Focal Adhesion Kinase (FAK) Inhibitor VS-6063 (defactinib) Preferentially Targets Cancer Stem Cells in Triple Negative Breast Cancer

Qunli Xu, Vihren N. Kolev, Quentin G. Wright, Jennifer E. Ring, Christian M. Vidal, Irina M. Shapiro, David T. Weaver, Mahesh V. Padval and Jonathan A. Pachter

Verastem Inc., Cambridge MA

ABSTRACT

Triple negative breast cancer (TNBC) is defined phenotypically as the lack of ER, PR and HER2 expression, and comprises a heterogeneous group of breast cancers. Cancer stem cells (CSCs), identified as either ALDH1+ or CD44+/CD24- subpopulations in breast cancer, have been shown to be resistant to standard chemotherapy and associated with poor clinical outcome. In recent reports, the presence of ALDH1+ (but not ALDH1-) cells in axillary lymph nodes following neoadjuvant chemotherapy was associated with poor overall survival. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that orchestrates cell signaling through integrins and growth factor receptors. In addition to regulating the proliferation, survival, invasion and metastasis of cancer cells, FAK also plays a critical role in the self-renewal and survival of CSCs. VS-6063 (defactinib) is a potent, selective, and orally bioavailable FAK inhibitor with demonstrated tolerability and preliminary signs of clinical activity as a single agent and in combination with paclitaxel in phase I clinical trials. We report here that VS-6063 preferentially targets CSCs in preclinical models of TNBC.

The effect of VS-6063 on CSCs was assessed in a panel of orthogonal assays. Treatment of human TNBC cells with VS-6063 in 3D matrigel reduced the percentage of Aldefluor+ CSCs, Hoechst dye-excluding side population (SP) CSCs, and tumour sphere-forming efficiency in a serial tumour sphere passaging assay, suggesting that VS-6063 preferentially inhibits CSCs. VS-6063 also reduced the proportion of Aldefluor+ CSCs in primary breast cancer tissue specimens cultured ex vivo. Consistent with the concept that the effect of VS-6063 on CSCs is mediated by FAK inhibition, silencing-mediated knockdown of FAK in SUM159 TNBC cells recapitulated the inhibitory effect of VS-6063 on CSCs. In contrast, the standard-of-care (SoC) chemotherapeutic agents paclitaxel and doxorubicin enriched the percentage of CSCs, suggesting that these SoC agents preferentially bulk tumor cells relative to CSCs. When administered in combination, VS-6063 attenuated the chemotherapy-induced enrichment of CSCs. Following oral administration of VS-6063 in the MDA-MB-231 human TNBC xenograft model in vivo, reduction of CSCs in tumors was evidenced by a decrease in secondary tumour sphere-forming efficiency. Importantly, cells dissociated from VS-6063-treated tumors displayed reduced tumor-initiating capability upon re-implantation into immunodeficient mice in limiting dilutions. In summary, our results indicate that the FAK inhibitor VS-6063 preferentially targets CSCs and support the clinical development of VS-6063 in combination with SoC agents, such as paclitaxel, to potentially improve clinical outcomes for patients with TNBC through simultaneous targeting of both CSCs and bulk tumor cells.

INTRODUCTION

Neoadjuvant chemotherapy in patients with TNBC increases the proportion of CSCs in primary breast tumors

Cancer stem cells in tumors quantified by (A) CD44+/CD24- cells or (B) efficiency of tumour sphere formation. (Li et al., JNCI 100:672, 2008)

Presence of ALDH1+ CSCs in residual axillary disease after neoadjuvant chemotherapy & surgery correlates with poor overall survival for patients with breast cancer

Fig 1: VS-6063 is a potent inhibitor of FAK and the closely related protein kinase PYK2

The potency of VS-6063 against FAK and PYK2 was quantified in (A) biochemical kinase assays and (B) cellular autophosphorylation assays. VS-6063 is -100 fold selective for FAK and PYK2 among a panel of 160 protein kinases. FAK has been reported to play an important role in CSC survival and tumor-initiating capability, and PYK2 has also been implicated in this biology.

RESULTS

Fig 2: VS-6063 preferentially reduces CSCs in multiple orthogonal assays in vitro

A. Aldefluor CSC Assay
B. 3-D Tumorsphere Formation
C. Hoechst Dye Exclusion CSC Assay

VS-6063 reduced the proportion of CSCs in vitro as assessed by (A) an Aldefluor ALDH enzymatic assay in SUM159 and MDA-MB-231 TNBC cell lines, FAK silRNA had the same effect. (B) secondary tumour sphere formation by SUM159 cells, and (C) Hoechst dye exclusion side population analysis in SUM159 cells.

Fig 3: VS-6063 Attenuates Chemotherapy Enrichment of CSCs

The percentage of Aldefluor-positive CSCs was dose-dependently increased by the standard-of-care chemotherapeutic agents (A) doxorubicin, (B) cisplatin and (C) paclitaxel. (D) Addition of VS-6063 (3 μM) blocked the induction of the percentage of CSCs by paclitaxel as assessed by the Aldefluor assay in SUM159 cells

SUMMARY

- Neoadjuvant chemotherapy has been shown to enrich CSCs and presence of CSCs in lymph nodes has been correlated with poor overall survival in patients with breast cancer
- VS-6063 is a potent, selective inhibitor of the FAK family kinases FAK & PYK2
- VS-6063 preferentially eliminates CSCs as assessed with multiple orthogonal assays in preclinical TNBC models both in vitro and in vivo
- In contrast, the standard-of-care chemotherapeutic agents doxorubicin, cisplatin and paclitaxel increase the percentage of CSCs
- These preclinical data provide rationale for the clinical development of VS-6063 in combination with chemotherapy for the neoadjuvant treatment of TNBC