DT095895, A SELECTIVE EP4 RECEPTOR ANTAGONIST WITH MONOTHERAPY EFFICACY IN SYNGENEIC MOUSE MODEL(S) AND BEST-IN-CLASS PROPERTIES

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Background Elevated levels of Prostaglandin E2 (PGE2), an eicosanoid notably synthesized by the cyclooxygenase-2 (COX-2), exert strong immunosuppressive effects in the tumor microenvironment. COX-2-positive solid tumors have the ability to use this pathway as a resistance mechanism, especially to escape from the host immune system, thus limiting the anti-tumor effects of immune checkpoint inhibitors (ICI). These immunosuppressive effects are largely mediated by the EP4 receptor, expressed on multiple immune cells.

Methods A novel series of EP4 receptor antagonists has been developed, with improved pharmacokinetic properties when compared to the EP4 receptor antagonists currently being evaluated in clinical trials. An intensive lead optimization program led to the identification of DT095895, a small molecule development candidate with a 'best-in-class' potential. DT095895 was assessed in multiple syngeneic mouse tumor models selected for their COX-2 expression profile.

Results DT095895 preclinical package will be presented in the poster. Efficacy was seen both in a monotherapy setting, as well as in combination with an ICI. Additionally, a specific biomarker program was implemented and validated in order to show target engagement. A phospho-flow murine whole blood assay was set-up to assess the ability of DT095895 to inhibit CREB phosphorylation induced by a selective EP4 receptor agonist in CD3+ cells. This biomarker was further developed for human whole blood to support Phase 1 and clinical trials studies.

Conclusions DT095895 is a selective EP4 receptor antagonist and demonstrates strong anti-tumor effects in multiple syngeneic mouse tumor models, both as a monotherapy and in combination with ICI, through the inhibition of the PGE2-induced immunosuppression. DT095895 progresses in regulatory development.

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SYNERGISTIC CANCER IMMUNOTHERAPY USING TUMOR TISSUE-DERIVED EXOSOMES AND ARTIFICIALLY PRODUCED BACTERIAL OUTER MEMBRANE VESICLES

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Background Checkpoint inhibitors work only in cancers that host inflammatory cells, and ‘cold’ tumors normally do not respond. Therefore, making ‘cold’ tumors ‘hot’ is required to increase the response rate to immunooncology therapies in general. Bacteria and bacterial products have been utilized for cancer immunotherapy for more than 100 years, but currently no such treatment is available because of the severe side effects that are observed. In this study, we produced artificial outer membrane vesicles (aOMVs) from Escherichia coli outer membrane, and injected them together with cancer tissue-derived exosomes to booster an immune response to the malignancy.

Abstract 816 Figure 1 Increase in memory CD8+ T cells in response to T-ALL
Changes to the T cell compartment were evaluated by transplanting primary T-ALL cells (CD45.2+) into immune-competent CD45.1 congenic recipient mice. T cells were then evaluated in the spleens at distinct stages of disease. As shown below, an increase in the frequency of CD8+ T cells that are memory (CD44+) and effector memory largely correlated with tumor burden in the spleens of transplanted mice that could indicate anti-leukemia T cell responses. Data is representative of a cohort from 1 of 3 independent experiments.

OX40 led to a drastic reduction in T-ALL burden. Importantly, control of tumor growth was accompanied by a concomitant increase in cytotoxic CD8+ T cells actively undergoing proliferation specifically in response to combination therapy. To gain better insight into T cell interactions with T-ALL, frozen tissue sections were used for comprehensive digital spatial profiling using NanoString’s GeoMX platform. This analysis revealed strong correlations between immune markers indicative of anti-leukemia responses as well as suppressive factors. Interestingly, regions enriched for activation markers were largely constrained to certain regions indicating the formation of ‘immunological hotspots’ in the context of T-ALL.

Conclusions The results from these studies suggest that T-ALL is recognized by T cells. As immune responses were not uniform within an organ, it will be important to specifically evaluate these ‘immunological hotspots’ in order to identify targets to activate T cells found in these regions. Ongoing studies are therefore aimed at comparing T cell interactions with T-ALL and their responses to immunotherapy between tissue types.

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Methods Outer membranes were obtained from E. coli by chemical means, followed by ionic stress and applied mild energy to generate aOMVs. The yield and purity of aOMVs were analyzed by nanoparticle tracking analysis and transmission electron microscopy. The protein and RNA contents were examined by label-free quantitative mass spectrometry and bioanalyzer. Inflammation was evaluated in macrophage cell line (RAW 264.7) and mice in vivo, and bone marrow-derived dendritic cells were used to assess the immunomodulatory functions of the aOMVs. For the study of antitumor activity, mice were subcutaneously inoculated with B16F10 cells and then subcutaneously immunized with aOMVs and melanoma exosomes five times at 3-day intervals. Also, anti-mouse PD-1 antibody was intraperitoneally injected into mice 1 day prior to immunization to investigate the effects of combination therapy. To elucidate the immunogenic mechanism, blood and spleen were obtained for antibody titer and splenocyte function study.

Results Bacterial aOMVs presented nanosized spherical shape with closed membranes and exhibited high yield and purity with very few cytosolic components. These aOMVs do not cause pro-inflammatory cytokine responses in RAW 264.7 cells and mice in vivo, despite high exposure levels. The aOMVs could be taken up by dendritic cells to stimulate cytokine and maturation marker expression. Co-immunization with aOMVs and melanoma tissue-derived exosomes elicited tumor regression in melanoma-bearing mice through Th-1 type T cell immunity and anti-tumor exosome IgG antibody production. Also, the immunotherapeutic effect of aOMVs was synergistically enhanced by anti-PD-1 inhibitor.

Conclusions Bacterial aOMVs can be produced in a large quantities with high purity, but are ‘detoxified’ compared to naturally released OMVs. The non-toxic aOMVs are powerful adjuvants for eliciting specific anti-tumor response, suggesting that aOMVs may be novel bacterial vesicle-mimetics clinically applicable as cancer treatment.

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819 TARGETING VASOACTIVE INTESTINAL PEPTIDE RECEPTOR SIGNALING: A NOVEL APPROACH TO ENHANCE ANTI-TUMOR RESPONSE IN PANCREATIC DUCTAL ADENOCARCINOMA

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Background Pancreatic ductal adenocarcinoma (PDAC), the fourth leading cause of cancer related death in the U.S, has a 5-year survival rate of only 10%.1 The paucity of T cells in the immune privileged tumor microenvironment (TME) is a major limitation in developing an effective immunotherapy against PDAC.2 The cancer genome atlas (TCGA) shows that human PDAC tumors express high levels of vasoactive intestinal peptide (VIP), an immunosuppressive neuropeptide (figure 1A), that inhibits effector T cell responses.3–4 We hypothesized that paracrine secretion of VIP in the TME is a targetable mediator of immune paralysis in PDAC, and that pharmacological inhibition of VIP receptor signaling could enhance anti-tumor responses in PDAC.

Methods VIP levels in plasma or cell culture supernatant was determined via VIP-specific enzyme immunoassay. Luciferase transfected KPC (KPC.luc) cells were injected subcutaneously or orthotopically into the pancreas of C57BL/6, CD4KO, or CD8KO mice from Jackson Laboratories. C57BL/6 mice T cell subsets in were depleted post tumor implantation with anti-CD4 and/or anti-CD8 antibodies. Tumor-bearing mice were treated daily with ANT008, a novel VIP receptor antagonist peptide, and/or anti-PD1 monoclonal antibody (MoAb) for 10 days, starting 7–10 days after implantation. T cells isolated from peripheral blood of PDAC patients were expanded 9 days ex vivo in anti-CD3 MoAb coated plates with 30U/ml IL-2 and either control peptide (scrambled VIP sequence) or

Abstract 819 Figure 1 Overexpression of VIP in PDAC tumors (A) VIP mRNA expression in several solid malignancies, with red arrow pointing at the levels in pancreatic cancer, as obtained from The Cancer Genome Atlas. (B) Blood collected from healthy volunteers (n=26) and consented untreated pancreatic cancer patients before surgery/chemotherapy (n=41) were quantified for levels of VIP. (C) Cell free supernatant collected from B16F10, SM1 and D4M (murine melanoma cell lines) or KPC, MT5 or Panc02 (murine PDAC cell lines) or 8XPC3 and Panc1 (human PDAC cell lines), were quantified for VIP (D) C57BL/6 mice were implanted with 1 million B16F10 cells (n=4), D4M cells (n=4) or MT5 cells (n=3) subcutaneously, or with KPC cells in the tail of the pancreas after laparotomy (n=3). When tumor volumes reached 500mm3 for subcutaneous tumors or the tumor flux reached 2 x 1010 photons/sec for orthotopic KPC tumors, mice were sacrificed and the concentration of VIP in the blood was determined. P value for B was calculated using student t test and C and D were calculated by one-way ANOVA followed by Tukey’s post-test. Error bars show mean with standard deviation. *p<0.0001

Abstract 819 Figure 2 PDAC cell lines express receptors for VIP (A) Western blot analysis showing constitutive expression of VPAC1, VPAC2 and PACAP receptors in murine and human PDAC cell lines and B16F10, a murine melanoma cell line. GAPDH was used as the loading control. (B) Percentage viability of KPC and Capan02 cells treated with varying concentrations of ANT008 (0.5-5μM) relative to cells treated with 0μM ANT008 for 24, 48 and 72 hours as measured by MTT assay.