The FAK Inhibitors VS-4718 and VS-5095 Attenuate Breast Cancer Stem Cell Function *in vitro* and Tumor Growth *in vivo*

LB-192

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ABSTRACT

As a key mediator of integrin signaling, focal adhesion kinase (FAK) regulates cellular responses to extracellular matrix interactions. Amplification and overexpression of FAK have been observed in aggressive human cancers including breast cancer. FAK has been implicated in multiple steps in carcinogenesis including tumor growth, metastasis and angiogenesis. We now demonstrate the importance of FAK in breast cancer stem cell function, and the reduction of cancer stem cell function by the selective FAK inhibitors VS-4718 and VS-5095.

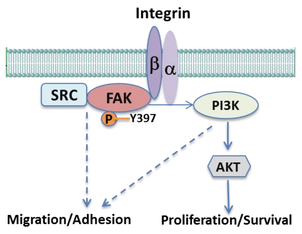
VS-4718 and VS-5095 are potent and selective FAK inhibitors which were optimized following high throughput screening. Both VS-4718 and VS-5095 block fibronectin-stimulated FAK autophosphorylation of Tyr397 with low nanomolar cellular potency and are highly selective for FAK among a panel of protein kinases. Consistent with their mechanism of action, VS-4718 and VS-5095 showed greater inhibitory potency on the growth of multiple cancer cell lines in 3D matrigel culture as compared to conventional 2D culture. To determine if FAK plays a role in the biology of breast cancer stem cells in addition to its reported function in normal mammary stem cell biology, the effects of these FAK inhibitors were characterized using two different *in vitro* assays. It was previously demonstrated that immortalized mammary epithelial cells (HMLEs) driven to undergo epithelial to mesenchymal transition (EMT) by knockdown of E-cadherin (HMLE-shECad) exhibit many of the characteristics of cancer stem cells and can be used to identify agents that selectively target cancer stem cells. VS-4718 exhibited greater potency against proliferation of mesenchymal HMLE-shECad cells as compared to epithelial HMLE-shGFP control cells, suggesting preferential effects on breast cancer stem cells. Furthermore, pre-treatment of SUM159 triple negative breast cancer cells with VS-5095 in matrigel attenuated secondary tumorsphere formation, suggesting that FAK is important for the self-renewal function of breast cancer stem cells. The role of FAK in breast cancer stem cell renewal was further corroborated by the observation that FAK shRNA inhibited tumorsphere formation by SUM159 cells. The *in vivo* efficacy of the FAK inhibitor VS-5095 was evaluated in a xenograft tumor model. By oral administration,

VS-5095 induced significant dose-dependent tumor growth inhibition. In summary, these results demonstrate the importance of FAK in the self-renewal of breast cancer stem cells, and support the clinical development of the selective FAK inhibitors VS-4718 and VS-5095 to target breast cancer stem cells for the treatment of triple negative breast cancer.

INTRODUCTION

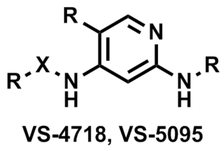
FAK has been implicated in the self-renewal of cancer stem cells (CSC) and breast cancer development

- 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)

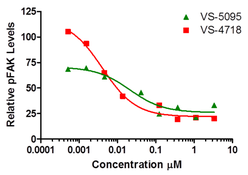


RESULTS

Fig 1: VS-4718 and VS-5095 are selective FAK inhibitors with potent cell-based activities



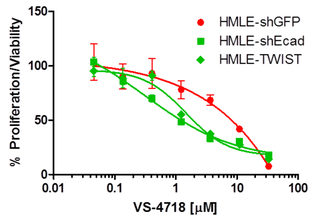
VS-4718, VS-5095



	VS-4718	VS-5095
EC_{50} (nM)	4	21

VS-4718 and VS-5095 were evaluated in a phospho- ELISA assay measuring FAK autophosphorylation on Tyr-397. Both VS-4718 and VS-5095 exhibit high selectivity for FAK relative to a panel of protein kinases.

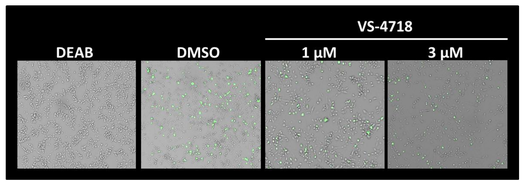
Fig 2: FAK inhibitor VS-4718 show preferential effects on mesenchymal HMLEs (CSCs)



	HMLE-shGFP	HMLE-shECad	HMLE-TWIST
EC_{50} (μM)	~ 10	0.31	1.5

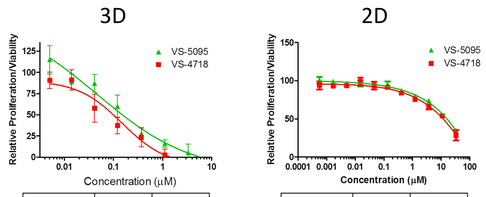
Derivatives of HMLE cells were treated with VS-4718 for 3 days and cell proliferation/viability was assessed using CellTiter Glo (Promega). HMLE-shGFP, HMLE containing shRNA of GFP; HMLE-shEcad, HMLE containing shRNA of CDH1; HMLE-TWIST, HMLE overexpressing TWIST.

Fig 3: FAK inhibitor VS-4718 reduce the % of Aldefluor positive cells in contrast to paclitaxel



SUM159 cells were treated with VS-4718 for 2 days, and Aldefluor assay was performed. Aldefluor positive cells are shown in green.

Fig 4: VS-4718 and VS-5095 inhibit the proliferation/survival of breast cancer cells in 3D matrigel but not in 2D cell culture plates

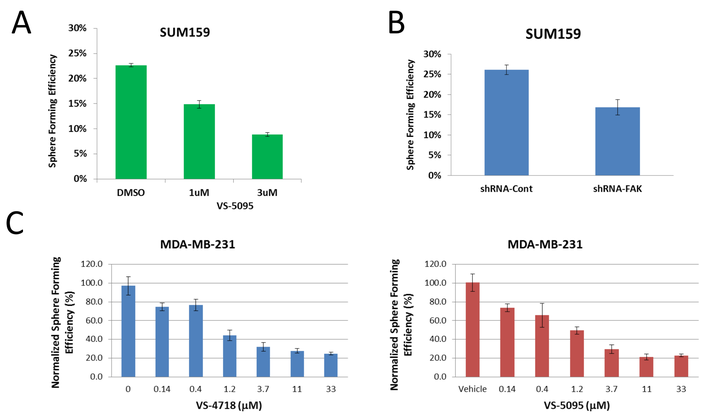


	VS-4718	VS-5095
EC_{50} (μM)	0.15	0.046

	VS-4718	VS-5095
EC_{50} (μM)	> 10	> 10

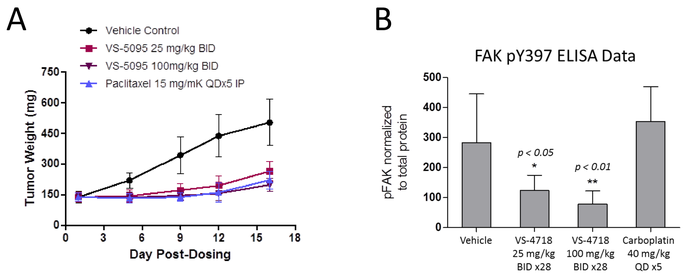
Cells grown in standard cell culture plates or 3D matrigel were treated with VS-4718 and VS-5095. Cell proliferation/viability was accessed using CellTiter Glo for 2D culture or MTS for 3D matrigel.

Fig 5: FAK is important for the self renewal of cancer stem cells *in vitro*



(A) SUM159 cells were treated with VS-5095 in matrigel for 5 days. (B) SUM159 cells harboring FAK shRNA or control shRNA were cultured without compound in matrigel for 5 days. Cells from (A) and (B) were then dissociated and plated on low adhesion plates in serum free medium for tumorspheres to form. (C) MDA-MB-231 cells were treated with compounds during tumorsphere formation (Experiment was performed by Epistem, Inc.) Both VS-4718 and VS-5095 inhibited sphere forming efficiency of MDA-MB-231 breast cancer cells.

Fig 6: Potent *in vivo* antitumor activity and inhibition of FAK in tumors



(A) ICR-scid mice bearing xenograft tumors were treated with VS-5095 and paclitaxel at the indicated doses and schedules (study was conducted by TGen.) (B) Autophosphorylation of FAK on Y397 in xenograft tumor tissues was determined using a phospho-FAK ELISA assay. Tumors were harvested 2h post a single dose of indicated compounds at the end of the study.

SUMMARY

Here we provide *in vitro* evidence that FAK inhibitors VS-4718 and VS-5095 target cancer stem cells. These results demonstrate the importance of FAK in the self-renewal of breast cancer stem cells and support the clinical development of the selective FAK inhibitors VS-4718 and VS-5095 to target breast cancer stem cells for the treatment of triple negative breast cancer.

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