Multi-omic multi-parameter circulating biomarker analysis in chemoimmunotherapy combinations identifies unique immune activation signatures in the pancreatic cancer setting.

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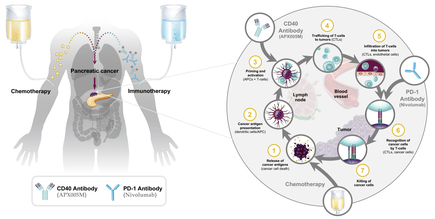
BACKGROUND



Gemcitabine/nab-paclitaxel ± sotigalimab ± nivolumab in first-line metastatic PDAC

Gemcitabine/nab-paclitaxel (GnP or chemo) is a standard of care chemotherapy regimen for first-line metastatic pancreatic ductal adenocarcinoma (mPDAC) and has a reporte1-year overall survival (OS) rate of approximately 35%.There is an urgent need for novel therapeutics and precision medicine approaches in mPDAC. PRINCE, a randomized phase 2 trial, evaluating two different immunotherapies d an increased 1-year OS relative to historical data, for patients treated with nivolumab (nivo) (PD-1/chemo) (57.3%, p = 0.007, n=34) and sotigalimab (sotiga) (APX005M; CD40/chemo) (48.1%, p = 0.062, n= 36)[1]. We performed deep immune profiling on all patients in the study to give unique insights into mechanisms of the immune therapies. Results from the CD40/PD-1/chemo cohort are not presented in this analysis.

Trial Registration: NCT03214250

METHODS

STUDY DESIGN: Phase 1b and Phase 2

- Phase 1b was a dose-ranging study to assess safety, clinical activity and to determine the recommended phase 2 dose of agonistic CD40 monoclonal antibody in combination with PD-1/chemo. The phase 1b results have been previously published.[2]
- In Phase 2, the first 12 participants were randomized 4:1:1 to cohort A1 (PD-1/chemo), cohort B2 (CD40/chemo), or cohort C2 (CD40/PD-1/chemo). The remaining participants were randomized in a 1:1:1 allocation. The 12 dose-limiting toxicity (DLT) evaluable participants from phase 1b (6 in B2 and 6 in C2) were included in phase 2 efficacy analyses (**Figure 1**).
- In Phase 2, the study was not powered to compare between treatment cohorts. This study did not enroll participants to a standard of care (chemo) cohort, but results were compared to historical control data.

ENDPOINTS: Phase 2

- Primary: 1-year overall survival (OS) rate compared with a 35% historical rate for chemotherapy.[3]
- Secondary: safety (adverse events [AEs], treatment-related adverse events [TRAEs]), objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), and duration of response (DOR).
- Exploratory: immune pharmacodynamics, associations between immune biomarkers and clinical outcomes.

ENROLLMENT: Phase 2

Participants were eligible for enrollment if they had:

- Histological or cytological diagnosis of metastatic pancreatic adenocarcinoma and Eastern Cooperative Oncology Group (ECOG) 0, or 1; no prior treatment for metastatic disease was permitted, nor was prior CD40, PD-1, PD-L1, CTLA-4 treatment in any setting.
- The enrollment period for phase 2 was from August 30, 2018 to June 10, 2019.

DOSING SCHEDULE: Phase 2

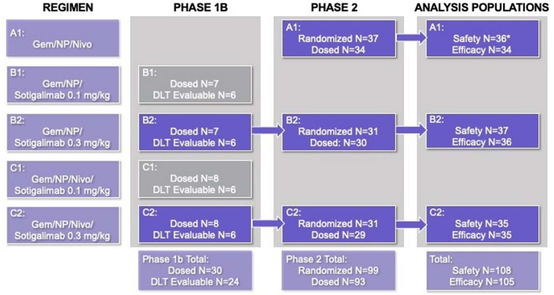
- For each 28-day cycle, on Day 1, Day 8, and Day 15 chemotherapy (Gem[1000 mg/m2]+nP [125 mg/m2]) was administered.
- For cohorts A1 and C2, on Day 1 and Day 15 nivo (240 mg) was administered.
- For cohorts B2 and C2, on Day 3 sotiga (0.3 mg/kg) was administered.

SAMPLE COLLECTION AND ANALYSIS: Phase 2

Baseline (Cycle 1 Day 1 (C1D1) or screening) and on-treatment (C1D15, C2D1, C4D1) blood samples were collected and analyzed for immune biomarkers using a variety of technologies. Immune population profiles were evaluated using frozen PBMC by CyTOF and features of T cell phenotype and function by multicolor flow cytometry. Soluble serum proteins were evaluated using two predefined Olink panels (immuno-oncology (IO) and Immune Response) along with an unbiased mass spectrometry proteomic approach (Biognosys) that identified circulating soluble proteins of significance.

TREATMENT COHORTS AND ANALYSIS POPULATIONS

Figure 1: PICI0002 Study Design.



*Two participants were randomized to cohort C2 but did not receive a dose of sotiga and are therefore listed under cohort A1 for this figure. Note B1/C1/C2 are not described in this poster.

RESULTS

- Phase 2 safety and efficacy were presented at ASCO 2021[1].
- The objective of this analysis was to further investigate unique mechanisms of action and relative contribution for PD-1/chemo and CD40/chemo
  - Results from the CD40/PD-1/chemo cohort are not presented in this analysis

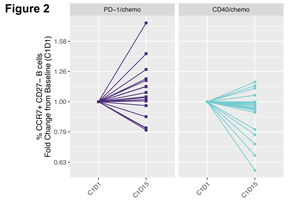


Figure 2: CCR7 expression decreases on CD27- B cells from patient PBMCs treated with CD40/chemo. CD40/chemo, but not PD-1/chemo, decreases the expression of CCR7 on B cells among individuals receiving CD40 relative to baseline as identified by CyTOF. (Samples included in analyses for PD-1/chemo; C1D1 n = 31, C2D1 n = 27; and CD40/chemo: C1D1 n =32, C2D1 n = 29)

RESULTS

Figure 3: Steady increases in proliferating, activated T cells are observed in circulation from patients treated with PD-1/chemo. 3A-F. PD-1/chemo, but not CD40/chemo, consistently increases the relative to baseline percentage of activated and proliferating effector memory CD4 and/or CD8 T cells (measured by Ki67+, HLD-DR+, CD38+, CD39+) in circulation across multiple timepoints as identified by X50 or CyTOF. (Samples included in analyses for PD-1/chemo: C1D1 n = 30, C1D15 n = 24, C2D1 n = 27, C4D1 n = 20; and CD40/chemo: C1D1 n = 31, C1D15 n = 25, C2D1 n = 29, C4D1 n = 20)

Figure 3

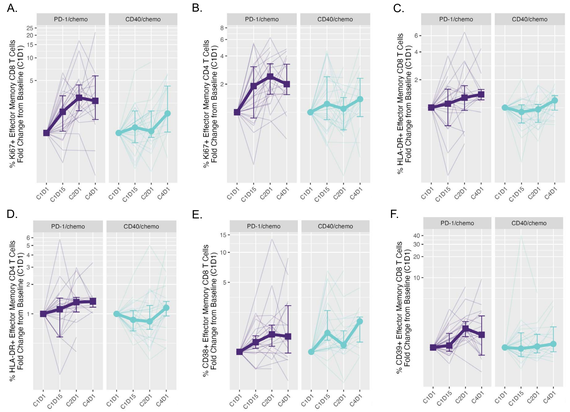


Figure 4: Early increases in soluble inflammatory proteins and type-1 skewing chemokines are observed in serum from patients treated with PD-1/chemo, while increases in soluble proteins and chemokines associated with DC maturation are observed in serum from patients treated with CD40/chemo. 4A-C. Proteins that changed significantly (p-value ≤ 0.05 as determined via t-test, and log2 expression fold change ≥ 0.5) from pretreatment (baseline) compared to PD-1/chemo treatment at C1D15, C2D1, and C3D1. Only highlighted proteins of interest are shown. PD-1/chemo treatment had significant increases in type II interferons (early effect), PD-1, IL-18. There was also significantly decreased levels of the immunosuppressive protein, arginase-1. **4D-E.** Volcano plots of serum proteins measured by the Olink platform. Proteins that changed significantly (p-value ≤ 0.05 as determined via t-test, and log2 expression fold change ≥ 0.5) from pre-treatment compared to CD40/chemo treatment at C1D15, C2D1, and C3D1. CD40/chemo treatment had earlier significant increases in soluble proteins associated with antigen presenting cell activation and Th1-inducing chemokines (LAMP3 and CXCL11 (early), respectively). IL-15 induction at C3D1 was unique to CD40/chemo treatment and may be produced by monocytes or macrophages, which can stimulate CD8 and NK cells.

4A-E. Common to both cohorts, were increases in type-1 cell mediated effector immunity proteins CXCL9, CXCL10, CXCL11. There were also decreases in known biomarkers prognostic to pancreatic cancer, such as K1C19 (ductal marker[3]) and CA-125[5,6], which tracked with tumor regression measurements. Decreases in various immunosuppressive factors were also observed in both cohorts (IL-8, MMP12). (Samples included in analyses for PD-1/chemo: C1D1 n = 24, C2D1 n = 27, C3D1 n= 25; and CD40/chemo: C1D1 n = 29, C2D1 n = 31, C3D1 = 25)

Figure 4

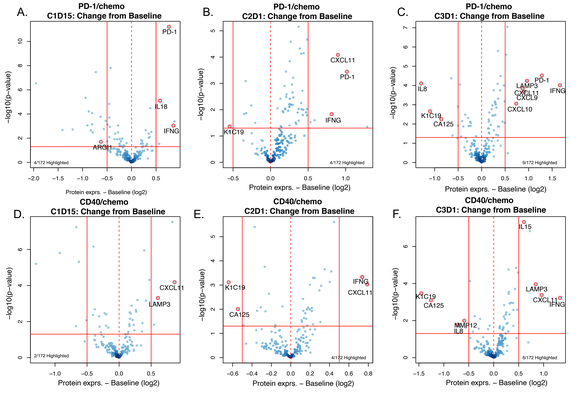


Figure 5 Increases in soluble proteins aiding in T cell activation and immune cell migration are observed in serum from patients treated with PD-1/chemo, while increases in soluble proteins associated with helper responses and innate immunity and chemokines are observed in serum from patients treated with CD40/chemo. 5A. Schematic of Biognosys biomarker identification and validation process using machine learning (ML) methods. The accuracy of the sPLSDA model was 62% for PD-1/chemo and 67% for CD40/chemo at predicting biomarkers for activation of dose related mechanisms. **5B-D.** Absolute scores plotted against fold changes of median signal at pretreatment (baseline) compared with PD-1/chemo treatment at C1D15, C2D1, and C3D1 allowed for identification of serum candidate biomarkers. Proteins associated with immune cell migration and T cell activation (GKN1, B3GN2, and PGRP1) . macrophage development (CSF1R), and immunosuppressive chemokine (CXCL7) were found to be significant in PD-1/chemo treatment, which mirrors the increases in T cell activation and decreases in immunosuppressive circulating factors from the Olink proteomics data. **5E-FG.** Absolute scores plotted against fold changes of median signal at pre-treatment (baseline) compared with CD40/chemo treatment at C1D15, C2D1, and C3D1. Soluble proteins essential for the activation of helper T cells/B cells (CCL15) and activated monocytes (GSHB) were identified as significant in CD40/chemo treated samples. Candidates of high importance (CRISP3 and ST3GAL6) have unclear mechanisms in pancreatic cancer and require further exploration. (Samples included in analyses for PD-1/chemo: C1D1 n = 30, C1D15 n = 23, C2D1 n = 19, C3D1 n = 18; and CD40/chemo: C1D1 n = 32, C1D15 n = 27, C2D1 n = 28, C3D1 n = 22)

RESULTS

Figure 5 (Legend Bottom of Middle Panel)

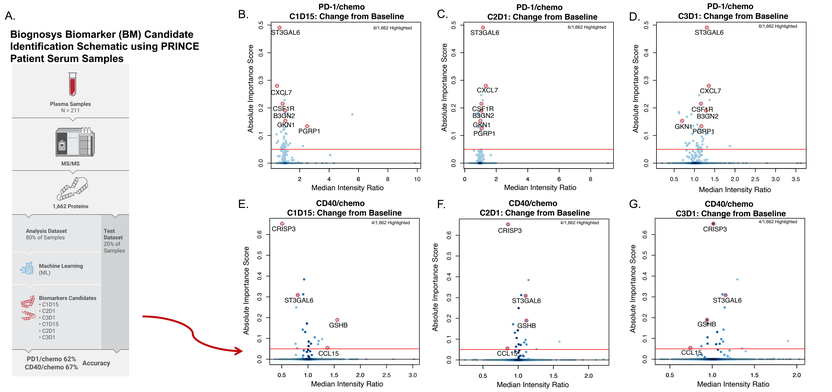


Figure 6

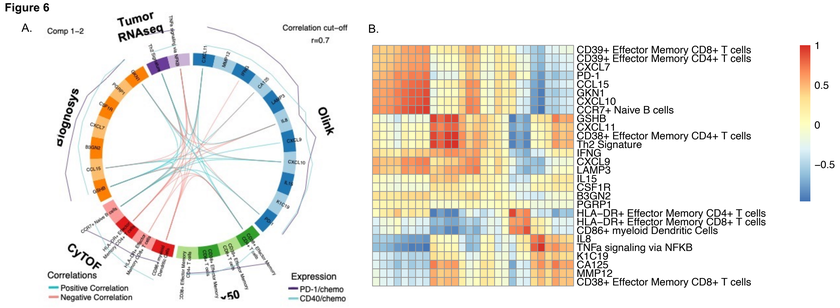


Figure 6: Integrated analysis of on-Treatment (C2D1) data of interest across the arms and assays reveals associations within treatment arms. 6A. Circos plot showing Pearson's correlations of selected molecules, gene signatures, and cell populations of interest. **6B.** Heatmap of correlated gene signatures, circulating factors, and cell populations of interest from 6A with respect to the effect of expression from PD-1/chemo or CD40/chemo. Data was normalized to baseline levels. The x-axis is a mirrored reflection of the y-axis. **6A-B.** Of interest, GKN1 and CCL15, chemoattractants for B cells, T cells, and/or monocytes correlate with LAMP3 and activated T cells (CD38+ and CD39+). Additionally, type 1 chemokines (CXCL10, CXCL9, CXCL11) are positively correlated to activated T cells (HLA-DR+, CD38+) and type-II interferons (IFNG).

CONCLUSIONS

This study is a first to use multi-omic minimally invasive biomarker approaches in mPDAC to demonstrate PD effects and immune modulation with immunotherapy/chemotherapy combinations. Orthogonal assays demonstrate that nivo(PD-1)/chemo and sotiga(CD40)/chemo elicit unique immune responses and the observed effects are consistent with their distinct mechanisms of action. These data suggest that multi-omic biomarker signatures identify clear mechanisms that reflect PD effect of both treatments which can be linked to response. Moreover, results from these analyses will support early phase clinical study development decisions, such as the ongoing PICI platform clinical trial in mPDAC, REVOLUTION (trial registration NCT04787991).

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ACKNOWLEDGMENTS

We extend our gratitude to the patients, their families, the clinical investigators, and their site staff members who are making this trial possible. We would also like to thank Sultan Nawabi at Parker Institute for Cancer Immunotherapy (PICI) for operations leadership of the trial. We thank Bristol Myers Squibb (BMS) and Apexigen for collaboration and study drugs. We would also like to thank and acknowledge Biognosys for partnering with us on this study, in part due to receiving the Biognosys Grant Program in 2020 for this study. The study was funded by Cancer Research Institute, BMS and PICI.