PCSK9 Inhibition Enhances Efficacy of Vemurafenib in Delaying Melanoma Tumor Growth

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Background BRAF mutations are common in melanoma, with more than 50% harboring activating BRAF mutations. Though BRAF inhibitors are highly efficacious for nearly half of patients with this BRAF-mutant melanoma, many develop secondary resistance shortly after start of therapy. PCSK9 activity has been shown to promote tumorigenesis in other cancers via activation of RAS/RAF/MAPK signaling pathway. However, PCSK9's relevance in BRAF activity and role in melanomagenesis of BRAF-mutant melanoma is poorly understood.

Methods PCSK9 association with BRAF was assessed with BRAF inhibition of BRAF-mutant melanoma *in vitro* and analyzed via western blot of PCSK9 expression levels. BRAF overexpression vectors were stably expressed in murine melanoma B16F10 cells and studied for impact on PCSK9 expression levels. *In vivo and in vitro* YUMM-1.7 was treated with combination vs monotherapy of evolocumab/PCSK9 small molecule inhibitor and vemurafenib using syngeneic C57BL/6 mice. *In vitro* cell viability was measured via MTT assay and *in vivo* tumor growth delay was measured. Intratumoral infiltration of lymphocytes was also assayed via flow cytometry.

Results PCSK9 protein downregulation was positively correlated with BRAF inhibition. PCSK9 protein levels were inversely increased in BRAF-OE models. PCSK9 and BRAF co-inhibition significantly decreased cell viability *in vitro*, caused significant tumor growth delay, and increased survival in mice. Combination therapy saw increases in $CD4^+$, $CD8^+$, granzyme-B expressing $CD8^+$, and NK cells without significant change in Treg/CTL ratio.

Conclusions Evolocumab and vemurafenib combination therapy showed significant synergistic effects in mouse models while PCSK9 small molecule inhibitor and vemurafenib combination therapy recapitulated these findings *in vitro* in human and mouse melanoma cell lines overexpressing BRAF, exhibiting strong clinical implications as a potential adjunct treatment to established targeted therapy against BRAF.

Acronyms

BRAF = v-Raf murine sarcoma viral oncogene homolog B. PCSK9 = Proprotein convertase subtilisin/kexin type 9. RAS = Rat sarcoma. RAF = Rapidly Accelerated Fibrosarcoma. MAPK = Mitogen-Activated Protein Kinase. OE = Overexpression. YUMM = Yale University Mouse Melanoma. MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide. NK = Natural Killer. Treg = Regulatory T cell. CTL = Cytotoxic T cells.