FAK Inhibitor VS-6063 (Defactinib) Targets Mesothelioma Cancer Stem Cells Which Are Enriched by Standard of Care Chemotherapy

INTRODUCTION

Malignant pleural mesothelioma (MPM) is an aggressive tumor in the lining of the lung often resulting from prior exposure to asbestos. Median overall survival with standard of care chemotherapy is only 12 months from diagnosis. This poor prognosis may be attributable at least in part to cancer stem cells (CSCs) which are resistant to chemotherapy and can mediate cancer recurrence and progression. Focal adhesion kinase (FAK) has been shown to play an essential role in the survival, self-renewal and tumor-initiating capability of CSCs. Accordingly, the FAK inhibitor VS-6063 is currently being tested in patients with mesothelioma following disease control on standard pemetrexed/platinum chemotherapy (COMMAND, ClinicalTrials.gov NCT01870609).

ABSTRACT

In preclinical models and mesothelioma patient biopsies, the standard of care agents, or cisplatin+pemetrexed for 2 weeks followed by oral pemetrexed- and VS-6063-treated mesothelioma cells. D. Mice bearing mesothelioma tumors in lungs treated orally with VS-6063 showed markedly reduced ALDH+ cancer stem cells. In contrast, the FAK inhibitor VS-6063 markedly reduced the proportion of CSCs. B. Paired tumor samples from mesothelioma patients post-pemetrexed/cisplatin also showed an increase in expression of CSC-related genes, such as CD133, measured by RT-PCR as compared to pretreatment biopsies.

RESULTS

A. MM87 mesothelioma were sorted to Aldefluor (ALDH activity) positive and negative populations and subjected to functional tests for CSC activity. B. ALDH+ cells showed enhanced CSC self-renewal in a tumor sphere assay in vitro. Upon implantation in limiting dilutions into immunodeficient mice, ALDH+ cells showed a 35-fold increase in tumor initiating frequency compared to ALDH- mesothelioma cells. 50 ALDH+ cells were sufficient to form a sizeable tumor within 3 weeks. These data validate ALDH as a CSC marker in mesothelioma, and indicate that mesothelioma CSCs are especially aggressive in tumor initiating capacity. In a patient-derived xenograft model of malignant mesothelioma (30 nM) or combination of both. 10,000 viable cells/mouse were implanted into BeigeXID mice and initiated new tumors in mice. Mesothelioma cells treated with VS-6063 or VS-6063+pemetrexed failed to initiate tumors in mice, indicating elimination of CSCs. C. Final tumor volumes with control, pemetrexed- and VS-6063-treated mesothelioma cells. D. Mice bearing mesothelioma tumors in lungs treated orally with VS-6063 showed marked reduction of ALDH+ CSCs by immunofluorescence. In patients with malignant pleural mesothelioma, mean tumor pFAK (Y927) was reduced by 66% in VS-6063-treated patients evaluated to date (E) and tumor cancer stem cells were decreased in 5 out of 7 patients as assessed by CD133 RNA expression in mesothelioma biopsies at day 12 of VS-6063 treatment compared to matched control biopsies (F). G. Tumor volumes from a human mesothelioma patient-derived xenograft (PDx) model treated with control (blue), cisplatin+pemetrexed for 2 weeks (green) or cisplatin+pemetrexed for 2 weeks followed by oral fak inhibitor (red). Tumor growth was rapid after cessation of cisplatin-pemetrexed, but was greatly delayed by addition of the FAK inhibitor consistent with the ability of the FAK inhibitor to reduce CSCs.

Fig 1: Schema: Importance of targeting cancer stem cells for a durable response

Fig 2: Validation of ALDH (Aldefluor) as Cancer Stem Cell Marker in Mesothelioma

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Fig 3: Standard of care chemotherapy enriches Cancer Stem Cells in mesothelioma cell lines and patient tumors

A. H2052 human mesothelioma cells were treated with cytotoxic chemotherapeutics used for mesothelioma treatment (pemetrexed/platinum typically used first line; gemcitabine & vinorelbine sometimes used second line). All of the cytotoxics increased the proportion of ALDH+ CSCs. In contrast, the FAK inhibitor VS-6063 reduced the proportion of CSCs. B. Paired tumor samples were collected from 11 mesothelioma patients pre- and post- pemetrexed/cisplatin treatment and assessed for CSCs by ALDH ICT. Chemotherapy induced an increase in ALDH+ CSCs (brown). C. Paired tumor samples from mesothelioma patients post-pemetrexed/cisplatin also showed an increase in expression of CSC-related genes, such as CD133, measured by RT-PCR as compared to pretreatment biopsies.

Fig 4: VS-6063 preferentially inhibits mesothelioma CSCs in vitro, in vivo and in tumors of patients with mesothelioma

A. H2B human mesothelioma cells were treated with DMSO (control), VS-6063 (1 μM), pemetrexed (30 μM) or combination of both. 10,000 viable cells/mouse were implanted into BeigeXID mice and tumor initiating capacity (CSC activity) was assessed. B. All control or pemetrexed treated cells initiated new tumors in mice. Mesothelioma cells treated with VS-6063 or VS-6063+pemetrexed failed to initiate tumors in mice, indicating elimination of CSCs. C. Final tumor volumes with control, pemetrexed- and VS-6063-treated mesothelioma cells. D. Mice bearing mesothelioma tumors in lungs treated orally with VS-6063 showed marked reduction of ALDH+ CSCs by immunofluorescence. In patients with malignant pleural mesothelioma, mean tumor pFAK (Y927) was reduced by 66% in VS-6063-treated patients evaluated to date (E) and tumor cancer stem cells were decreased in 5 out of 7 patients as assessed by CD133 RNA expression in mesothelioma biopsies at day 12 of VS-6063 treatment compared to matched control biopsies (F). G. Tumor volumes from a human mesothelioma patient-derived xenograft (PDx) model treated with control (blue), cisplatin+pemetrexed for 2 weeks (green) or cisplatin+pemetrexed for 2 weeks followed by oral fak inhibitor (red). Tumor growth was rapid after cessation of cisplatin-pemetrexed, but was greatly delayed by addition of the FAK inhibitor consistent with the ability of the FAK inhibitor to reduce CSCs.

SUMMARY

• Mesothelioma cancer stem cells aggressively drive tumor formation
• In preclinical models and mesothelioma patient biopsies, the standard of care agents, pemetrexed & platinum, enrich the proportion of CSCs
• The FAK inhibitor VS-6063 can reduce the tumor-initiating cells (CSCs) in vitro, in vivo and in tumors of VS-6063-treated mesothelioma patients
• FAK inhibitor treatment delayed tumor regrowth following cisplatin+pemetrexed treatment in a patient-derived xenograft model of malignant mesothelioma
• These data provide strong rationale for the current clinical testing of VS-6063 (defactinib) following treatment with pemetrexed plus platinum to potentially prolong response to front line chemotherapy in patients with mesothelioma. (COMMAND, NCT01870609)