Abstract: 4127

Combined inhibition of RAF, MEK, and FAK attenuates melanoma brain metastases and prolongs overall survival in preclinical models

Karly Stanley[1], Jared Almazan[1], Tursun Turapov[1], David A. Kircher[1], Gennie L. Parkman[2], MiKaela N. Field[1], Katie M. Culver[1], Christopher M. Stehn[1], Silvia Coma[3], Jonathan A. Pachter[3], Howard Colman[1], and Sheri L. Holmen[1]

[1]Huntsman Cancer Institute , Salt Lake City, UT, [2]Weber State University, Ogden, UT, [3]Verastem Oncology, Needham, MA

BACKGROUND

Despite promising results from recent FDA-approved therapies, many advanced melanoma patients develop resistance to both immunotherapy and targeted therapy. A common resistance mechanism to targeted therapy is upregulation of the PI3K/AKT signaling pathway, which has also been shown to promote the development of melanoma brain metastases. Historically, AKT inhibitors have failed in the clinic due to their limited efficacy or intolerable toxicity[1]. Proteomic analysis comparing non-metastatic with brain metastatic primary tumors revealed focal adhesion kinase (FAK) as an AKT1-specific effector and potential alternative therapeutic target[2]. FAK is a non-receptor tyrosine kinase that localizes primarily to focal adhesions to regulate cell migration. To determine whether targeting FAK alone or in combination with the RAF/MEK clamp avutometinib reduces brain metastases and improves overall survival, we utilized autochthonous, syngeneic, and xenograft melanoma mouse models.

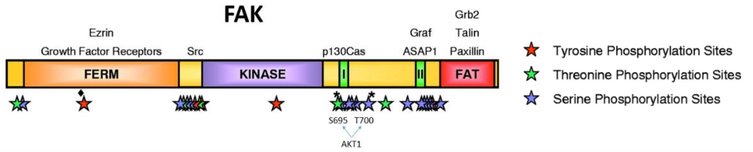


Figure 1: Schematic of FAK protein structure demonstrating its binding and kinase domains (Quispe et al., *Drug Discovery Today,* 2022).

HYPOTHESIS

Combined RAF/MEK/FAK inhibition attenuates melanoma brain metastases and prolongs overall survival in preclinical models.

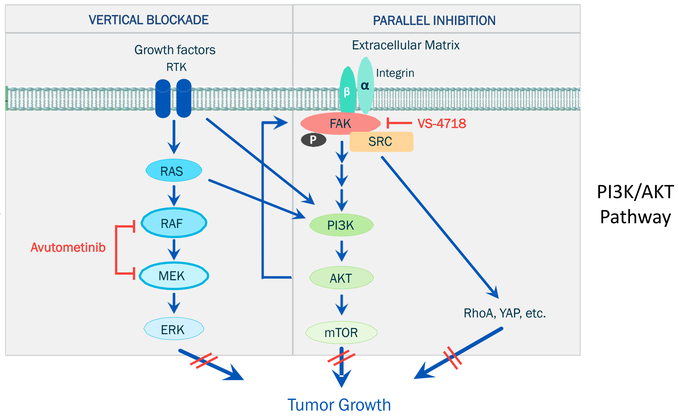


Figure 2: Schematic showing pharmacological inhibition of RAF/MEK and FAK signaling. Figure courtesy of Verastem Oncology.

RESULTS

Pharmacological inhibition of FAK does not delay tumor onset but significantly prevents the development of brain metastases

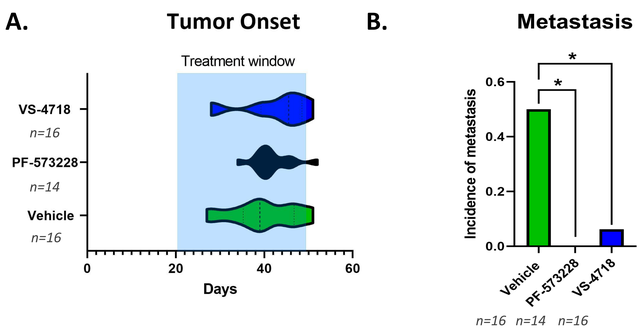


Figure 3: Tumor onset (A) and incidence of brain metastasis (B) from *Dct::Tva;*BRAF^{CA}*;Cdkn2a*$^{lox/lox}$*;Pten*$^{lox/lox}$ mice that were injected with RCAS-myrAKT1 and RCAS-Cre upon birth to induce tumor growth. Mice were treated for 28 days (treatment period shaded in blue) with FAK inhibitors PF-573228 or VS-4718 . *P < 0.05 compared with vehicle.

Combined RAF/MEK/FAK inhibition in established tumors significantly reduces tumor growth and brain metastasis *in vivo*

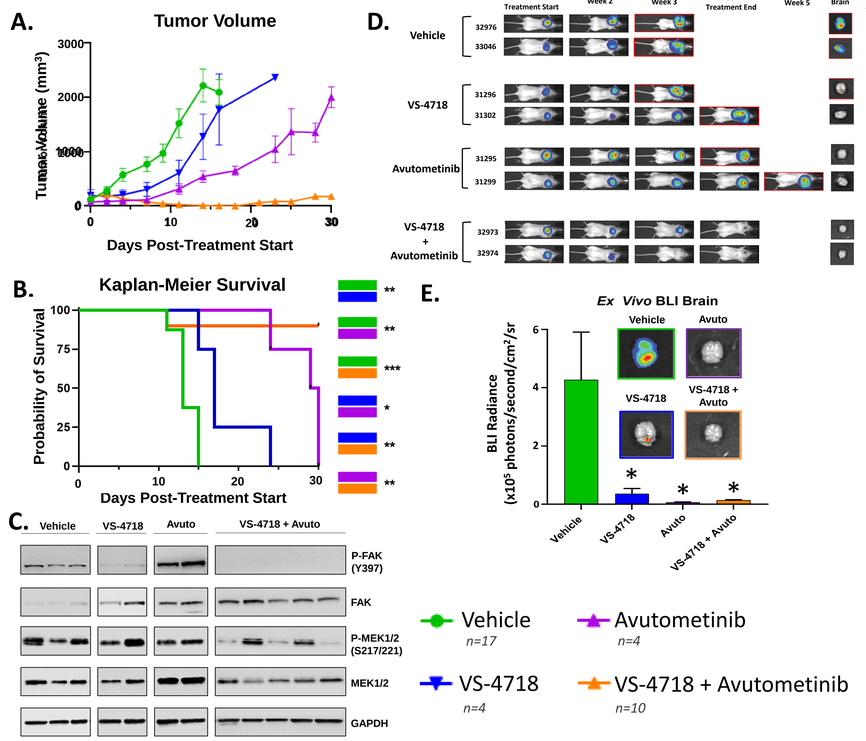


Figure 4: Tumor volume measurements (A), survival curves (B), and protein expression (C) of primary tumors from syngeneic mice injected subcutaneously with YUMM3.2 cells (BRAF^{V600E} and *Cdkn2a*-null and rendered PTEN-null through CRISPR-mediated deletion) engineered to express luciferase and GFP on the same vector as HA-AKT1-E17K for bioluminescent imaging (D). Mice were treated with VS-4718 (FAKi) and/or avutometinib (RAF/MEKi) for 28 days. Bioluminescent radiance was measured in the brain (E) and presented as x10^5 photons/second/cm^2/sr. *P < 0.05 compared with vehicle.

Inhibition of RAF/MEK/FAK in mice with established brain metastases significantly prolongs survival

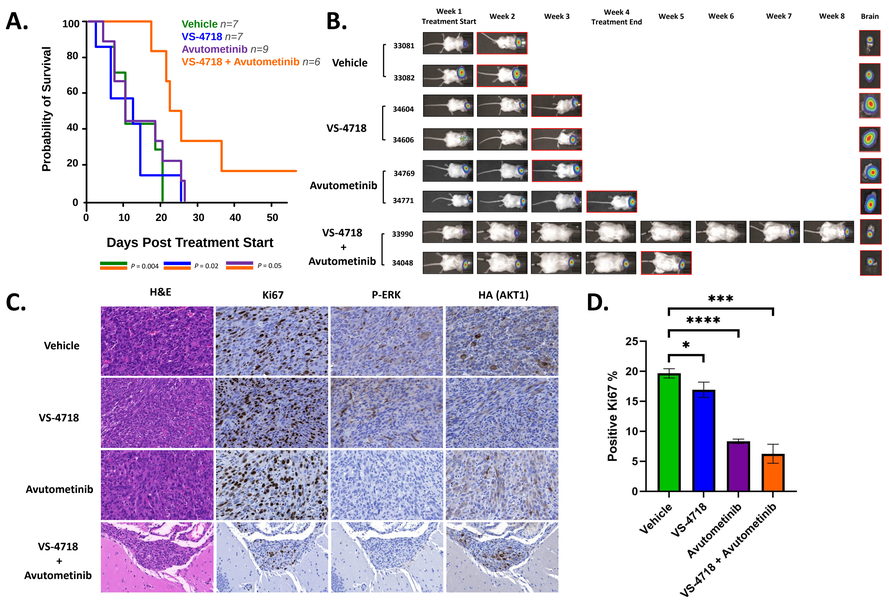


Figure 5: Kaplan-Meier survival curves from syngeneic mice injected intracranially with YUMM3.2 cells described above (A). Mice were treated with VS-4718 and/or avutometinib for 28 days once a signal was detected by bioluminescent imaging (B). Immunohistochemistry on brain tissue (C) shows RAF/MEK inhibition or combined RAF/MEK/FAK inhibition significantly reduces signal of P-ERK and Ki67 (D).

Dabrafenib/trametinib resistant BRAF-mutant melanoma PDX models are sensitive to combined RAF/MEK/FAK inhibition +/- BRAF inhibition (encorafenib)

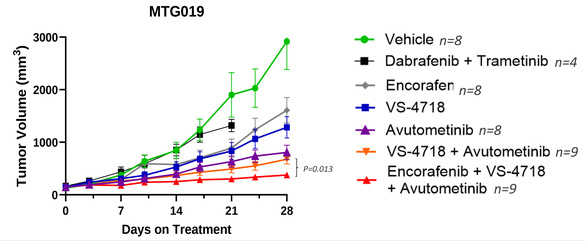


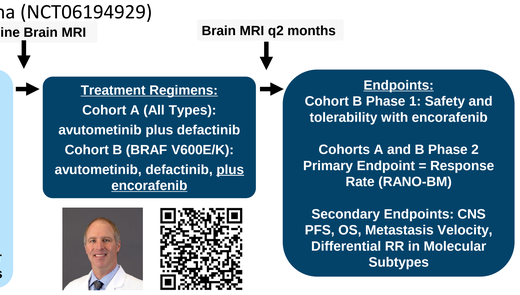
Figure 6: Example of tumor growth measurements from BRAF-mutant PDX melanoma resistant to dabrafenib and trametinib. Mice were treated with the drugs indicated for 28 days.

CONCLUSIONS

- FAK inhibition prevents the development of brain metastases despite lack of tumor onset delay
- RAF/MEK/FAK inhibition reduces the incidence of lung and brain metastases in mice with established tumors, promotes the regression of established brain metastases, and prolongs overall survival in preclinical models
- Dabrafenib/trametinib resistant BRAF-mutant PDX melanoma models are sensitive to combined RAF/MEK/FAK inhibition +/- BRAF inhibition (encorafenib)

FUTURE DIRECTIONS

- Assess non-canonical roles of FAK in modulating the tumor microenvironment to determine if RAF/MEK/FAK inhibition enhances efficacy of immune checkpoint inhibition
- Investigate if RAF/MEK/FAK inhibition prevents brain metastases and improves overall survival in mutant NRAS and NF1 melanoma mouse models
- Ongoing clinical study evaluating defactinib and avutometinib, with or without encorafenib, for the treatment of patients with brain metastases from cutaneous melanoma (NCT06194929)



References:

1. Parkman, Gennie L et al. "Genetic Silencing of AKT Induces Melanoma Cell Death via mTOR Suppression." *Molecular cancer therapeutics* vol. 23,3 (2024): 301-315. doi:10.1158/1535-7163.MCT-23-0474
2. Kircher, David A et al. "AKT1^{E17K} Activates Focal Adhesion Kinase and Promotes Melanoma Brain Metastasis." *Molecular cancer research : MCR* vol. 17,9 (2019): 1787-1800. doi:10.1158/1541-7786.MCR-18-1372

Acknowledgements:

- This work was funded by: NIH/NCI grant R01CA121118, Huntsman Cancer Institute Melanoma Disease Center, Huntsman Cancer Foundation, University of Utah/HCI, USTAR, Melanoma Research Alliance, Melanoma Research Foundation, and the Stowell family
- Verastem Oncology generously provided avutometinib and VS-4718
- HCI Preclinical Research Resource assisted with PDX studies

UNIVERSITY OF UTAH HUNTSMAN CANCER INSTITUTE
HEALTH UNIVERSITY OF UTAH