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.¹ 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.^{2,3} 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 immunocompetent 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.⁴⁻⁷ 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 monotherapy 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,⁸ 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 1 VIP is over-expressed by PDAC. (A) VIP mRNA expression levels in various solid malignancies, as obtained from TCGA. (B) Representative images of human PDAC tumor stained with antibodies to VIP or CK19, showing VIP co-expression in islets (black arrow) and cancer epithelial cells (red arrow). Levels of VIP in (C) culture supernatants collected from murine and human PDAC cell lines cultured for 24 hours ($n=3$ per cell line) were compared to culture supernatants from B16F10 and D4M melanoma cells; (D) plasma of mice bearing melanoma or PDAC tumors ($n=5$) compared to plasma of non-tumor-bearing mice; (E) plasma of PDAC patients ($n=19$) compared to that from healthy volunteers ($n=26$). Statistical differences in C and D were performed by ANOVA followed by Dunnett's post-test and in E were performed by student's t-test. Error bars show mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

Abstract 748 Figure 2 VIP-R antagonists improve responses to anti-PD-1. KPC.Luc, MT5 or Panc02 cells were subcutaneously implanted in immunocompetent C57BL/6 mice. About one week after tumor implantation, when the tumors were palpable, mice were randomized into treatment groups and treated with VIP-R antagonist and/or anti-PD-1 as described in methods. (A) KPC.Luc, MT5 and Panc02 tumor volumes as measured by Vernier calipers on day 22 after subcutaneous tumor implantation. (B) Kaplan-Meier survival plots of C57BL/6 mice with subcutaneously implanted KPC.Luc, MT5 or Panc02 tumors stratified by treatment. Kaplan-Meier survival plots of (C) C57BL/6 mice receiving monoclonal CD4 and/or CD8 monoclonal antibodies (D) CD4KO or (E) CD8KO mice compared to wild-type CD57BL/6 mice with subcutaneously implanted KPC.Luc tumors, stratified by treatment. Statistical differences in A were calculated by ANOVA followed by Dunnett's post-test. Solid line shows mean with in each treatment group. Statistical differences in B-E are calculated via Log-rank test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, **** $p < 0.0001$.

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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. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Acknowledgements The authors thank healthy volunteers and patients for blood and/or tissue samples. The authors also thank the shared resources at Emory University, namely the Emory Integrated Genomics Core (EIGC), Emory Flow Cytometry Core (EFCC), Cancer Animal Models Shared Resource (CAMS), Cancer Tissue Pathology Core (CTP), Bio-statistics Shared Resource (BSR) and Integrated Cellular Imaging Core (ICI), that provided services or instruments at subsidized cost to conduct some of the reported experiments. BioRender was used to make figure 4A and 5C. This work was supported in part by Katz Foundation funding and Emory School of Medicine Dean's Imagine, Innovate and Impact (I3) venture award to Edmund K. Waller and NIH R01 CA207619 awarded to Susan N. Thomas. Part of the cost for the immunohistochemistry staining of tissues was covered by Winship Cancer Institute Development Discovery and Therapeutic Program Pilot funding to Sruthi Ravindranathan.

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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.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.748>