The cancer stem cell inhibitors VS-6063 (defactinib) and VS-5584 exhibit synergistic anticancer activity in preclinical models of mesothelioma

Abstract# 446

Qunli Xu[1], Winnie F. Tam[1], Christian M. Vidal[1], Vihren N. Kolev[1], Yuwaraj Kadariya[2], Craig W. Menges[2], Joseph R. Testa[2], and Jonathan A. Pachter[1]

[1]Verastem Inc., Boston, MA; [2]Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA

ABSTRACT

Malignant pleural mesothelioma is an aggressive cancer of the lining of the lung in which cancer stem cells may drive resistance to current chemotherapy. There is only one treatment regimen approved for use, pemetrexed plus a platinum agent, which is used as front-line therapy and results in median overall survival of only 12 months. Unfortunately, there are no approved second line options for patients with actively progressing disease after front-line therapy.

VS-6063 (defactinib) is an oral small molecule that targets cancer stem cells through the inhibition of focal adhesion kinase (FAK). FAK is a cytoplasmic tyrosine kinase which orchestrates cell signaling through integrins and growth factor receptors and has been shown to be critical for tumor initiation and cancer stem cell function (Luo et al., Cancer Res, 2009; Shibue et al., Cancer Disc, 2012; Kolev et al., Cancer Res, 2013, 73(8 Suppl): 236). VS-6063 has demonstrated tolerability, target inhibition, and preliminary signs of clinical activity as a single agent and in combination with paclitaxel in Phase 1 clinical trials (Jones et al., J Clin Oncol 29: 2011 (suppl; abstr 3002); Patel et al., J Clin Oncol 32:5s, 2014 (suppl; abstr 5521)). Based on these preclinical and clinical data, VS-6063 is being tested in a registration-directed, randomized, double-blind, placebo-controlled trial in malignant pleural mesothelioma immediately following front-line therapy (COMMAND Trial, NCT01870609).

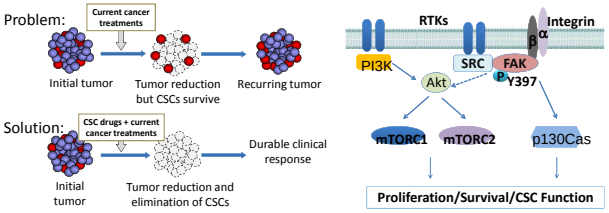
In an effort to expand the mesothelioma patient population that may potentially benefit from drugs targeting cancer stem cells, we sought to identify agents that show synergistic anticancer activity with VS-6063. An *in vitro* combination screen was carried out evaluating VS-6063 in combination with 20 anticancer agents including both cytotoxic drugs and targeted agents. Several anticancer agents, including the dual PI3K/mTOR inhibitor VS-5584, showed synergistic activity with VS-6063. We report here characterization of the combination activity of VS-6063 and VS-5584 in mesothelioma models *in vitro* and *in vivo*.

VS-6063 and VS-5584, alone and in combination, reduced the proportion of mesothelioma cancer stem cells. In addition, single agent treatment with VS-6063 or VS-5584 reduced the viability of mesothelioma cells cultured in 3D matrigel, and the combination of VS-6063 and VS-5584 displayed synergistic reduction in cell viability based on multiple combination analysis models. When tested *in vivo* for reduction of mesothelioma tumor growth, VS-6063 and VS-5584 were each active as single agents. In combination, VS-5584 further enhanced the antitumor efficacy of VS-6063 in this model.

The combination of VS-6063 and VS-5584 represents a novel therapeutic approach and these data support the clinical evaluation of the combination of VS-6063 and VS-5584 in patients with actively progressing mesothelioma following front-line therapy.

INTRODUCTION

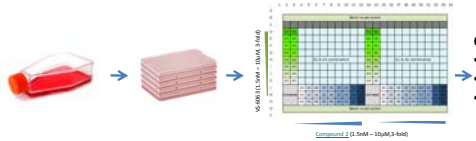
Fig 1: Critical to target cancer stem cells for a durable clinical response



- Both VS-6063 and VS-5584 effectively target CSCs
- VS-6063 inhibits bulk tumor in Merlin-low mesothelioma cells: *Shapiro et al. (2014) Sci Transl Med*
- FAK & PI3K/mTOR inhibition may combine for more robust shut down of AKT survival signaling

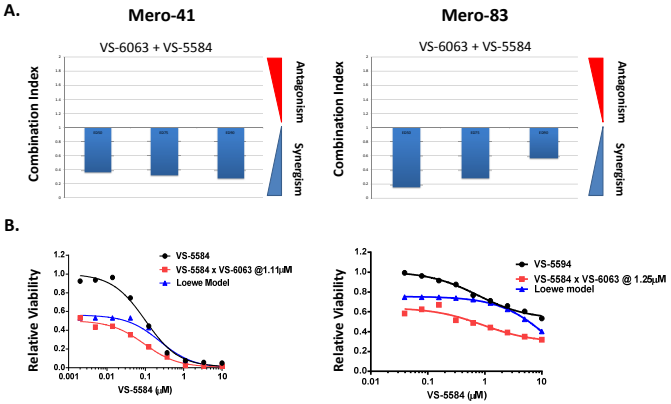
RESULTS

Fig 2: Screening for drugs exhibiting synergistic anticancer activity with VS-6063.



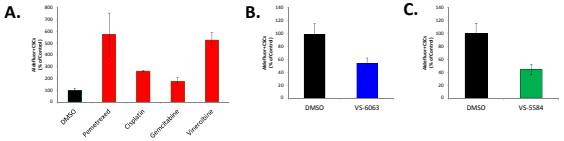
An *in vitro* combination screen was carried out to identify anticancer drugs that exhibit synergistic anticancer activity with VS-6063. Multiple cell lines were treated with VS-6063 and 20 anticancer agents in matrigel with a full 10x10 matrix format. Combination activity was assessed using Highest single agent (HSA) , Loewe additivity and Bliss models.

Fig 3: PI3K/mTOR inhibitor VS-5584 synergistically enhances the efficacy of VS-6063 in mesothelioma in vitro.



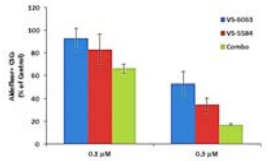
Mesothelioma cell lines were treated with combination of VS-6063 and VS-5584 in 10x10 concentration matrix in matrigel for 4 days and cell viability was measured by CellTiter-Glo (Promega). A. Combination index analysis using CalcuSyn (Biosoft, Cambridge, UK) at effective dose (ED) 50, 75, or 90 for both agents are shown. B. Cell viability measured by Cell Titer-Glo (Promega) at selected concentrations of VS-5584 and in combination with VS-6063 , as well as the expected cell viability predicted by Loewe models are presented.

Fig 4: VS-6063 and VS-5584 preferentially inhibit mesothelioma CSCs *in vitro,* in contrast to standard chemotherapy.



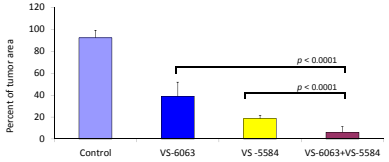
A. Mesothelioma cell was treated with standard chemotherapy (A), VS-6063 in 3D matrigel (B) or VS-5584 (C) and percentage of Aldefluor+ CSCs was measured.

Fig 5: VS-5584 further enhances anti-CSC effect of VS-6063 *in vitro*.



Mesothelioma cells Mero-14 were treated with DMSO, VS-6063, VS-5584 or combination of both at 0.1 μM or 0.3 μM and percentage of Aldefluor+ cells was determined.

Fig 5: Combination of VS-5584 and VS-6063 has stronger anti-tumor effect than single agents *in vivo*.



Mesothelioma tumors were generated in the lungs through tail vein injection of MM87 cells. Dosing started 11 days after cell implantation at 50 mg/kg bid for 14 days for VS-6063 and 20 mg/kg MWF for 2 weeks for VS-5584.

SUMMARY

- **VS-6063 (defactinib) is a potent/selective FAK kinase inhibitor.**
- **VS-5584 is a potent/selective inhibitor of PI3K & mTORC1/2.**
- **Both agents preferentially target CSCs in preclinical mesothelioma models.**
- **Synergistic activity of VS-6063 & VS-5584 on CSCs & bulk tumor has been observed in preclinical models.**
- **These data provide preclinical rationale for a planned Phase I combination study of VS-6063 & VS-5584 in patients with relapsed mesothelioma.**

Verastem
novel drugs targeting cancer stem cells

Verastem, Inc. | 117 Kendrick Street | Suite 500| Needham| Massachusetts | 02494