FAK Inhibition Targets Cancer Stem Cells

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ABSTRACT

Targeting cancer stem cells (CSCs) holds promise to address key challenges of cancer treatment: chem- and radiation therapy resistance, metastasis, and recurrence. Increased CSC abundance after neoadjuvant chemotherapy has been associated with a significantly worse outcome. Combining CSC-targeted agents with chemotherapy may lead to more durable response with increased overall survival. Focal adhesion kinase (FAK), a non-receptor tyrosine kinase, mediates signal transduction by integrins and growth factor receptors to regulate cellular adhesion, proliferation, migration, and survival. Several studies have demonstrated that FAK expression and kinase activity are necessary for survival and maintenance of CSCs. FAK is also upregulated in many epithelial tumors and associated with poor patient prognosis and therefore has been pursued as a promising therapeutic target for cancer. vs-6063 and VS-4718 are orally bioavailable small molecules that impede CSCs through the inhibition of FAK. Both vs-6063 and VS-4718 preferentially kill CSCs in multiple cancer models, including models of breast, ovarian, SCLC, and mesothelioma, and these agents are in clinical development. We demonstrate that treatment of cancer cell lines or mice bearing xenograft tumors with vs-6063 or VS-4718 decreases CSCs as assessed by decreased sphere formation in limiting dilution assays. Both FAK inhibitors decrease significantly CSCs to a varying degrees across multiple tumor models. Furthermore, treatment of mice with either FAK inhibitor following cessation of chemotherapy delayed tumor regrowth in xenograft and PDX models of TNBC, SCLC, and mesothelioma. A mechanistic investigation in breast cancer cell lines revealed an important crosstalk between FAK and the Wnt/b-catenin pathway, whereby FAK inhibition blocked Notch phosphorylation and activation. Importantly, a constitutively active mutant form of b-catenin “rescued” CSCs, suggesting that preferential targeting of CSCs by FAK inhibitors is mediated, at least in part, through attenuation of downstream b-catenin activation. In breast cancer, CSCs are identified by the expression of aldehyde dehydrogenase 1 (ALDH1) or CD44 high/CD24 low markers. To probe the CSC populations in breast cancer patients, we developed a multiplex immunofluorescence assay with three CSC markers (ALDH1, CD44, and CD24) and an epithelial marker (pan-cytokeratin) for FFPF tissue. Automated image analysis with pathology review was used to evaluate single CSCs within the tumor. This assay may provide a means of monitoring a neoadjuvant CSC-targeted treatment in breast cancer FFPF samples in clinical trials.

INTRODUCTION

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

Importance of targeting cancer stem cells for a durable response

RESULTS

Fig 1: VS-4718 and VS-6063 target CSCs in breast, ovarian, small cell lung cancers and mesothelioma xenograft tumors as measured in vivo limiting dilution assay

Fig 2: VS-4718 and VS-6063 extend the response to chemotherapy in breast cancer, SCLC and mesothelioma models

Fig 3: FAK inhibition of CSCs is associated with a Wnt/b-Catenin-dependent mechanism

Fig 4: Identification of CSCs in primary TNBC tumors

SUMMARY

• Cancer stem cells are increased by standard chemotherapy.
• Oral small molecule inhibitors of FAK/PYK2 decrease CSCs and tumor initiating capability in multiple models.
• FAK inhibitors VS-4718 and VS-6063 block tumor regrowth in various xenograft models when administered with, or following, chemotherapy.
• FAK inhibitors attenuate CSC self renewal through a Wnt/b-Catenin-dependent mechanism

CSCs in breast cancer have been identified as ALDH1+ or CD44hi/CD24int subpopulations. Sections from primary TNBC tumors were analyzed for expression of Cytokeratin, ALDH1A1, CD44 and CD24 by Opal (Perkin Elmer) multiplex assay.