The RAF/MEK clamp avutometinib (VS-6766) induces an immunogenic tumor microenvironment and potentiates the efficacy of anti-PD-1

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BACKGROUND

The RAS/RAF/MEK/ERK (MAPK) pathway is one of the most commonly mutated oncogenic pathways in human cancers (1). Although RAS, RAF and MEK have been validated as anticancer targets with approval of KRAS G12C, BRAF and MEK inhibitors, combination strategies with chemotherapy, targeted therapies and/or immune checkpoint inhibitors may be optimal for deep and durable response (2, 3). Indeed, the combinations of the KRAS G12C inhibitors (G12Ci) sotorasib or adagrasib with anti-PD-1 have been clinically evaluated in first-line KRAS G12C non-small cell lung cancer (NSCLC) (4, 5) with adagrasib + anti-PD-1 showing a manageable safety profile and encouraging clinical activity.

Avutometinib is a unique RAF/MEK clamp that potently inhibits MEK kinase activity and induces dominant negative RAF/MEK complexes, preventing phosphorylation of MEK by ARAF, BRAF and CRAF (Figure 1) (6-8). Preclinically, avutometinib potentiates G12Ci efficacy in KRAS G12C NSCLC models *in vivo* (Figure 2) (8) and two clinical studies of avutometinib in combination with sotorasib (NCT05074810) or adagrasib (NCT05375994) for patients with KRAS G12C NSCLC are ongoing. Here, we tested the immune modulatory effects of avutometinib on tumor cells and tumor-infiltrating immune cells and assessed anti-tumor efficacy in mice treated with avutometinib ± G12Ci in combination with an anti-PD-1 antibody.

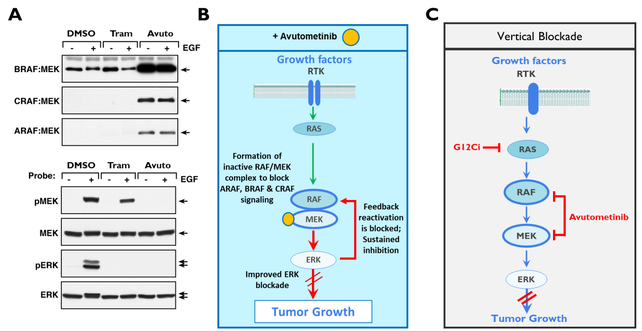

Figure 1. (A) Western blot analyses in HeLa cells treated with 1 μM avutometinib (Avuto) or 1 μM trametinib (Tram) for 3 hours (8). **(B)** Schematic showing that avutometinib is a unique RAF/MEK clamp that induces inactive complexes of MEK with ARAF, BRAF and CRAF. **(C)** Addition of avutometinib to G12Ci might improve MAPK pathway blockade and anti-tumor efficacy through vertical inhibition of RAS, RAF and MEK.

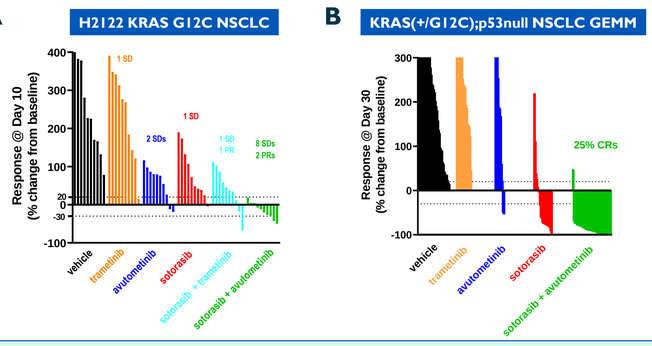

Figure 2. (A) Changes in tumor volume in H2122 KRAS G12C NSCLC tumor-bearing mice treated with avutometinib (0.3 mg/kg PO QD) ± sotorasib (30 mg/kg PO QD) (8).Trametinib was tested at 0.3 mg/kg PO QD. N=10 mice/group. SD: stable disease; PR: partial response. **(B)** Changes in tumor volume in KRAS(+/G12C);p53null NSCLC GEMM mice treated with avutometinib (0.1 mg/kg PO QD) ± sotorasib (100 mg/kg PO QD) (8).Trametinib was tested at 0.1 mg/kg PO QD. N=5-15 mice/group. CR: Complete response.

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RESULTS

Avutometinib upregulates antigen presentation machinery (MHC-I) in KRAS and BRAF mutant cancer models *in vitro* and *in vivo*

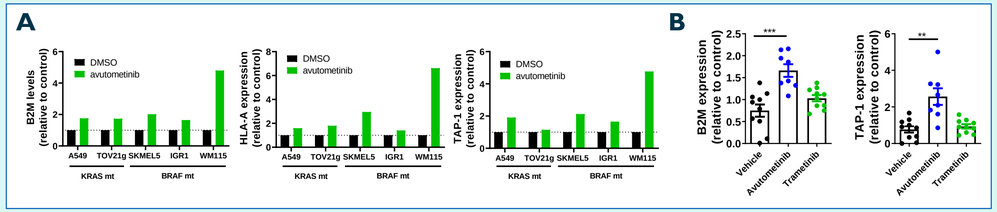

Figure 3. (A) A panel of KRAS and BRAF mutant cancer cell lines were treated with avutometinb for 48h. Avutometinib increased the expression of MHC-I complex genes including B2M, HLA-A and TAP-1. **(B)** CT26 KRAS mutant CRC tumor-bearing mice were treated with avutometinib or trametinib at 0.3 mg/kg QD for 9 days. Avutometinib upregulated B2M and TAP-1 in the CT26 model. p value calculated using t test: ** = p<0.01; *** < p<0.001

Combination of avutometinib + anti-PD-1 induces durable immune memory in the CT26 KRAS mutant syngeneic colorectal cancer model

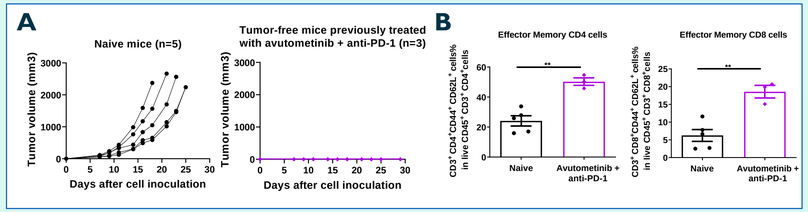

Figure 6. CT26 KRAS mutant CRC tumor-bearing mice that were tumor-free after previous treatment with the combination of avutometinib + anti-PD-1 were subsequent re-challenged with CT26 cells with no further treatment. Naïve mice were used as positive control. Tumor volume changes **(A)** and percentage of memory CD4+ and CD8+ T cells in spleen **(B)** are shown.

Avutometinib induces an immunogenic tumor microenvironment in the CT26 KRAS mutant syngeneic colorectal cancer model

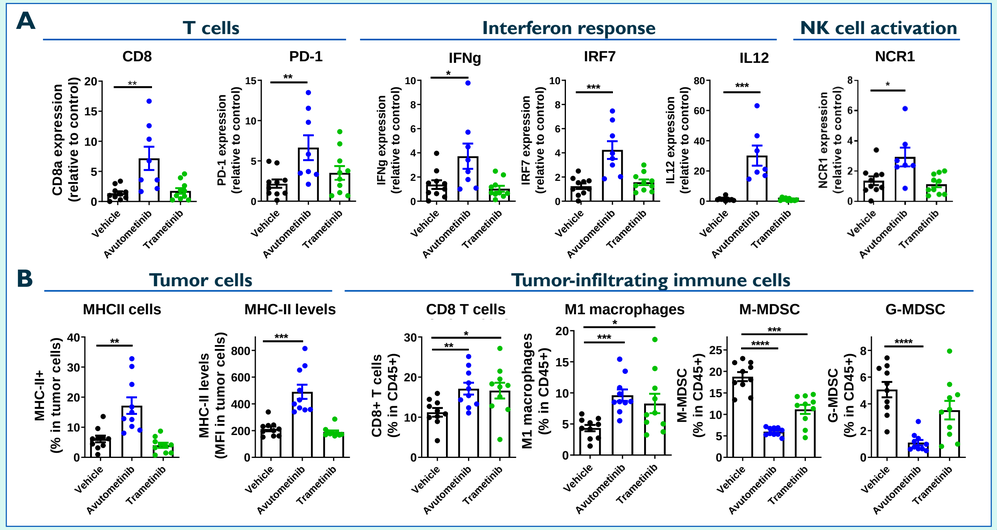

Figure 4. CT26 KRAS mutant CRC tumor-bearing mice were treated with avutometinib or trametinib at 0.3 mg/kg QD for 9 days. **(A)** Avutometinib upregulated the expression of markers of T cells, interferon response, and NK cell activation. **(B)** Avutometinib increased MHC-II expression by tumor cells, increased the numbers of CD8 T cells and M1 macrophages, and decreased the number of monocytic and granulocytic MDSCs. p value calculated using t test: * = p<0.1; ** = p<0.01; *** < p<0.001; **** < p<0.0001

In the CT26 KRAS mutant syngeneic colorectal cancer model, combination of avutometinib with anti-PD-1 results in increased antitumor efficacy and prolonged survival relative to single agents

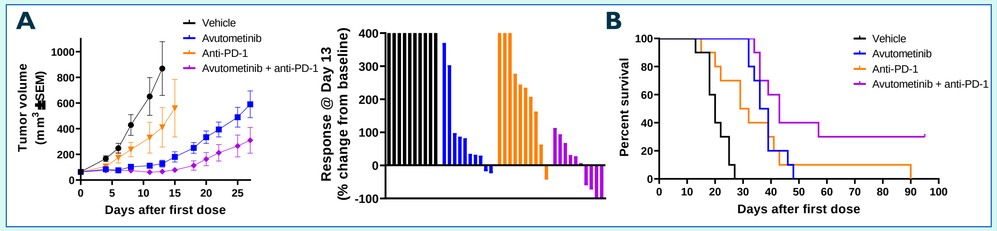

Figure 5. CT26 KRAS mutant CRC tumor-bearing mice were treated with avutometinib (0.5 mg/kg PO QD x28 days), anti-PD-1 antibody (3 mg/kg IP BIW x4 doses), avutometinib + anti-PD-1 or vehicle. Tumor volume changes **(A)** and Kaplan-Meier survival curve **(B)** are shown.

Avutometinib + adagrasib (KRAS G12C inhibitor) induces an immunogenic tumor microenvironment and improves survival in the KPAR^{G12C} orthotopic lung cancer model

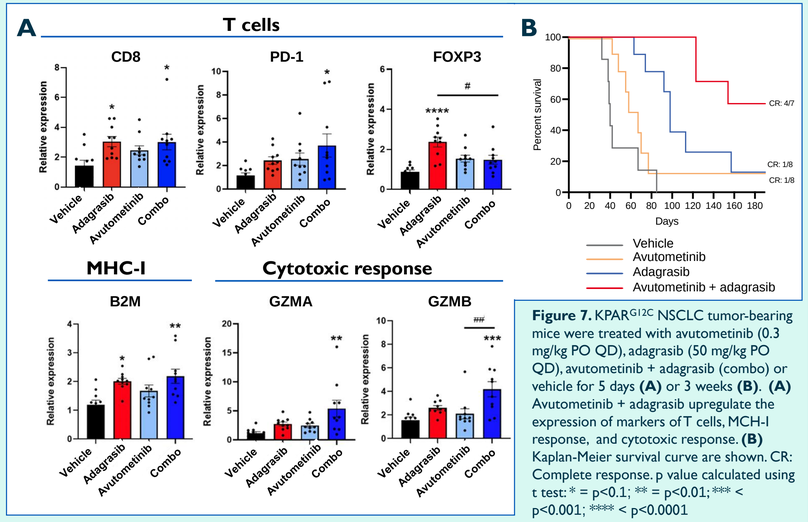

Figure 7. KPAR^{G12C} NSCLC tumor-bearing mice were treated with avutometinib (0.3 mg/kg PO QD), adagrasib (50 mg/kg PO QD), avutometinib + adagrasib (combo) or vehicle for 5 days **(A)** or 3 weeks **(B)**. **(A)** Avutometinib + adagrasib upregulate the expression of markers of T cells, MCH-I response, and cytotoxic response. **(B)** Kaplan-Meier survival curve are shown. CR: Complete response. p value calculated using t test: * = p<0.1; ** = p<0.01; *** < p<0.001; **** < p<0.0001

CONCLUSIONS

- The RAF/MEK clamp avutometinib induced an immunogenic tumor microenvironment including upregulation of MHC-I and MHC-II antigen presentation machinery, and upregulation of markers of T cell and NK cell activation and interferon response. Furthermore, avutometinib significantly increased the numbers of CD8 T cells and M1 macrophages with a concomitant decrease in monocytic and granulocytic MDSCs.
- Combination of avutometinib with anti-PD-1 increased antitumor efficacy and prolonged survival relative to either agent alone in the CT26 KRAS mutant colorectal cancer model. Furthermore, avutometinib + anti-PD-1 treatment induced durable immune memory.
- Combination of avutometinib with adagrasib (KRAS G12C inhibitor) improved the immunogenic tumor microenvironment relative to adagrasib alone. This included increased granzyme production and reduced Tregs along with increased survival in the KPAR^{G12C} orthotopic lung cancer model.
- Preclinical evaluation of the triplet avutometinib + adagrasib + anti-PD-1 is currently ongoing.
- These results support clinical evaluation of avutometinib with anti-PD-1 for treatment of patients with MAPK pathway-dependent tumors as part of a doublet (e.g. avutometinib + anti-PD-1 in BRAF mutant melanoma) or triplet (e.g. avutometinib + adagrasib + anti-PD-1 in front-line KRAS G12C NSCLC).