FAK Inhibitor VS-4718 Preferentially Attenuates Growth of Malignant Mesotheliomas with NF2 Mutation: Role of Cancer Stem Cells

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ABSTRACT

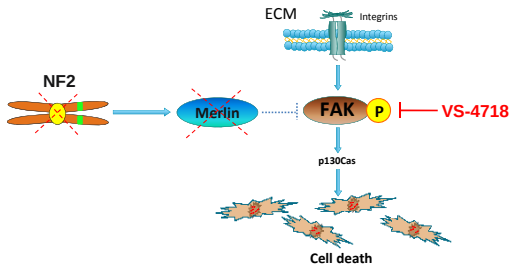
Malignant pleural mesothelioma (MPM) is an aggressive tumor in the pleural lining of the lung often caused by asbestos exposure. MPM patients are usually diagnosed at an advanced stage of the disease and the prognosis is poor. Median survival after diagnosis is 9 to 12 months and standard-of-care agents such as cisplatin and pemetrexed are relatively ineffective in increasing median survival time for MPM patients. New therapeutic modalities are urgently needed to improve the prognosis of MPM patients.

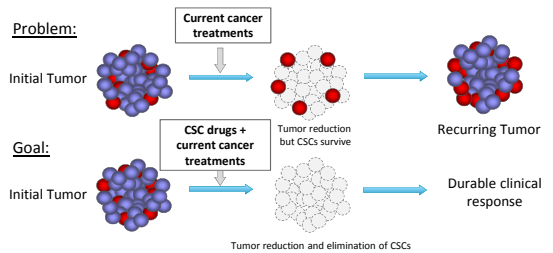
Neurofibromatosis 2 (NF2) is a tumor suppressor gene that encodes the protein Merlin. Biallelic inactivation of NF2 by mutation and/or deletion occurs in ~40% of MPMs leading to inactive Merlin. Merlin has been demonstrated to play roles in cell adhesion, invasion and cell motility in tumor cell lines partially through regulation of focal adhesion kinase (FAK) which in turn mediates signal transduction by integrins and growth factor receptors. Increased activation of FAK has been demonstrated in NF2-mutated mesothelioma cells, indicating that FAK may represent an important therapeutic target for MPM.

VS-4718, a selective FAK kinase inhibitor was evaluated in a panel of MPM cell lines with wild-type or mutated NF2. Mutant NF2 MPM cells were found to be especially sensitive to the FAK inhibitor VS-4718 with EC50 values below 100 nM, in contrast to wild type NF2 MPM cell lines which were less sensitive with EC50 values above 1 µM. Ectopic expression of a non-phosphorylatable artificial mutant of NF2 (NF2-S518A) in NF2 mutant MPM cells abolished the enhanced sensitivity to VS-4718, confirming the hypothesis that Merlin loss mediates sensitivity to VS-4718. Interestingly, MPM cell lines were found to have sub-populations of Aldefluor+ cancer stem cells (CSCs). Furthermore, the FAK inhibitor VS-4718 induced a significant reduction in the percentage of CSCs in contrast to the standard-of-care agent pemetrexed which enriched the CSC population.

In summary, our results indicate that the FAK inhibitor VS-4718 is especially potent in NF2-mutated MPM tumor cells, and that NF2 status may be a valuable stratification marker for VS-4718 response. Furthermore, cancer stem cells in NF2 mutant mesothelioma appear to be particularly resistant to pemetrexed, but sensitive to VS-4718. We believe that these data support the clinical development of a selective FAK inhibitor such as VS-4718 for treatment of NF2-mutated malignant mesothelioma.

INTRODUCTION





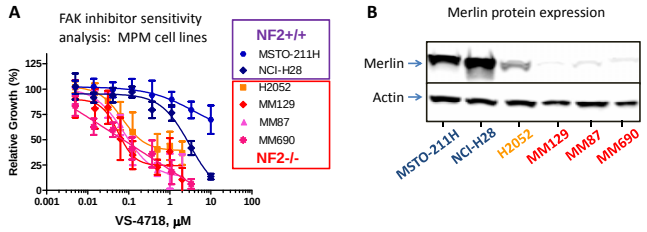
METHODS

Aldehyde dehydrogenase (ALDH) – cancer stem cell marker:

- A detoxifying enzyme
- Oxidizes intracellular aldehydes
- Plays a role in stem cell differentiation through metabolism of retinal to retinoic acid
- Activity is assessed by the fluorescent Aldefluor assay

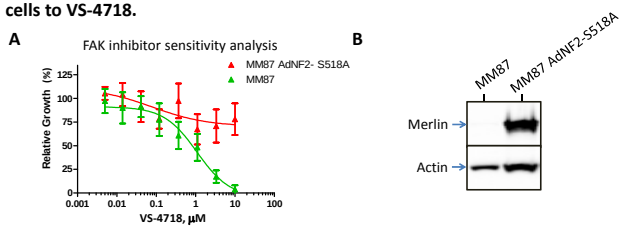
RESULTS

Fig 1. Mesothelioma cell lines lacking NF2/Merlin are especially sensitive to VS-4718 in 3D culture.



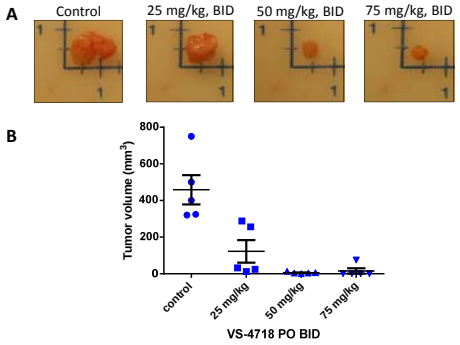
A) Cell viability analysis of mesothelioma cell lines in 3D Matrigel culture treated with VS-4718 for 4 days.
B) Western blot analysis of MPM cell lines probed with anti-Merlin antibody. Actin was used as a loading control.

Fig 2. Ectopic expression of AdNF2-S518A abolishes sensitivity of mesothelioma cells to VS-4718.



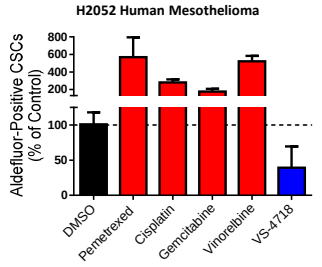
A) Cell viability analysis of mesothelioma cell lines MM87 and MM87-AdNF2-S518A in a 3D matrigel culture treated with FAK inhibitor VS-4718 as indicated.
B) Western blot analysis of protein lysates from A) probed with anti-Merlin antibody. Actin was used as a loading control.

Fig 3. VS-4718 inhibits growth of Merlin-negative Mesothelioma tumors.



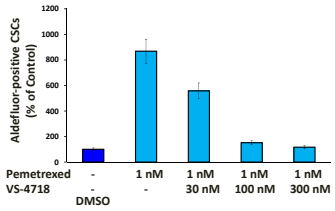
A) Representative pictures of the mouse mesothelioma xenografts. MM87 cells were injected into interperitoneal cavity. Tumors were grown for 2 weeks. VS-4718 FAK inhibitor was administered orally BID for 10 days.
B) Dot plot demonstrating compound effect on tumor growth.

Fig 4. VS-4718 inhibits mesothelioma Cancer Stem Cells while standard of care agents induce them.



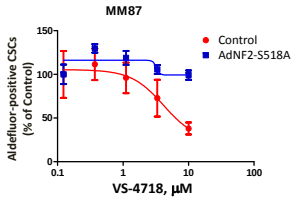
Aldefluor assay was used to measure percent Aldefluor positive cells in NF2-mutant H2052 cells treated with VS-4718 or standard of care agents, as indicated. Concentrations of the compounds used in the study: Pemetrexed 1nM, Cisplatin 2nM, Gemcitabine 1 nM, Vinorelbine 1 nM, VS-4718 300 nM.

Fig 5. FAK inhibitor VS-4718 inhibits Cancer Stem Cells in combination with pemetrexed.



NF2-mutant H2052 cells were treated with VS-4718 and pemetrexed for 4 days in 3D Matrigel culture. Aldefluor assay was used to determine proportion of Aldefluor positive cells.

Fig 6. Expression of AdNF2-S518A abolishes increased sensitivity of Cancer Stem Cells to FAK inhibitor VS-4718.



MM87 cells were infected with AdNF2-S518A, treated with FAK inhibitor VS-4718 in culture for 4 days and analyzed for the proportion of Aldefluor positive cells.

CONCLUSIONS

- The selective FAK inhibitor VS-4718 shows increased potency in mesothelioma cell lines lacking NF2/Merlin.
- Mesothelioma cancer stem cells (CSCs) are stimulated by standard-of-care agents, but ameliorated by VS-4718.
- VS-4718 significantly inhibits growth of Merlin-negative mesothelioma tumors in a mouse orthotopic model.
- NF2/Merlin status may be a valuable stratification marker for FAK inhibitor response.
- These data provide rationale for clinical trials of a FAK inhibitor in patients with malignant mesothelioma.

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