Combined inhibition of PI3K isoforms and mTOR kinase is critical for cancer stem cell inhibition by VS-5584

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ABSTRACT

Cancer stem cells (CSCs) represent a subpopulation of cancer cells that have tumor-initiating capability, are particularly resistant to chemotherapy, and can mediate tumor recurrence both locally and at metastatic sites. As such, these cells represent a critical challenge for effective treatment of cancer. High-throughput screening for small molecules that preferentially target CSCs identified inhibitors of the PI3K/mTOR pathway, suggesting the importance of this signaling pathway for CSC biology. Here we demonstrate that inhibition of multiple PI3K isoforms by isoform-specific siRNAs is not sufficient to reduce the proportion of CSCs. In contrast, combined knock down of PI3K isoforms and mTOR effectively reduced the proportion of CSCs in tumor cell lines. VS-5584 is a potent and selective PI3K/mTOR inhibitor with equipotency against all four human Class I PI3K isoforms and the mTOR kinase. We demonstrate that VS-5584 preferentially targets CSCs in multiple orthogonal assays both in vitro and in human tumor xenograft models. Cancer stem cells express high levels of aldehyde dehydrogenase, and an Aldefluor assay that measures activity of this enzyme was used to identify CSCs. VS-5584 decreased the percentage of Aldefluor-positive cells across multiple breast cancer cell lines. We demonstrated that VS-5584 preferentially induced apoptosis in Aldefluor-positive SUM159 cells relative to Aldefluor-negative cells as measured by Annexin V and Caspase 3/7 assays. In contrast, paclitaxel induced more apoptosis in Aldefluor-negative than Aldefluor-positive cells and enriched the percentage of CSCs. Another characteristic of CSCs is their enhanced ability to efflux cytotoxic agents. This CSC population, which is drug resistant and is called side population (SP), can be monitored by exclusion of Hoechst dye. VS-5584 effectively eliminated the SP CSCs across multiple cancer types, while cisplatin and etoposide increased this subpopulation. Furthermore, ex vivo treatment of primary breast and ovarian tumor specimens with VS-5584 decreased the proportion of CSCs as measured by the Aldefluor assay and cell surface markers. Significantly, VS-5584 also targets CSCs in vivo in MDA-MB-231 triple negative and MC27 ER+ breast cancer xenograft models as evidenced by decreases in the percentage of Aldefluor-positive cells, tumourosphere-forming efficiency, and tumor-initiating capability in an in vivo limiting dilution re-implantation assay. Consistent with the notion that combined inhibition of PI3K isoforms and mTOR is critical for exerting a strong anti-CSC effect, the mTORC1-selective inhibitor everolimus did not reduce CSCs in the MC27 xenograft model. The potent anti-CSC activities in primary patient cancer tissue and in xenograft models provide strong rationale for the clinical development of VS-5584 in combination with agents targeting the bulk tumor to achieve durable clinical responses for cancer patients.

INTRODUCTION

Structure & selectivity profile of VS-5584

Biochemical Potencies (IC50, nM)*

<table>
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<tr>
<th>Kinase</th>
<th>VS-5584</th>
<th>mTOR</th>
<th>PI3Kβ</th>
<th>PI3Kα</th>
<th>PI3Kμ</th>
<th>PI3Kδ</th>
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<tbody>
<tr>
<td>IC50 (nM)</td>
<td>2.6</td>
<td>3.4</td>
<td>3.3</td>
<td>21</td>
<td>2.7</td>
<td>3</td>
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</tbody>
</table>
* Data on file Verastem

Selective for these kinases among a panel of over 400 kinases
(Hart et al., Mol Cancer Ther. 2013 Feb;12(2):151-61.)

RESULTS

Fig 1: Combined inhibition of PI3K Isoforms and mTOR kinase by VS-5584 preferentially targets CSCs in vitro.

A. SUM159
B. MCF7
C. HS578T

Fig 2: VS-5584 preferentially targets CSCs in vivo and delays tumor regrowth after chemotherapy in small cell lung cancer models.

A. NCI-H841
B. NCI-H841
C. NCI-H841
D. NCI-H841

Fig 3: VS-5584 reduces CSCs and tumor initiating frequency in an ER+ breast cancer xenograft model in vivo.

A. MC27 Tumor bearing mouse
B. MC27 Tumor bearing mouse
C. MC27 Tumor bearing mouse
D. MC27 Tumor bearing mouse

Fig 4: VS-5584 reduces CSCs in primary human ovarian and breast tumors in ex vivo culture.

A. Patient 1
B. Patient 1
C. Patient 1
D. Patient 1

Fig 5: VS-5584 induces apoptosis preferentially in CSCs

A. Palcitaxel
B. Palcitaxel
C. Palcitaxel
D. Palcitaxel

SUMMARY

• VS-5584 is a highly selective dual PI3K/mTOR inhibitor that potently inhibits mTORC1, mTORC2 and all Class I PI3K isoforms.

• VS-5584 preferentially inhibits cancer stem cells as assessed in orthogonal assays in vitro, in vivo and ex vivo using human primary cancer specimens. In contrast, everolimus – a mTORC1 inhibitor does not have an effect on CSCs and the cytotoxic agent paclitaxel enriches for CSCs.

• Consistent with its preferential targeting of CSCs, VS-5584 delays tumor regrowth after treatment with the cytotoxic agent cisplatin in a SCLC xenograft model.

• VS-5584 is currently being evaluated in a Phase 1 clinical trial in patients with solid tumors and lymphoma.