PCSK9 INHIBITION ENHANCES EFFICACY OF VEMURAFENIB IN DELAYING MELANOMA TUMOR GROWTH

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INTRODUCTION

- BRAF activating mutations are seen in more than 50% of melanomas.
- V600E mutation accounts for more than 90% of all BRAF mutant melanomas
- Vemurafenib specifically targets BRAF V600E and is effective for nearly half of patients but develops secondary resistance soon after start of therapy.
- PCSK9 has been shown to be involved in various cancers via activation of RAS/RAF/MAPK signaling pathway.
- PCSK9's role in melanomagenesis of BRAF-mutant melanoma is poorly understood, despite data suggesting BRAF mutants have a distinct lipid metabolic profile compared to WT.
- Here, I aim to demonstrate PCSK9 inhibition as a synergistic target with BRAF inhibition in melanoma treatment.

METHODS

1. PCSK9 association with BRAF was assessed with BRAF inhibition of BRAF-mutant mouse YUMM-1.7 and human A375 melanomas in vitro and analyzed via western blot to quantify PCSK9 expression levels.
2. BRAF overexpression vectors were stably expressed in murine melanoma B16F10 cells via lentiviral infection and cell lysates were run on western blot and assayed for PCSK9 protein expression levels.
3. In vivo and in vitro YUMM-1.7 was treated with combination vs monotherapy of evolocumab/PCSK9 small molecule inhibitor or vemurafenib using syngeneic C57BL/6 mice. In vitro cell viability was measured via MTT assay and in vivo tumor growth delay was measured via digital caliper.
4. Tumors were harvested, homogenized, and assayed with fluorescent antibodies against surface and intracellular lymphocyte markers and assayed via flow cytometry to quantify tumor infiltrating lymphocytes.

RESULTS

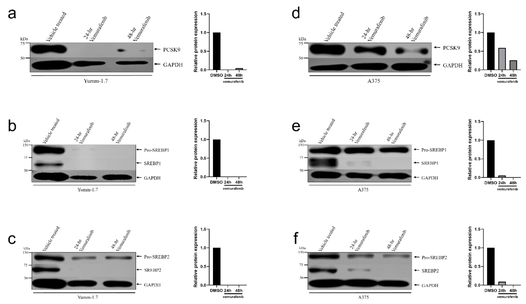

Fig. 1

BRAF inhibition downregulates PCSK9 and lipid metabolism transcription factors

a-c. Western blot quantification of PCSK9 (**a**), SREBP1 (**b**), and SREBP2 (**c**) protein expression in YUMM-1.7 cells treated at 24 and 48 hours. Graph on the right represents quantitative estimates of expression levels based on western blot images to the left. **d-e.** Western blot quantification of the same proteins in **a-c** respectively in A375 cells. Graph on the right represents quantitative estimates of expression levels based on western blot images to the left.

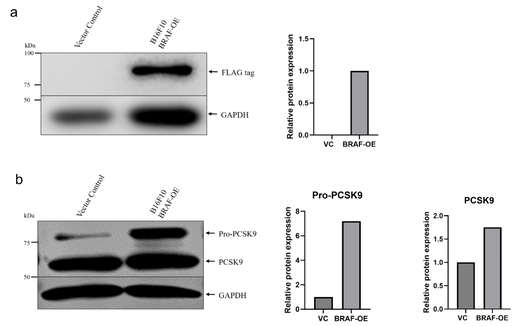

Fig. 2

BRAF overexpression increases mature and precursor PCSK9

a. Western blot quantification of FLAG tagged BRAF in vector control and BRAF-OE B16F10 cells. Graph on the right represents quantitative estimate of expression level compared to VC based on western blot images on the left. **b.** Western blot of PCSK9 expression levels in vector control vs BRAF-OE B16F10 cells. Quantification of Pro-PCSK9 and PCSK9 on the right.

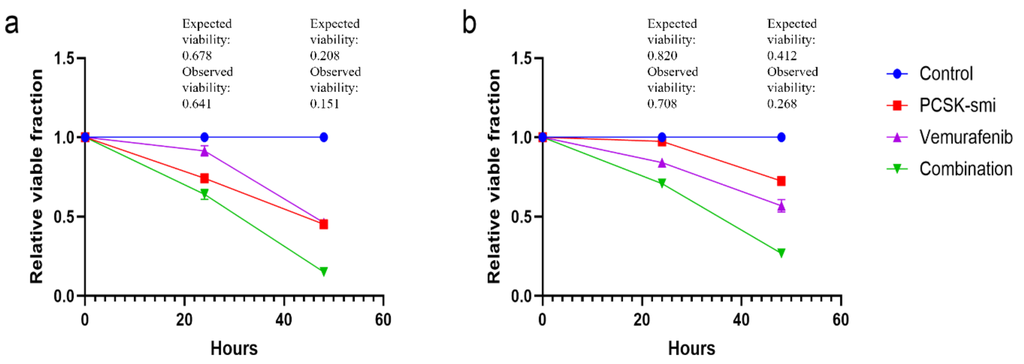

Fig. 3

Combination therapy exhibits increased cytotoxicity *in vitro*

a. YUMM-1.7 cells or **b.** A375 cells were treated with PCSK9-smi PF-06446846 or vemurafenib and MTT assay performed at 24 and 48 hours. n=6 for each group at each time point. Values for each time point represent cell viability as a relative fraction of control. Bliss Independence Model was used to analyze the interaction between the two compounds. I performed these experiments and analyzed the data myself.

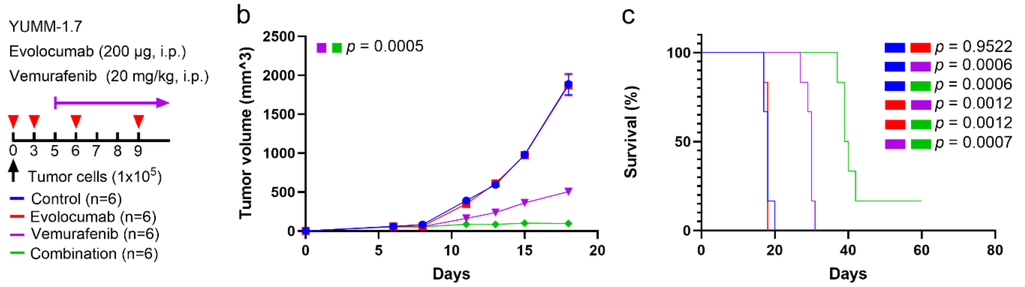

Fig. 4

Combination therapy significantly delays tumor growth and increases survival.

a-c. Treatment of wild type YUMM-1.7 melanoma with an anti-PCSK9 antibody (evolocumab) in combination with a BRAF kinase inhibitor (vemurafenib) in syngeneic mice. Experiment protocol (**a**), tumor growth curve (**b**), and overall survival (**c**) were shown. n=6 in the four groups, respectively. Shown were combined results from two separate experiments. Error bars represent mean ± S.E.M. *P* values were calculated by two-way ANOVA in **b** and significant main differences were further analyzed using a post-hoc test (Tukey's HSD) to identify specific group difference between vemurafenib and combination. Survival curves were analyzed using logrank test in **c**.

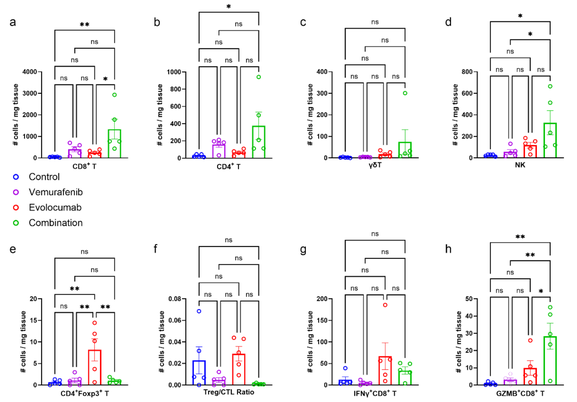

Fig. 5

Combination therapy enhances select cytotoxic lymphocytes

a-e. Quantitative estimate of immune effector cells per mg of YUMM-1.7 tumor tissue treated *in vivo* with vehicle control, monotherapy, or combination therapy as determined by flow cytometry. n=5 tumors per group. **f.** Ratio of $CD4^+Foxp3^+$ Treg / $CD8^+$ T cells, n=5 per group. **g-h.** Average numbers of tumor-infiltrating $IFN\gamma^+$ $CD8^+$ T (**g**) and $Gzmb^+CD8^+$ T (**h**) cells per mg of tumor tissue, n=5 tumors per group. Error bars represent mean ± S.E.M. throughout the figure. *P* values in **a-h** were calculated by one-way ANOVA and significant main differences were further analyzed using a post-hoc test (Tukey's HSD) to identify specific group differences. One asterisk (*) identifies adjusted P values of 0.01 to 0.05., two asterisks (**) identify adjusted P values between 0.01 and 0.001.

Conclusions

1. BRAF expression is necessary and sufficient to upregulate PCSK9 expression.
2. PCSK9 inhibition in addition to BRAF inhibition affords significant added cytotoxicity to tumors in vitro
3. Combination therapy with evolocumab and vemurafenib causes significant delay in tumor growth in a manner suggesting enhanced efficacy of innate immune response

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