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

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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 capability when implanted in limiting dilutions into immunodeficient mice. Indeed, ALDH-positive cells were sufficient to generate sizeable tumors in 3 weeks illustrating the aggressive nature of MPM CSCs. Treatment with a human MM87 cell line with 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 (IHC) 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 low-MMP and H28 high-MMP MPA 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 tumors 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) models 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/cisplatin 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

- Pemetrexed + Platinum
- Disease control but CSCs are enriched
- More durable clinical response

H28 Merlin-low 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 pemetrexed. Graph depicts % tumor-free mice over time. Final tumor volumes after implantation of pre-treated H28 Merlin-high (B) or MM87 Merlin-low (C) cells. B. 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).

Despite 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

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 free of evidence of disease (oPDED) and post-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)