The RAF/MEK clamp avutometinib as the backbone of therapy for pancreatic cancer: Novel combinations with standard of care chemotherapy, FAK inhibitors, KRAS G12D inhibitors and/or autophagy inhibitors

Silvia Coma[1], Xiuting Liu[2], Noah L. Pieper[3], Wen-Hsuan Chang[3], Kirsten L Bryant[3,4], Channing J Der[3,4], David G DeNardo[2], Jonathan A Pachter[1]

[1]Verastem Oncology, Needham, MA, USA; [2]Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA; [3]Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA;

[4]Dept. of Pharmacology, University of North Carolina at Chapel Hill, NC, USA

BACKGROUND

KRAS mutations (mt) occur in up to 98% of pancreatic ductal adenocarcinoma (PDAC) and represent a key initiating event in PDAC carcinogenesis[1, 2]. Therapeutic efforts targeting the RAS/RAF/MEK/ERK (MAPK) pathway with MEK-only inhibitors have been unsuccessful in substantially modifying PDAC prognosis[3-6]. Thus, novel strategies are needed to overcome putative mechanisms of resistance to MEK inhibition such as focal adhesion kinase (FAK) pathway activation[7]. Another hallmark of PDAC is its high stromal density, which is thought to limit the penetration of cytotoxic drugs and T cells into the tumor and has been shown to be correlated with FAK hyperactivation[8, 9], altogether supporting co-targeting the MAPK and FAK pathways to achieve deep and durable responses for patients with PDAC.

Avutometinib is a novel RAF/MEK clamp that potently inhibits MEK kinase activity and induces dominant negative complexes of ARAF, BRAF and CRAF with MEK[10, 11] (Figure 1A, B). This unique mechanism allows avutometinib to block MEK signaling without the compensatory re-activation of MEK that appears to limit the efficacy of MEK-only inhibitors. Furthermore, addition of FAK inhibition to avutometinib has been shown to block acquired resistance to MAPK inhibition (Figure 1C). Preclinically, avutometinib has shown strong anti-proliferative potency across tumor cell lines carrying KRAS mt including PDAC cell lines (Figure 2). Clinically, defactinib + gemcitabine + pembrolizumab was safe, showed responses, decreased stromal density, and increased CD8+ T cell infiltration in PDAC (NCT02546531)[12].

Here, we tested the combination of avutometinib, with standard of care chemotherapy, FAK inhibition, KRAS G12D inhibition and/or autophagy inhibition in KRAS mutant pancreatic cancer models (Figure 1C).

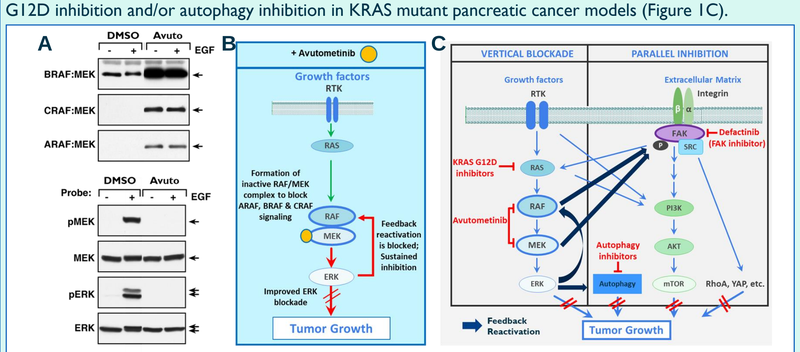

Figure 1. (A) Western blot analyses in serum-starved HeLa cells treated with 1 μM avutometinib (Avuto) for 3 hours and with EGF for 5 minutes. (B) Schematic showing that avutometinib is a unique RAF/MEK clamp that blocks both MEK kinase activity and the ability of RAF to phosphorylate MEK. (C) Establishing avutometinib as the backbone of targeted therapy for treatment of pancreatic cancer. Novel combinations with FAK inhibition, KRAS G12D inhibition and autophagy inhibitors.

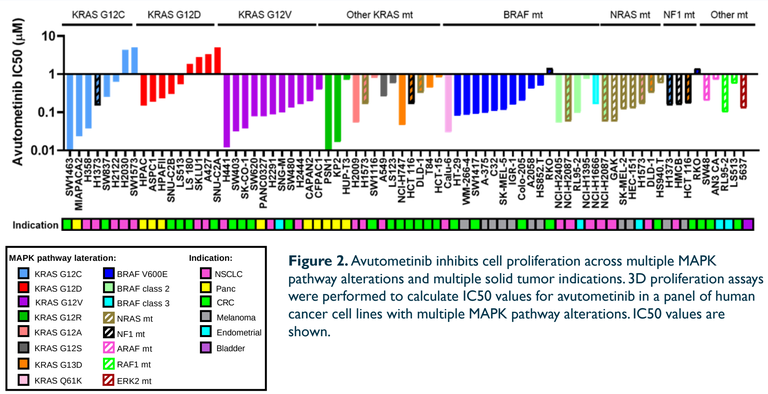

Figure 2. Avutometinib inhibits cell proliferation across multiple MAPK pathway alterations and multiple solid tumor indications. 3D proliferation assays were performed to calculate IC50 values for avutometinib in a panel of human cancer cell lines with multiple MAPK pathway alterations. IC50 values are shown.

REFERENCES

1. Cox, Drug discovery2014
2. Fan, Transl Cancer Res 2018
3. Bodoky, Investig New Drugs2012
4. Infante, Eur J Cancer 2014
5. Van Laethem, Target Oncol 2017
6. Van Cutsem, Int J Cancer 2018
7. Paradis, Clin Cancer Res 2021
8. Beatty, Genes Dev 2021
9. Jiang, Nat Med 2016
10. Ishii, Cancer Res 2013
11. Lito, Cancer Cell 2014
12. Wang-Gillam, Clin Cancer Res 2022

RESULTS

1. Combination of avutometinib and FAK inhibition with standard of care chemotherapy induces tumor regression and improves survival

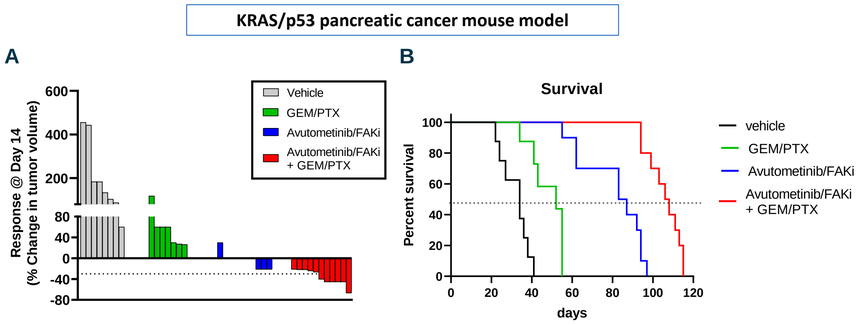

Figure 3. The combination of avutometinib + FAK inhibition (FAKi) induces tumor inhibition and increases survival but does not induce tumor regression. Addition of chemotherapy (gemcitabine + paclitaxel as a surrogate for nab-paclitaxel) to avutometinib/FAKi induces tumor regression and further improves survival. Changes in tumor volume (A) and survival (B) in KP2-OVA KRAS G12D/p53 pancreatic cancer tumor-bearing mice treated with avutometinib (0.3 mg/kg PO, QD 5 days on/2 days off) + FAKi (VS-4718; 50 mg/kg PO, BID) ± gemcitabine/paclitaxel (GEM/PTX; 75 mg/kg/5 mg/kg IV every 5 days; x5) are shown. Paclitaxel was used as a surrogate for nab-paclitaxel in mice. N = 10 mice/group.

2. Avutometinib enhances anti-tumor activity of KRAS G12D inhibitor *in vitro* & *in vivo*

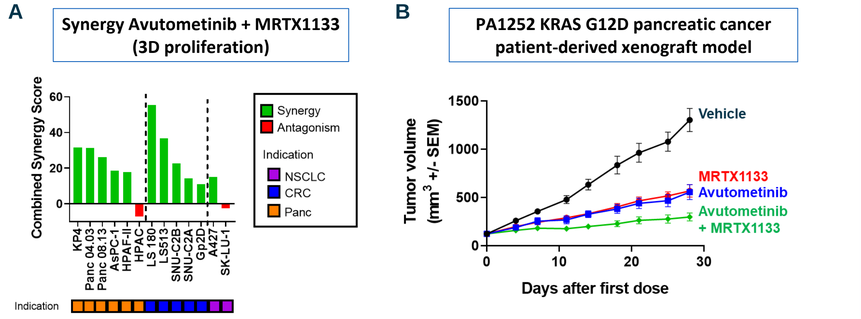

Figure 4. (A) In 3D culture, avutometinib is synergistic with the KRAS G12D inhibitor (G12Di) MRTX1133 in reducing viability of a panel of KRAS G12D pancreatic cancer cell lines. Waterfall plots summarize the combination synergy results for avutometinib with MRTX1133 in lung cancer (NSCLC), colorectal cancer (CRC) and pancreatic cancer (Panc) cell lines. Bliss, Loewe, HSA and ZIP synergy analyses were performed to generate a composite synergy score. (B) In mouse models, avutometinib enhances anti-tumor efficacy of MRTX1133 in a KRAS G12D pancreatic cancer patient-derived xenograft model. Changes in tumor volume in PA1252 KRAS G12D pancreatic cancer tumor-bearing mice treated with avutometinib (0.3 mg/kg PO, once daily 5 days on/2 days off) ± MRTX1133 (30 mg/kg PO, twice daily twice per week) are shown (collaboration with Mirati). N = 10 mice/group.

3. Combination of avutometinib + KRAS G12D inhibitor increases MAPK pathway inhibition, autophagy flux and induces synergistic anti-proliferative activity

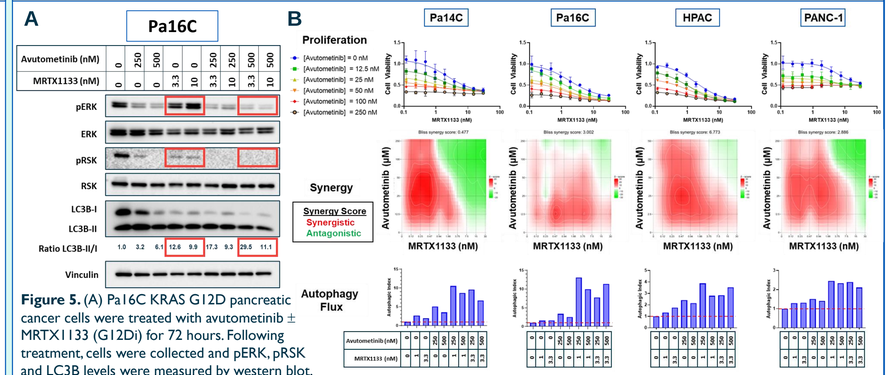

Figure 5. (A) Pa16C KRAS G12D pancreatic cancer cells were treated with avutometinib ± MRTX1133 (G12Di) for 72 hours. Following treatment, cells were collected and pERK, pRSK and LC3B levels were measured by western blot. (B top) KRAS G12D pancreatic cancer cells were treated with avutometinib ± MRTX1133 for 72 hours. Following drug treatment, live cell counting was performed and plotted to quantify effects on proliferation. Heatmaps indicating BLISS synergy scores are shown. (B bottom) KRAS G12D pancreatic cancer cells stably expressing a tandem fluorescent GFP-LC3-RFP-LC3ΔG reporter were treated with avutometinib ± MRTX1133 for 72 hours. Following drug treatment, autophagic flux was quantified by calculating the ratio of RFP/GFP in cells tested.

4. Addition of an autophagy inhibitor to avutometinib + KRAS G12D inhibitor further increases anti-proliferative synergy

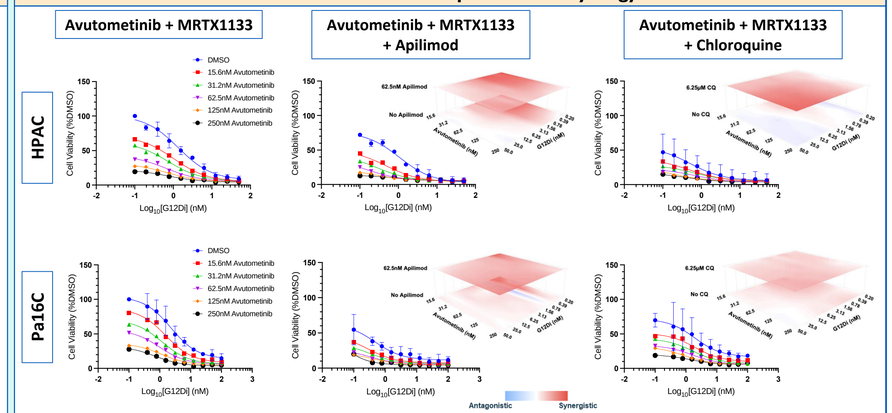

Figure 6. Human KRAS G12D pancreatic cancer cells were treated with avutometinib + MRTX1133 (G12Di) ± the autophagy inhibitors apilimod (62.5 nM) or chloroquine (CQ; 6.25 μM) for 72 hours. Following drug treatment, live cell counting was performed and plotted to quantify effects on proliferation. Insets are heatmaps indicating BLISS synergy scores ± autophagy inhibitor.

CONCLUSIONS

- In a KRAS/p53 pancreatic cancer mouse model, we found that the combination of avutometinib + FAKi induced tumor inhibition and increased survival. Addition of chemotherapy (gemcitabine + paclitaxel as a surrogate for nab-paclitaxel) to avutometinib/FAKi induced tumor regression and further improved survival.
- In 3D culture, avutometinib was synergistic with the G12Di MRTX1133 in reducing viability of a panel of KRAS G12D cell lines including PDAC. In mouse models, avutometinib enhanced anti-tumor efficacy of MRTX1133 in a KRAS G12D pancreatic cancer patient-derived xenograft model.
- In KRAS mt PDAC cell lines, treatment with avutometinib ± MRTX1333 decreased proliferation and increased autophagic flux in KRAS G12D PDAC cell lines. Accordingly, avutometinib ± MRTX1333 was synergistic with the autophagy inhibitors apilimod or chloroquine.
- These results support the ongoing clinical study of avutometinib and defactinib in combination with standard of care chemotherapy (gemcitabine/nab-paclitaxel) for patients with front-line metastatic PDAC (RAMP 205; NCT05669482). Furthermore, these results support testing the combination of avutometinib with G12Di, autophagy inhibitor or the triple combination in patients with KRAS G12D pancreatic cancer.
- In summary, avutometinib is a unique RAF/MEK clamp with the potential to become a backbone of therapy for treatment of pancreatic cancer.

AACR Special Conference in Cancer Research: Pancreatic Cancer; Boston September 27-30, 2023