The Pan-PI3K/mTOR Kinase Inhibitor VS-5584 Preferentially Targets Cancer Stem Cells in Breast Cancer Models

Jonathan A. Pachter, Vihren N. Kolev, Alissa A. Neill, Quentin Wright, Mahesh V. Padval & Qunli Xu
Verastem, Inc., Cambridge, MA

ABSTRACT

The PI3-kinase/mTOR/AKT pathway is well established to play a central role in tumor cell proliferation and survival. Depending on the mode of pathway activation, different PI3K isoforms and mTOR complexes have been shown to play important roles in oncogenesis. VS-5584 is an ATP-competitive inhibitor which selectively inhibits PI3K/mTOR signaling with equivalent low nanomolar potency against all human Class I PI3K isoforms and mTOR kinase. In a high-throughput screen designed to identify compounds that selectively target cancer stem cells (CSCs), we identified PI3K/mTOR signaling as a critical node. Using HMLE immortalized human mammary cells driven through epithelial-mesenchymal transition (EMT) by knock down of E-cadherin, the PI3K/mTOR inhibitor VS-5584 was approximately 10-fold selective for CSCs with an EC50 value of 15 nanomolar. Accordingly, VS-5584 preferentially decreased CD44+/CD24- cells in an HMLER immortalized mammary cancer cell line in contrast to paclitaxel which increased the proportion of CD44+/CD24- CSCs in this model. The effect of VS-5584 on CSCs was also measured by assessing Aldefluor-positive subsets of SUM159 triple negative breast cancer cells. VS-5584 dose dependently decreased the percentage of Aldefluor-positive cells, in contrast to paclitaxel which again enriched CSCs in this model. Finally, CSCs are known to have an enhanced ability to exclude cytotoxic agents. This drug-resistant CSC population, termed the side population, can be monitored by exclusion of Hoechst dye. In SUM159 cells, VS-5584 effectively eliminated the CSC side population, while paclitaxel increased this population. Upon oral dosing, VS-5584 induced tumor regression in mice and strongly reduced Aldefluor-positive CSCs in tumors. These data provide rationale for the clinical development of VS-5584. We observed clear inhibition of CSCs by VS-5584 in contrast to a consistent increase in the proportion of CSCs following treatment with cytotoxic standard-of-care agents such as paclitaxel. Therefore, combination treatment with standard-of-care agents to de-bulk the tumor and VS-5584 to reduce CSC burden may be an ideal drug regimen to achieve durable responses in patients with triple negative breast cancer or other solid tumors.

INTRODUCTION

Fig 1: Structure & selectivity profile of VS-5584

RESULTS

Fig 2: VS-5584 preferentially inhibits Cancer Stem Cells in HMLE breast cancer cells while paclitaxel increases the percentage of Cancer Stem Cells

Fig 3: VS-5584 reduces the number of Aldefluor-positive Cancer Stem Cells while paclitaxel increases the percentage of Cancer Stem Cells

Fig 4: VS-5584 reduces the percentage of Cancer Stem Cells (side population) in a Hoechst dye exclusion assay

Fig 5: Oral treatment of tumor bearing mice with VS-5584 reduces Cancer Stem Cells analyzed from extracted tumors

Fig 6: Oral VS-5584 induces tumor regression in a docetaxel-resistant patient-derived breast cancer model

SUMMARY

- VS-5584 is a selective dual PI3K/mTOR inhibitor that potently inhibits mTOR kinase and all Class I PI3K isoforms
- VS-5584 preferentially inhibits Cancer Stem Cells in vitro as assessed in multiple CSC assays
- In contrast, taxane treatment increases the percentage of CSCs
- In mice bearing triple negative breast cancer tumors, oral dosing of VS-5584 decreases tumor CSCs & induces tumor regression in taxane-resistant models
- These data support the clinical development of VS-5584 for treatment of breast and other cancers