Targeting Vasoactive Intestinal Peptide Receptor Signaling in Pancreatic Ductal Adenocarcinoma for Enhanced Anti-Tumor Response to Checkpoint Blockade

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Background: Paucity of T cells in the immune privileged tumor microenvironment (TME) of pancreatic ductal adenocarcinoma (PDAC) is a major reason that PDAC is refractory to immune checkpoint blockade. In this study, we show that human PDAC tumors over-express vasoactive intestinal peptide (VIP), an immunosuppressive neuropeptide, that inhibits effector T cell responses and regulates chemokine receptor expression on activated T cells. We thus hypothesized that pharmacological inhibition of VIP receptor signaling could enhance anti-tumor responses in PDAC.

Methods: VIP levels in plasma were determined via VIP-specific enzyme immunoassay and confirmed with immunohistochemistry (IHC) of tissue sections. VIP receptor (VIP-R) signaling in C57BL/6 immune competent murine models of KPC, MT5 or Panc02 pancreatic cancer was inhibited by daily sub-cutaneous treatment with ANT008 or ANT308, two novel VIP-R antagonists with predicted high binding affinities to VIP receptors. Multiplex IHC or flow cytometry detected frequencies and phenotypes of intra-tumoral T cells across treatment groups.

Results: Human PDAC tumors expressed VIP by immunohistochemistry, and PDAC patients had significantly elevated plasma VIP levels when compared to healthy volunteers (p<0.01, figure 1). Inhibiting VIP-R signaling in combination with anti-PD-1 monoclonal antibody (MoAb) synergistically enhanced T-cell dependent anti-tumor responses in murine PDAC resulting in elimination of tumors in up to 30% of the animals and increased intratumoral CD4+ or CD8+ T cell density in orthotopic murine PDAC (figure 2). VIP-R antagonist+anti-PD-1 combination therapy significantly increased intratumoral T cell activation and the proportion of tumor specific CD8+ T cells when compared to control (p<0.01, figure 3–5). Furthermore, tumor-free mice that had been treated with VIP-R antagonist and anti-PD-1 MoAb remained tumor-free upon tumor rechallenge, indicating that combination treatment induced robust immunological memory. Interestingly, anti-PD-1 monoclonerotherapy increased expression of CXCR4 on T cells in tumor draining lymph nodes, a chemokine receptor that has been shown to trap T cells in the extracellular tumor matrix. On the other hand, combination therapy with VIP-R antagonists and anti-PD1 MoAb significantly decreased CXCR4 expression and promoted homing of adoptively-transferred GFP+ T cells into the tumors.

Conclusions: VIP-R antagonists represent a novel approach to treat PDAC. VIP and VIP-R sequences are highly conserved between humans and mice, and human T cells are activated in vitro following treatment with VIP-R antagonists. Thus, we predict comparable anti-tumor activity of the combination of VIP-R antagonist and anti-PD-1 MoAb in human PDAC patients. Further clinical development of this novel concept will require appropriate pre-clinical pharmacokinetic and toxicology studies.
Abstract 748 Figure 3  Enhanced T cell response with combination therapy. mRNA expression in T cells isolated from subcutaneous KPC. Luc tumors in C57BL/6 mice treated with ANT008 and/or anti-PD-1 (n=3 per treatment group), were analyzed via Nanostring metabolism panel. Volcano plot showing differential expression of genes in T cells from (A) ANT008+ isotype IgG (IgG) vs scrambled peptide (Scram) + isotype IgG, (B) scrambled peptide +anti-PD-1 vs scrambled peptide + isotype IgG and (C) ANT008+anti-PD-1 vs scrambled peptide + isotype IgG (n=3 mice per treatment group). Genes that are associated with TCR activation and co-stimulation and are at levels significantly higher when compared to Scram + isotype IgG (FDR<0.1) are labeled in red. (D) Heat map showing gene expression changes in genes associated with TCR activation and co-stimulation. (E) TCR activation and co-stimulation pathway score between the T cells in tumors of mice from the different treatment groups. Cross symbol represents mice that were euthanized before day 25 due to ulceration of the tumor and circle symbol represent mouse that were imaged on day 26 via MRI imaging shown in supplementary figure S5. (D) Bar graph showing weight of pancreas on day 25 when the mice were euthanized. ‘Star’ shaped data points indicate tumor free mice and dotted horizontal line represents the average weight of healthy pancreas from naïve mice. (E) Representative multiplex IHC images (right) showing pancreatic tumors stained for DAPI (blue), CD4 (yellow), CD8 (red) and Ki67 (cyan) and trichrome staining (left) with black arrows showing blue collagen stain in the tissue. XY plot showing the correlation between number of (F) CD4+ or (G) CD8+ T cells/mm2; and (H) Ki67+ CD4+ or (I) Ki67+ CD8+ T cells/mm2 with weight of the pancreas with n=4 to 6 mice per group. P values in panel D were calculated using student ANOVA followed by Dunnett’s post hoc test (comparing each treatment group with Scram + IgG). Error bars show mean ± SEM. *p<0.05, **p<0.01.
Abstract 748 Figure 5  Increased T cell homing with combination therapy. KPC.Luc tumors were subcutaneously implanted in C57BL/6 mice and treated with VIP-R antagonist and/or anti-PD-1 checkpoint therapy for 10 days after the tumors were palpable. Tumor draining lymph nodes were then analyzed for percentage of (A) CXCR4+CD69+ and (B) CXCR4+Ki67+ cells in CD4+ (left) and CD8+ (right) subsets of T cells. In a separate experiment, on day 15 after subcutaneous implantation of KPC.Luc tumors, GFP+ T cells from enhanced GFP transgenic mice (C57BL/6 background) were adoptively transferred (via tail vein injections) and treated with ANT308± aPD-1 for 3 days. (C) Schematic showing GFP+ T cell transfer and treatment strategy in mice with subcutaneous KPC.Luc tumors. (D) Representative Hoescht (blue for nucleus) stained tumor tissues from tumors of each treatment group. Two regions of interest (ROI) in ANT308+aPD-1 treated tumors are shown at higher magnification. Statistical differences in A and B were determined via repeated measures ANOVA and Dunnett’s post-test with n=4–5 mice per group. *p<0.05, **p<0.01, ***p<0.001, p<0.0001.

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REFERENCES

Ethics Approval All experimental procedures involving mice were approved by the Institutional Animal Care and Use Committee (IACUC) at Emory University. De-identified blood samples from consented patients with PDAC (IRB 00087397) or healthy volunteers (IRB 00046063) were obtained with approval from Institutional Review Boards.

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