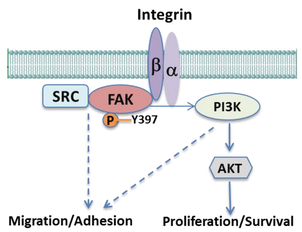
Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that mediates signal transduction by integrins as well as growth factor receptors. FAK has been implicated in multiple steps of carcinogenesis including tumor initiation, growth and metastasis. Amplification and overexpression of FAK have been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

VS-4718 is a potent and selective FAK kinase inhibitor that blocks fibronectin-stimulated FAK autophosphorylation at Tyr397 at low nanomolar concentrations. To determine if FAK plays a role in the biology of breast cancer stem cells, VS-4718 was evaluated in a multitude of cancer stem cell assays both *in vitro* and *in vivo*. Treatment of SUM159 triple negative breast cancer cells *in vitro* with FAK shRNA inhibits tumorsphere formation, and therefore indicates a role of FAK in breast cancer stem cell renewal. Similarly, pre-treatment of SUM159 cells with VS-4718 in matrigel attenuated secondary tumorsphere formation. Furthermore, VS-4718 reduced the side population (SP) and the percentage of Aldefluor+ cancer stem cells in SUM159 breast cancer cells *in vitro*. In direct contrast, standard-of-care agents such as paclitaxel and cisplatin increased the percentage of Aldefluor+ cancer stem cells under equivalent conditions.

The effect of VS-4718 on cancer stem cells *in vivo* was evaluated in SUM159 and MDA-MB-231 human triple negative breast cancer xenograft models. Following systemic administration, VS-4718 induced significant reduction of cancer stem cells in tumors as assessed by a decrease in Aldefluor+ cells and tumorsphere-forming efficiency relative to vehicle-treated tumors.

In summary, our results indicate the importance of FAK in the self-renewal of 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.

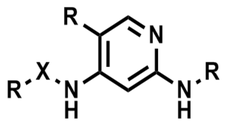
INTRODUCTION



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)

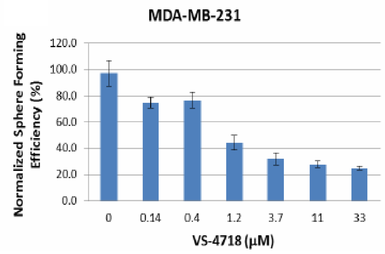
VS-4718 is a potent and selective FAK kinase inhibitor



Cellular pFAK EC_{50} = 4 nM

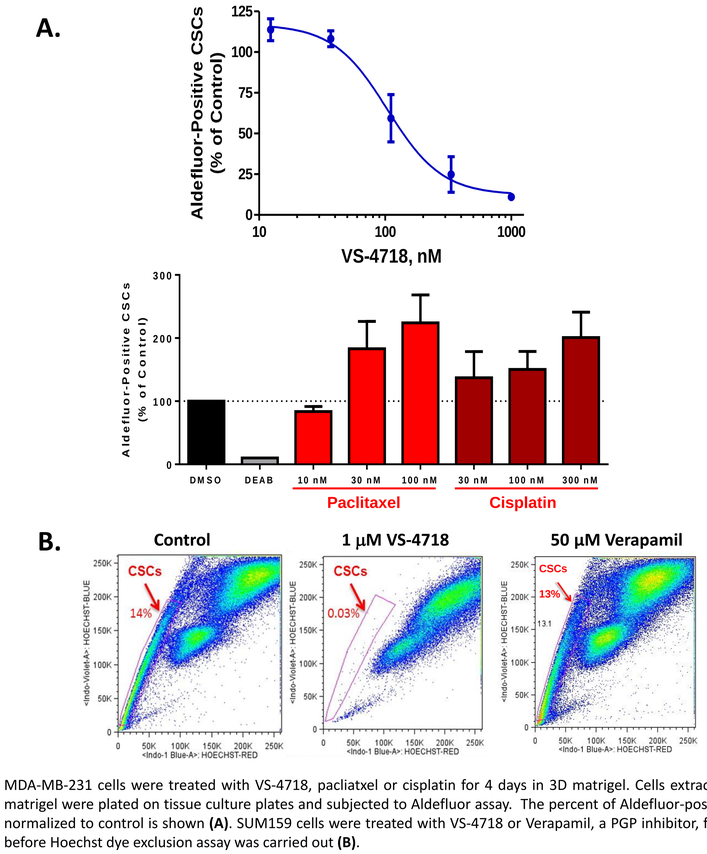
RESULTS

Fig 1: FAK is important for the self renewal of CSCs *in vitro*



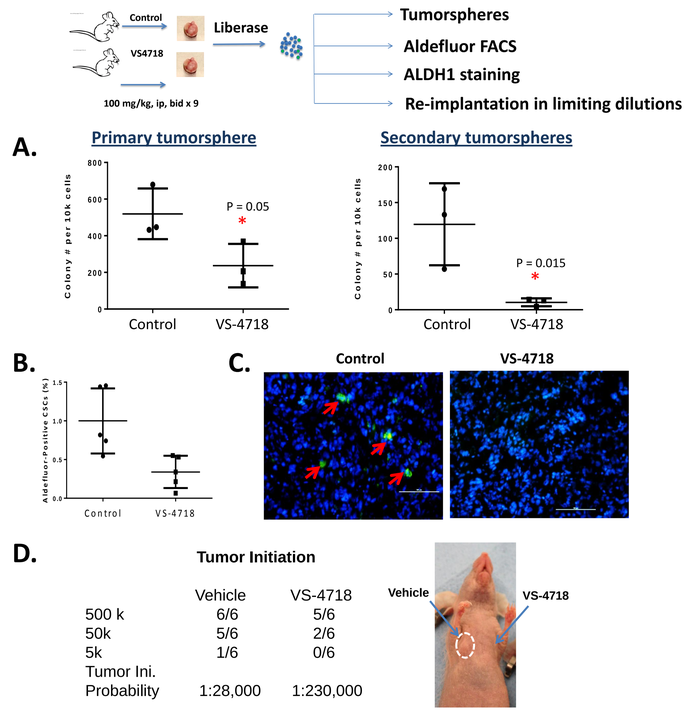
MDA-MB-231 cells were treated with VS-4718 in tumorsphere formation assay. VS-4718 inhibited sphere forming efficiency of MDA-MB-231 breast cancer cells in a dose-dependent manner.

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



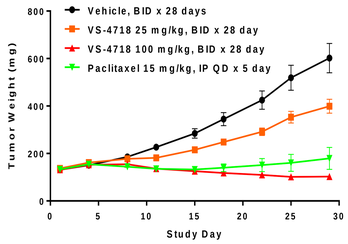
MDA-MB-231 cells were treated with VS-4718, pacliatxel or cisplatin for 4 days in 3D matrigel. Cells extracted from matrigel were plated on tissue culture plates and subjected to Aldefluor assay. The percent of Aldefluor-positive cells normalized to control is shown **(A)**. SUM159 cells were treated with VS-4718 or Verapamil, a PGP inhibitor, for 4 days before Hoechst dye exclusion assay was carried out **(B)**.

Fig 3: VS-4718 preferentially reduces CSCs in xenograft tumors *in vivo*



Tumor bearing mice were treated with VS-4718 at the indicated schedules. Tumors from Sum159 xenograft model were dissociated 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)**. Cells from dissociated MDA-MB-231 tumors were xenografted in limiting dilutions in SHrN mice **(D)**.

Fig 4: 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.

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* as assessed with multiple CSC assays
3. VS-4718 effectively inhibits breast cancer tumor growth *in vivo*
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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