FAK Inhibitor VS-6063 (Defactinib) Targets Mesothelioma Cancer Stem Cells, which are Enriched by Standard of Care Chemotherapy

Abstract # 4236

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ABSTRACT

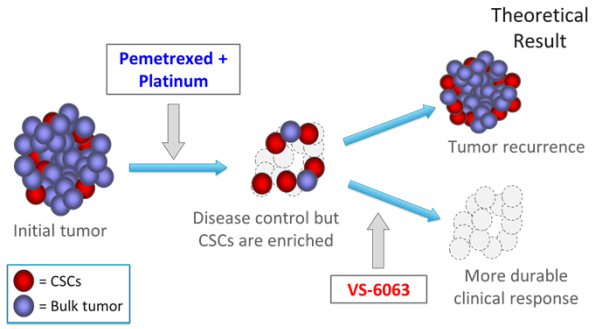
Malignant pleural mesothelioma (MPM) is an aggressive tumor in the lining of the lung often resulting from prior exposure to asbestos. Median overall survival with standard of care chemotherapy is only 12 months from diagnosis. This poor prognosis may be attributable at least in part to cancer stem cells (CSCs) which are resistant to chemotherapy and can mediate cancer recurrence, progression and metastasis. 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 MPM following disease control on standard pemetrexed/platinum chemotherapy (COMMAND, ClinicalTrials.gov NCT01870609).

Aldehyde dehydrogenase (ALDH) activity was validated as a CSC marker in MPM by assessment of tumor-initiating capability in mice. As compared to ALDH-negative cells, sorted ALDH-positive MM87 MPM cells showed 35-fold greater tumor initiating capacity when implanted in limiting dilutions into immunodeficient mice. Indeed, 50 ALDH-positive cells were sufficient to generate sizeable tumors in 3 weeks illustrating the aggressive nature of MPM CSCs. Treatment of a human MPM cell line with pemetrexed enriched ALDH-positive CSCs 6-fold, with similar CSC enrichment by cisplatin. In direct contrast, the FAK inhibitor VS-6063 markedly reduced the proportion of CSCs. The enrichment of MPM CSCs was similarly observed in samples from 11 tested patients following first line chemotherapy. Patient specimens post treatment with pemetrexed and cisplatin showed an elevated ALDH immunohistochemistry H-score and an increase in expression of CSC genes such as CD133 as compared to matched MPM biopsies taken from the same patients prior to chemotherapy. To assess drug effects on tumor-initiating capacity, MM87 merlin-low MPM and H28 merlin-high MPM cell lines were treated in vitro with VS-6063, pemetrexed or the combination and subsequently implanted into mice. While control or pemetrexed-treated MPM cells showed robust tumor initiation, cells treated with VS-6063 alone or VS-6063 plus pemetrexed showed little or no tumor initiating capacity. Accordingly, in tumor biopsies from MPM patients treated for 12 days with VS-6063, tumor pFAK (Y397) and expression of CSC genes such as CD133 were reduced. MPM patient-derived xenograft (PDX) tumors were employed to model the clinical scenario. Compared to control, tumor growth was blocked by 2-week treatment with pemetrexed/cisplatin. Tumors then grew rapidly upon cessation of pemetrexed/platinum treatment, whereas tumor growth was substantially delayed by FAK inhibitor treatment after cessation of chemotherapy.

These data provide strong rationale for the current clinical testing of VS-6063 following treatment with pemetrexed plus platinum to potentially delay time to progression in patients with mesothelioma.

INTRODUCTION

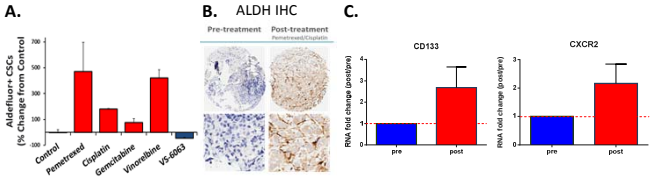
Fig 1: Importance of targeting cancer stem cells for a durable response



Standard of care chemotherapy controls the disease, but enriches for Cancer Stem Cells (CSCs), such that disease is poised to recur. Use of a FAK inhibitor VS-6063 to target residual CSCs after chemotherapy may potentially increase duration of anti-tumor response.

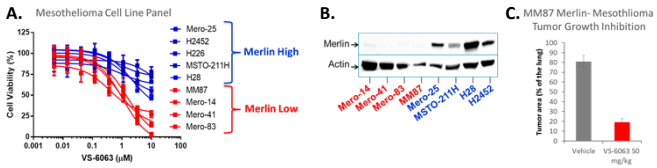
RESULTS

Fig 2: Standard of care chemotherapy enriches for Cancer Stem Cells in mesothelioma cell lines and patient tumors



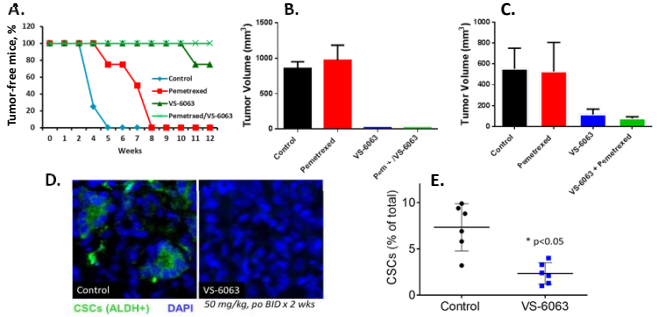
A. Cytotoxic chemotherapeutics increased the proportion of ALDH+ CSCs in H2052 human mesothelioma cells while the FAK inhibitor VS-6063 reduced the proportion of CSCs. **B.** Paired tumor samples from 11 mesothelioma patients pre- and post- pemetrexed/cisplatin treatment demonstrate an increase in ALDH+ CSCs by IHC (brown staining). **C.** Paired tumor samples from mesothelioma patients demonstrated increased expression of CSC-related genes post-pemetrexed/cisplatin. CD133 and CXCR2 RNA were measured by RT-PCR.

Fig 3: VS-6063 inhibits bulk tumor cells in Merlin-low mesothelioma models



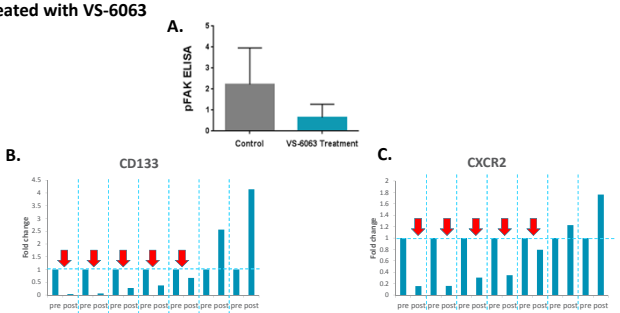
A. Among a panel of mesothelioma cell lines grown in 3D Matrigel culture, proliferation of cell lines with low expression of the tumor suppressor Merlin was especially sensitive to VS-6063.
B. Differential expression of Merlin protein in mesothelioma cells lines measured by western blot.
C. Growth of MM87 Merlin-low mesothelioma in the lungs of mice was reduced by oral administration of VS-6063.

Fig 4: VS-6063 reduces CSCs and tumor-initiating potential in both Merlin-low and Merlin-high mesothelioma tumor models



H28 Merlin-high mesothelioma cells (**A, B**) or MM87 Merlin-low mesothelioma cells (**C**) were treated *in vitro* with DMSO (control), VS-6063 (1 μM), pemetrexed (30 nM) or combination of both. 10,000 viable cells per mouse from each treatment were implanted into immunodeficient mice and tumor initiating capability (CSC activity) was assessed. **A.** Reduced tumor-initiating capability of H28 cells following *in vitro* treatment with VS-6063 or VS-6063 + pemetrexed. Graph depicts % tumor-free mice over time. Final tumor volumes after implantation of pretreated H28 Merlin-high (**B**) or MM87 Merlin-low (**C**) cells. **D,E.** Immunofluorescence of CSCs in MM87 tumors grown in lungs of mice. Tumors in mice treated orally with VS-6063 showed marked reduction of ALDH+ cells (CSCs).

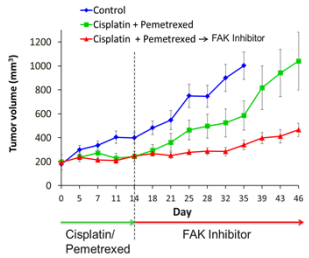
Fig 5: Reduction of pFAK and CSC markers in tumors of mesothelioma patients treated with VS-6063



* pre- and post- are matched samples from individual patients

Mesothelioma patients were treated for 12 days with VS-6063, and pre and post-treatment biopsies were taken. **A.** Mean tumor pFAK (Y397) was reduced by 70% in the VS-6063-treated patients. pFAK (Y397) ELISA was performed on pre- and post-treatment matched samples. Cancer stem cells were decreased in 5 out of 7 patient tumors assessed by CD133 (**B**) and CXCR2 (**C**) RT-PCR in mesothelioma biopsies at day 12 of VS-6063 treatment compared to matched pre-treatment biopsies.

Fig 6: FAK inhibitor extends tumor growth inhibition in a maintenance setting after cessation of cisplatin/pemetrexed treatment in a mesothelioma patient-derived xenograft model



Tumor growth inhibition in a human Merlin-low 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 treatment with cisplatin plus pemetrexed, but was greatly delayed by subsequent addition of the FAK inhibitor consistent with the ability of the FAK inhibitor to reduce CSCs.

SUMMARY

- The standard of care agents, pemetrexed & platinum, enrich the proportion of CSCs in preclinical models and mesothelioma patient biopsies as assessed by multiple markers
- The FAK inhibitor VS-6063 reduces mesothelioma bulk tumor growth especially when expression of the tumor suppressor Merlin is low
- VS-6063 reduces tumor-initiating cells (CSCs) in both Merlin-low and Merlin-high mesothelioma preclinical models
- In mesothelioma patients treated with VS-6063 for 12 days, a reduction of pFAK (pharmacodynamic marker) and CSC RNAs (CD133, CXCR2) was observed in tumors
- FAK inhibitor treatment delayed tumor growth following cisplatin+pemetrexed treatment in a patient-derived xenograft model of malignant mesothelioma
- These data provide a strong rationale for the current clinical testing of VS-6063 (defactinib) in a maintenance setting to potentially prolong response to front line chemotherapy in patients with mesothelioma. (COMMAND, NCT01870609)