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The impact of *KRAS* mutations on the clinical outcome and immune response following immunotherapy for pancreatic cancer

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Abstract

Background: Pancreatic ductal adenocarcinoma (PDAC) is predicted to be the second leading cause of cancer-related death by 2030. This is driven by a high case-fatality rate with most patients even with radiologically localized PDAC at diagnosis ultimately relapsing with metastatic disease. *KRAS* mutations present in 90% to 95% of PDAC drive these poor statistics through its role in driving cellular growth, inhibition of apoptosis, and immunosuppression. The recent development of *KRAS* inhibitors has increased interest in understanding key molecular differences between the

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different *KRAS* codon changes seen in PDAC and other malignancies and how this might alter therapeutic decision making.

Methods: To understand how mutant *KRAS* influences the PDAC tumor microenvironment (TME) and cytokine signaling, we evaluated patients enrolled on [NCT02451982](#) (A Platform Study of Combination Immunotherapy for the Neoadjuvant and Adjuvant Treatment of Patients with Surgically Resectable Adenocarcinoma of the Pancreas). Interleukin 8 levels were measured using ELISA, these levels were compared with previously determined *KRAS* mutation status using next generation sequencing, tumor immune microenvironment populations quantified using multiplex immunohistochemistry, and survival outcomes.

Results: We identified a total of 30 patients from cohorts A: GVAX, an allogeneic whole cell cancer vaccine (n=16) and B: GVAX + anti-PD-1 (nivolumab) (n=14) with known *KRAS* mutation status and survival outcomes. Twenty-six of these tumors were *KRAS* mutant (G12C: 1, G12D: 11, G12R: 4, G12V: 10) and four were *KRAS* wild type. As *KRAS* G12D was the most commonly identified mutation and has been associated in some cohorts with worse outcomes, this was evaluated as a separate subgroup. *KRAS* G12D mutant PDAC had decreased disease-free survival (P=0.01) and a trend towards inferior overall survival in patients treated with GVAX alone (P=0.14) or GVAX plus anti-PD-1 (P=0.17) which became significant when combining both treatment groups (P=0.04). Looking at the relationship between *KRAS* status and the immune composition of the TME, patients with *KRAS* mutant PDAC had a trend towards decreased CD8⁺ T lymphocyte (P=0.06) following treatment with GVAX compared to *KRAS* wild type tumors. With the addition of anti-PD-1 in Arm B, patients with *KRAS* G12D mutant disease had a lower ratio of CD8⁺ GZMB⁺/CD8⁺ T lymphocytes (P=0.005).

Conclusions: *KRAS* G12D mutated PDAC represents a unique subtype of disease with decreased survival and lower ratio of activated CD8⁺ T lymphocytes as denoted by granzyme B (GZMB) positivity following GVAX/aPD-1 treatment.

Keywords

KRAS mutations; pancreatic adenocarcinoma; interleukin 8 (IL-8); tumor microenvironment (TME); *KRAS* G12D

Introduction

Background

Pancreatic ductal adenocarcinoma (PDAC) is predicted to become the second leading cause of cancer related death in the United States by 2030 with one of the high case-fatality rates of any tumor type (1). Resectable PDAC represents the earliest stage of PDAC and is classically treated with a combination of surgical removal and increasingly intensive chemotherapy (2–4). However despite this aggressive approach, the majority of patients with resectable PDAC will ultimately relapse with systemic disease (4,5). To improve on these stark statistics, the pancreatic cancer multidisciplinary team at Johns Hopkins University has developed a resectable PDAC platform trial in which patients receive two weeks of a novel therapeutic combination prior to surgery followed by additional cycles in the adjuvant setting along with standard of care combination chemotherapy (Figure S1) (6). In Arm A of

this platform, we tested our GVAX vaccine, an allogeneic whole cell cancer vaccine given before and after surgery. Correlative analyses on pre- and post-treatment tumors uncovered the induction of lymphoid aggregates and found that the programmed cell death protein 1/programmed cell death protein ligand 1 (PD-1/PD-L1) pathway was upregulated on the T cell and monocyte populations, respectively. This observation inspired Arm B which tested nivolumab (aPD1) in combination with GVAX two weeks before surgery, and continued with the same treatment schedule as in Arm A. Pre-treatment plasma, peripheral blood mononuclear cells (PBMCs), and biopsy specimens were obtained. Surgical specimens along with blood were also collected at the time of surgery.

Rationale and knowledge gap

This trial design has been successful in elucidating the biologic impact of these novel treatment strategies through interrogating surgical resection specimens removed after 2 weeks of neoadjuvant treatment (7,8). Many of the investigations to this point have focused on better understanding the on-target effects of these drugs on the tumor and surrounding tumor microenvironment (TME) but these samples also provide the opportunity to define the effect of clinical and molecular differences on the TME and treatment outcomes.

KRAS mutations present in 90% to 95% of PDACs represent one of the initiating events in tumor development and contribute to biologic aggressiveness through increasing cellular proliferation, inhibiting apoptosis, and angiogenesis (9). In addition to these roles in driving the formation and growth of PDAC, its role in suppressing a productive immune response has become increasingly appreciated. Emerging data has implicated mutant *KRAS* in increasing the expression of immunosuppressive cytokines to modify circulating and intratumoral immune cell populations. Specifically, *KRAS* has been implicated in enhancing interleukin 8 (IL-8) production, an immunosuppressive cytokine linked with recruitment of myeloid cells into the TME and conversion to immunosuppression phenotypes (10–12). These elevated circulating IL-8 levels have been previously correlated with worse clinical outcomes with immunotherapy in other tumor types highlighting their clinical importance (11,12). *KRAS* has also been associated with induction of IL-10 and transforming growth factor- β (TGF- β) which encourages production of a more fibrotic and immunosuppressive TME with conversion of CD4+ T lymphocytes into regulatory T cells (Tregs) (13). In addition to these immunosuppressive cytokine changes, *KRAS* mutations have been implicated in the downregulation of major histocompatibility complex (MHC) class I molecules limiting the ability of the immune system to detect tumor associated neoantigens (14).

Objective

In this investigation we will explore the influence of *KRAS* mutations, with a particular focus on *KRAS* G12D mutant disease, to understand the influence of these molecular alterations on survival outcomes, the TME composition and cytokine levels. We present this article in accordance with the CONSORT reporting checklist (available at <https://apc.amegroups.com/article/view/10.21037/apc-24-2/rc>).

Methods

IL-8 measurement

De-identified human plasma samples were archived from the [NCT02451982](#) clinical trial (Figure S1). This study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). The study was approved by the Johns Hopkins Medical Institution Institutional Review Board (IRB)-approved protocol with the approval number IRB00050517 and informed consent was obtained from all individual participants. Plasma samples were collected and stored according to the standard procedure. The concentration of the plasma IL-8 was measured by Quantikine ELISA (R&D Systems, Minneapolis, MN, USA, Cat #: D8000C). In brief, 100 μ L of assay diluent RD1-85 was added to each well followed by addition of 50 μ L plasma samples or IL-8 standard. The IL-8 standard was prepared according to manufacturer protocol. The plate was then incubated at room temperature for 2 hours followed by four washes using wash buffer supplemented by the ELISA kit. After washing, the plate was inverted and blotted it against clean paper towels to remove residual wash buffer. 100 μ L of human IL-8 conjugate was added to each well. The plate was then incubated for 1 hour at room temperature. The washing step was repeated. 200 μ L of substrate solution was added to each well with an incubation step for 30 minutes at room temperature in the dark followed by addition of 50 μ L of stop solution. The optical density of each well was read using a microplate reader at 450 nm wavelength. A standard curve was generated by using a four-parameter logistic curve fit (GraphPad Prism, Graphpad Boston, MA, USA). The concentration of IL-8 in plasma was measured in duplicate and calculated according to the standard curve.

Multiplex immunohistochemistry (mIHC)

mIHC was performed on resection specimens for patients enrolled on [NCT02451982](#) as part of our previous correlative investigations (6). For this analysis we included the 19 patients treated on this study with GVAX +/- aPD1 that had resection samples that were adequate for mIHC staining/analysis and had at least 2 years of clinical follow up for overall survival. In brief, these tissues samples were obtained 2 weeks after cycle 1 of immunotherapy (6). Formalin-fixed paraffin-embedded (FFPE) tissue blocks from these resection specimens were sectioned into 5- μ m thick slides before staining with hematoxylin and scanning using NanoZoomer (Hamamatsu, Shizuoka, JPN). Following antigen retrieval by microwave treatment with the antigen retrieval citra, sequential multiple iterative immunohistochemistry (IHC) cycles involving staining, scanning, and antibody/chromogen stripping, were performed. Negative control images were taken after the last antibody and chromogen stripping. These samples were stained with two immune panels. The first panel designed for CD8⁺ T-cell markers, included CD45, CD3, CD8, PD-1, CD137, and granzyme B (GZMB). The second staining panel designed for myeloid cell markers and Treg markers, included CD45, CD3, CD8, CD4, CSF-1R, CD68, CD163, CD66b, Foxp3, and TIGIT. Digitized images obtained with NanoZoomer were co-registered via the CellProfiler pipeline (version 2.1.1). Three rectangle regions of interest (ROI)s of ~3,000×3,000 pixels each containing one tumor lymphoid aggregate and epithelial neoplastic cells in the vicinity were chosen for analysis. Immune cell subtypes were defined by their immune cell marker signature as denoted in prior studies. Immune cell density was defined as the percentage

of a specific immune cell subtype among all cells within that sample's tertiary lymphoid aggregates (TLAs). Multiplex IHC analysis was repeated twice with consistent results.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism software (GraphPad) and R version 4.2.3. Disease-free survival and overall survival, starting from the first dose of study treatment, for each patient subgroup were estimated using Kaplan-Meier curves. Survival comparisons were made between patient subgroups using Log-rank tests. Multivariable analysis was performed using Cox proportional hazards model to account for the treatment groups. Comparisons between *KRAS* mutation status with respect to IL-8 levels and different immune cell densities was performed using a two-tailed Mann-Whitney test for evaluations of two groups and Kruskal-Wallis when comparing three groups. As *KRAS* G12D was the most commonly identified mutation and has been associated in some cohorts with worse outcomes, this was evaluated as a separate subgroup. The number of samples in each subgroup is listed in the associated figure legends. A P value less than 0.05 was considered statistically significant.

Results

KRAS mutations and survival outcomes

We identified a total of 30 patients from the [NCT02451982](#) clinical trial with known *KRAS* mutation status and survival outcomes who were treated on Arms A and B of this platform trial. *KRAS* mutation status was determined using next generation sequencing (NGS). For Arm A, 16 patients were identified, 2 with *KRAS* wild type disease and 14 with *KRAS* mutations (G12D: 6, G12R: 2, G12V: 6). For Arm B, 14 patients were identified, 2 with *KRAS* wild type disease and 12 with *KRAS* mutations (G12C: 1, G12D: 5, G12R: 2, G12V: 4). Survival for each cohort was estimated using Kaplan Meier survival curves in Figure 1. This demonstrated a worse disease-free survival in the *KRAS* G12D mutant tumors treated with GVAX alone (P=0.01). Overall survival was also compared between these cohorts revealing a trend towards worse overall survival with *KRAS* G12D mutant disease in Arm A (P=0.13) and Arm B (P=0.17). We used a multivariable analysis to understand the influence of *KRAS* G12D status in the combined cohort controlling for treatment arm which identified a significantly worse survival in patients with *KRAS* G12D disease (P=0.04). We also examined whether *KRAS* G12D mutations might co-associate with other factors that would influence survival outcomes and/or immune infiltration and impact these associations. We compared age, sex, tumor differentiation (well or moderately differentiated versus poorly differentiated), nodal status (N0 versus N+), stage (1/2 versus 3), TP53 status, CDKN2A status, and SMAD4 status between patients with *KRAS* G12D mutant PDAC and those without this alteration. For age, we used a student's *t*-test to compare these groups and for the other variables we used Fisher's exact testing. For these variables, there was a trend towards older age (*KRAS* G12D mutant: mean =70, median =69 years versus not *KRAS* G12D mutant: mean =65, median =67 years, P=0.11) and increased rate of poorly differentiated tumors in the *KRAS* G12D mutated cohort (*KRAS* G12D mutant: 46% versus not *KRAS* G12D mutant: 15%, P=0.06).

IL-8 levels and KRAS mutation status

Previously reports have suggested IL-8 to be highly immunosuppressive through its recruitment of myeloid cells into the TME and conversion into a more immunosuppressive population. To determine the relationship between IL-8 levels and survival outcomes, baseline plasma IL-8 levels were measured on a total of 17 patients (*KRAS*G12D: 7, *KRAS* nonG12D: 8, *KRAS* wild type: 2). The patients with wild type *KRAS* PDAC were found to have lower C1D1 IL-8 levels than patients with *KRAS* mutated disease ($P=0.03$). To determine the influence of IL-8 levels on survival outcomes, patients were stratified by whether their IL-8 levels were above the median value. Disease-free survival and overall survival for the IL-8 high and IL-8 low groups was estimated via Kaplan-Meier curves. There was a statistically significant ($P=0.01$) longer overall survival for the group of patients with lower baseline IL-8 levels and a trend ($P=0.07$) towards longer progression-free survival (Figure 2).

KRAS and TME

KRAS mutations have previously been associated with a number of immunosuppressive functions that would be predicted to significantly alter the TME including IL-8 production. To understand the influence of *KRAS* mutations on the TME of resectable PDAC, we compared *KRAS* mutation status with previously obtained mIHC from the PDAC resection specimens. Expressed immunohistochemistry markers were used to identify CD8⁺ T lymphocytes, CD8⁺GZMB⁺ T lymphocytes, CD8⁺CD137⁺ T lymphocytes, CD4⁺ T lymphocytes, regulatory T cells, M1-like macrophages, M2-like macrophages, myeloid derived suppressor cells (MDSCs). Given the profound effects of anti-PD-1 therapy on the TME, arms A and B were analyzed separately for the effects of *KRAS* mutations. For cohort A, in which patients were treated with GVAX alone, *KRAS* mutant tumors had a trend towards lower densities of CD8⁺ T lymphocytes ($P=0.07$). With the addition of anti-PD-1 treatment to GVAX in Arm B, there was a notably lower percentage of activated CD8⁺ T lymphocytes as denoted by GZMB⁺ in the *KRAS* G12D tumors ($P=0.005$) (Figure 3). There were no significant association between *KRAS* mutation status and macrophage or MDSC populations although this may have been limited by small sample size.

Discussion

Key findings

This investigation adds to the growing list of studies reporting on the influence of *KRAS* mutations on the TME and clinical outcomes. Notably, this investigation identified *KRAS* mutant resectable PDAC had higher IL-8 levels than *KRAS* wild type disease which is consistent with prior investigations identifying IL-8 as being upregulated by *KRAS* mutations (10,14). Higher IL-8 levels were also associated with decreased overall survival in this cohort. These observations raise enthusiasm for therapeutically targeting IL-8 in PDAC and other tumor types. To this end, we have an ongoing investigation evaluating inhibition of IL-8 or its C-X-C chemokine receptor 1/2 (CXCR1/2) receptors in combination with PD-1 blockade which seeks to reverse the deleterious effects of this IL-8 upregulation. Uncovering the TME changes produced by this approach will help inform future strategies combining these agents with other therapies targeting the TME.

The influence of *KRAS* mutations on the TME was also assessed in resection specimens. This revealed a trend towards reduced CD8⁺ T lymphocytes in *KRAS* mutant tumors following treatment with GVAX. As there was only one *KRAS* G12D tumor in Arm A that had undergone TME assessment this could not be independently assessed. In Arm B, the *KRAS* G12D mutant tumors had a lower percentage of CD8⁺ T lymphocytes that were activated as defined by GZMB⁺. The lack of other significant relationship may have been the result of small sample size or our neoadjuvant treatment approaches. Larger studies would be beneficial in better assessing this question.

Comparisow with similar fiwdiwgs

Previous reports have suggested that tumors with *KRAS* G12D mutations have a particularly poor prognosis with shorter survival following curative intent resection than other *KRAS* subtypes (15). While the underlying rationale for these inferior outcomes with *KRAS* G12D mutant disease is poorly understood, prior investigations using cancer cell lines have suggested that *KRAS* G12D tumors have increased phosphatidylinositol 3-kinase/mitogen-activated protein kinase signaling and less dependency on genes at the G2 and M DNA damage checkpoint than tumors with other *KRAS* mutations (16,17). Given this observation, we sought to further investigate potential explanations for these outcome differences by assessing IL-8 levels and the TME composition in *KRAS* G12D mutant PDACs. Looking at the TME, we observed these tumors had a lower percentage of activated CD8⁺ T lymphocytes as defined by GZMB⁺ following anti-PD-1 and GVAX treatment. This observation indicates this might be a particularly challenging population to treat with immune checkpoint therapy +/- cancer vaccination, which might be reversed through direct targeting of the *KRAS* G12D mutation or downstream effectors.

Implications

Targeting mutant *KRAS* was previously hindered by excess toxicity and rapid development of resistance. More recently *KRAS* G12C inhibitors have shown promising activity in pancreatic cancer and other G12C mutated tumors (18). While this have also suffered from rapid development of treatment resistance, preclinical studies have identified anti-tumor immune changes including induction of MHC1 expression and shift towards a more anti-tumor immune microenvironment with these inhibitors (19). As pan-*KRAS* inhibitors and *KRAS* G12D specific inhibitors become available this will become an increasingly attractive opportunity to combine these targeted therapies with immunotherapy to improve treatment durability.

Strengths and limitations

This paper has the strength that it contains a cohort of patients that have undergone neoadjuvant immunotherapy with cancer vaccination +/- anti-PD-1 therapy in the absence of chemotherapy or radiation therapy that might otherwise complicate the TME. This allows for more precise evaluation of the influence of immunotherapy on the TME and the role that *KRAS* alterations might play in this interaction. This investigation also has several limitations that require consideration when interpreting the results. Most notably this investigation was performed retrospectively on a relatively small number (n=30) of resectable pancreatic cancer specimens with only some of these having plasma for IL-8

level assessment (n=17). These numbers became even smaller when we stratified by *KRAS* status and treatment group for comparison of the TME limiting our ability to make strong conclusions regarding the influence of *KRAS* mutation status on TME following neoadjuvant treatments. Another factor is that these samples were from patients enrolled on a clinical trial for resectable PDAC and may not be representative of other PDAC populations including those with metastatic disease or with resectable disease treated in other clinical settings.

Conclusions

In conclusion, this investigation evaluated the influence of *KRAS* mutations on clinical outcomes, circulating IL-8 levels, and the TME composition in resectable PDAC. This uncovered that *KRAS* mutated PDACs in this cohort had a trend towards lower CD8⁺ lymphocyte infiltration following treatment with GVAX. Looking specifically at *KRAS* G12D tumors, these tumors had a decreased disease-free survival and trend towards a worst overall survival in this cohort compared to patients with *KRAS* wild type PDAC or other mutations in *KRAS* and reduced CD8⁺ lymphocyte activation following GVAX and anti-PD-1 treatment. Further defining the influence of different *KRAS* mutations on the PDAC TME will help inform future therapeutic strategies integrating inhibition of mutant *KRAS* or its downstream effectors with immunotherapy to enhance outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Sharing Statement:

Available at <https://apc.amegroups.com/article/view/10.21037/apc-24-2/dss>

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Highlight box**Key findings**

- *KRAS* G12D mutant tumors were associated with worse survival outcomes in this resectable pancreatic adenocarcinoma cohort.
- *KRAS* G12D mutant tumors had lower rates of CD8+ T cell activation following treatment with anti-programmed cell death protein 1 (PD-1) and GVAX treatment.

What is known and what is new?

- *KRAS* mutations have been increasingly appreciated as mediators of immunosuppression through their influence on the tumor microenvironment and cytokine levels.
- *KRAS* mutant tumors had higher levels of systemic interleukin 8
- than those without mutations in this resectable pancreatic ductal adenocarcinoma (PDAC) cohort.
- This cohort confirmed the inferior survival outcomes with *KRAS* G12D mutant disease seen in other tumor types.

What is the implication, and what should change now?

- This investigation emphasizes the importance of taking into account *KRAS* mutation status when treating patients with resectable PDAC.
- The development of *KRAS* mutation specific inhibitors may offer the opportunity to reverse the unique immunosuppressive features of *KRAS* mutant tumors.

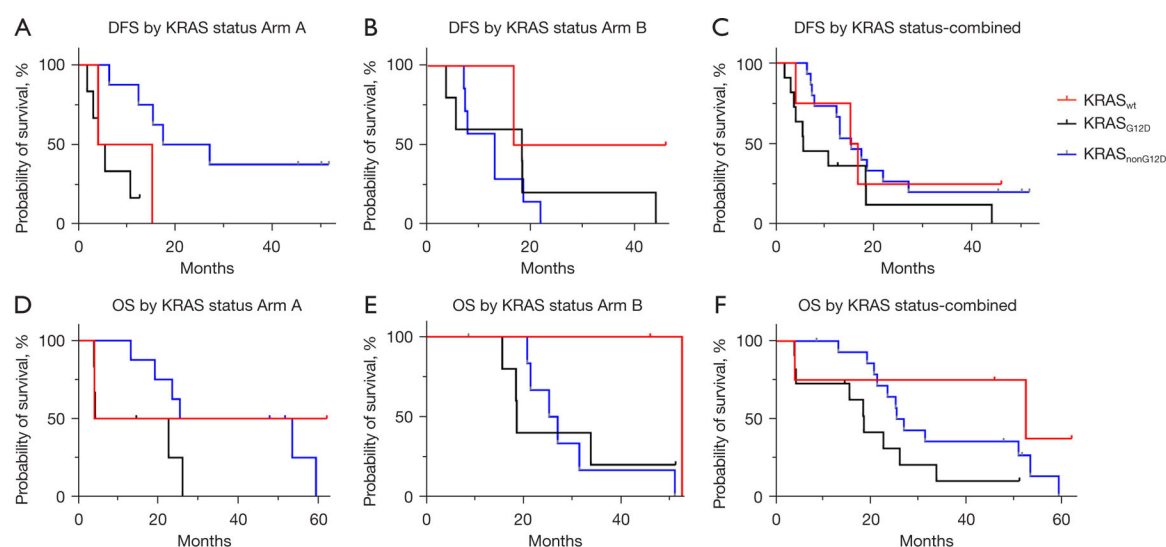


Figure 1.

Defining the relationship between KRAS mutation status and survival outcomes. To determine the influence of KRAS mutations and in particular KRAS G12D mutations on survival outcomes, KRAS status was assessed using next generation sequencing in patients undergoing treatment on [NCT02451982](#), a neoadjuvant-adjuvant resectable pancreatic adenocarcinoma platform trial (n=30). Of these, 4 were KRAS wild type and 26 were KRAS mutant with 11 of those 26 being *KRAS* G12D. Patients were stratified by whether they were *KRAS* wild type, *KRAS* G12D mutant or *KRAS* non-G12D mutant. These groups were then also compared for DFS (panels A-C) for Arm A, B, and both arms respectively and OS (panels D-F) for Arm A, B, and both arms respectively using Kaplan-Meier curves. There was a shorter DFS ($P=0.01$) and OS ($P=0.14$) for patients with *KRAS* G12D mutant PDAC treated on Arm A and OS ($P=0.17$) for patients with *KRAS* G12D mutant PDAC treated on Arm B. We used a multivariate analysis to understand the influence of *KRAS* G12D status in the combined cohort controlling for treatment arm which identified a significantly worse survival in patients with *KRAS* G12D disease ($P=0.04$). Kaplan-Meier survival statistics were performed using Log-Rank test on GraphPad prism. Multivariate analysis was performed using Cox proportional hazards model on R version 4.2.3. DFS, disease-free survival; OS, overall survival.

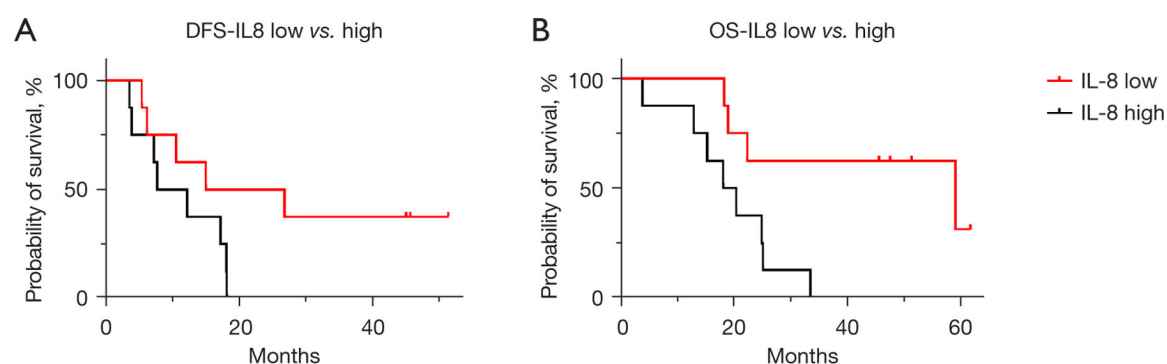


Figure 2.

IL-8 predicts disease-free survival and overall survival in patients with resectable pancreatic adenocarcinoma. To assess the influence of interleukin 8 on DFS and OS, we measured baseline plasma IL-8 via ELISA (n=17) in patients undergoing treatment on [NCT02451982](#), a neoadjuvant-adjuvant resectable pancreatic adenocarcinoma platform trial. Patients were stratified based on their IL-8 levels into “High IL-8” and “Low IL-8” subgroups and disease-free survival (A) and overall survival (B) between these two groups was compared using Kaplan-Meier curves. This identified patients with lower IL-8 levels had significantly longer overall survival ($P=0.01$) and trend towards longer disease-free survival ($P=0.07$). Statistics were performed using Log-Rank test on GraphPad prism. DFS, disease-free survival; IL-8, interleukin 8; OS, overall survival; ELISA, enzyme-linked immunosorbent assay.

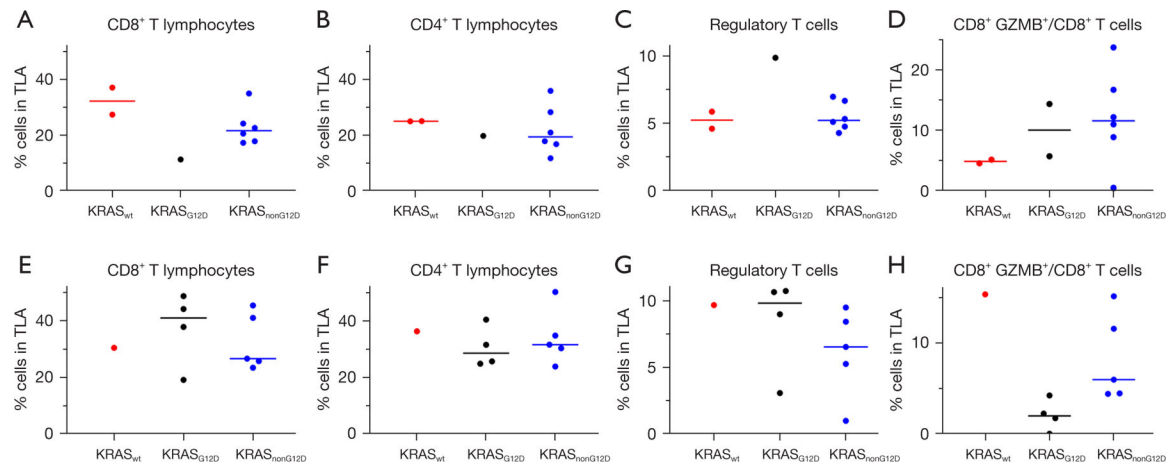


Figure 3.

Comparing *KRAS* mutation status, and infiltrating immune cell populations in resectable PDAC. To determine the influence of *KRAS* mutations and in particular *KRAS* G12D mutations on survival outcomes, *KRAS* status was assessed using next generation sequencing in patients undergoing treatment on [NCT02451982](#), a neoadjuvant-adjuvant resectable pancreatic adenocarcinoma platform trial (n=19). For this analysis each treatment arm was considered separately given the significant effects of anti-PD-1 therapy (Arm B) on the TME. A total of 9 patients had TME data available for Arm A: *KRAS* wild type: 2, *KRAS* G12D: 1, *KRAS* nonG12D mutant: 6. For Arm B, 10 patients had TME data available: *KRAS* wild type: 1, *KRAS* G12D: 4, *KRAS* nonG12D mutant: 5. Concentration of each immune cell population was denoted as a percentage of total cells within that patient's TLA. Differences between groups was assessed using Kruskal Wallis testing. Panels (A-D) show the density CD8⁺ T lymphocytes (A), CD4⁺ T lymphocytes (B), regulatory T cell (C), and proportion of CD8⁺ T lymphocytes that are also GZMB⁺ (D) in TLAs for patients treated on Arm A with GVAX alone. These evaluations demonstrated a trend towards lower CD8⁺ T lymphocyte concentrations in the TLAs of *KRAS* mutant tumors compared to those that are *KRAS* wild type (P=0.06). Panels (E-H) show the density CD8⁺ T lymphocytes (E), CD4⁺ T lymphocytes (F), regulatory T cell (G), and proportion of CD8⁺ T lymphocytes that are also GZMB⁺ (H) TLAs for patients treated on Arm B with GVAX plus nivolumab. These evaluations demonstrated a statistically lower proportion of CD8⁺ T lymphocytes that are GZMB⁺ (P=0.005) in *KRAS* G12D tumors compared to *KRAS* non-G12D mutant tumors. There were no other statistically significant differences between these molecular subgroups. Statistics were performed using Kruskal-Wallis testing on GraphPad prism as data may not be normally distributed. PDAC, pancreatic ductal adenocarcinoma; PD-1, programmed cell death protein 1; TME, tumor microenvironment; TLAs, tertiary lymphoid aggregates; GZMB⁺, granzyme B positive.