Lessons learned by features of pancreatic ductal adenocarcinoma and its tumor microenvironment

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Pancreatic ductal adenocarcinoma (PDAC) is a notoriously treatment resistant malignancy with high rates of mortality (1). While complete surgical resection is the only current option for cure, chemotherapy remains the bedrock for disease management across all disease presentations (1-3). The molecular profiles of individual cancers can be utilized to direct therapeutic selection and improve survival, such as hormonal therapy for breast cancer or checkpoint inhibitors in microsatellite instability high colon cancers (4,5). Unfortunately, current oncologic guidelines fail to account for molecular tumor heterogeneity and patient individuality when selecting therapeutic regimens for PDAC.

Significant efforts have been undertaken to begin to classify the molecular hallmarks of individual PDAC beyond the known driver mutations in an attempt to serve as a guide for precision therapy. Multiple molecular subtypes have been previously defined including: classical, quasi-mesenchymal and exocrine-like by Collisson *et al.*; basal-like, classical, normal and activated stromal subtypes by Moffit *et al.*; and squamous or basal-like, pancreatic progenitor or classical, exocrine-like, and immunogenic by the International Cancer Genome Consortium (6-8). To date this characterization process has led to challenges preventing its routine implementation, including the need for fresh frozen bulk tumor tissue, and thus has failed to yet make a significant impact on the guidance of PDAC therapy. Furthermore, molecular classification of solely the PDAC tumor tissue itself is likely inadequate as the stroma and immune cells composing the tumor microenvironment are pivotal role-players for disease progression and treatment resistance.

"Stratification of Pancreatic Ductal Adenocarcinomas Based on Tumor and Microenvironment Features", published in *Gastroenterology* by Dr. Puelo and colleagues, aims to redefine PDAC subtypes while also characterizing immune and stromal patterns for prognostication of disease (9). To answer these questions, a multi-institutional, prospective study was performed on 309 consecutives resected PDAC formalin fixed and paraffin embedded (FFPE) samples. The authors confirmed that RNA expression-determined subtypes can capture molecular diversity of PDAC and correlate with patient survival outcomes. Furthermore, they truly attempt to analyze the biology as a whole considering the tumor, stroma and immune cell components. This is thus a unique expansion upon previous existing classification systems that did not completely consider all tumor microenvironment components.

Following analysis of gene expression data with consensus clustering, five distinct PDAC subtypes were identified in this study based on tumor, stroma and immune cell microenvironment derived signatures. The previously defined classical/pancreatic progenitor and basal-like/squamous subtypes were again described coupled with low stromal signals. High stromal content was then identified

in 3 classes: immune classical, desmoplastic and stroma activated subtypes. The basal-like/squamous subset was associated with a significantly worse prognosis than the classical/pancreatic progenitor subtype (9). These findings are similar to previous molecular classifications, however expand upon these further by thoroughly incorporating the signals from the tumor microenvironment. Furthermore, by utilizing FFPE samples, this study offers improved feasibility for future clinical application.

Puelo and colleagues present a well-performed, preclinical study addressing an important question in a field undergoing intense study and revolution with tremendous future clinical implications. Nevertheless, a series of questions are posed. Firstly, patients who received preoperative chemotherapy were excluded. In the modern age of PDAC management, neoadjuvant chemotherapy and or radiation is of growing importance. More and more borderline or locally advanced pancreatic cancers are being resected following neoadjuvant therapy with encouraging survival results at least similar to that of upfront resectable PDAC (10,11). Furthermore, the opportunity for a pathologic complete response and exceptional survival has particularly influenced our group into increasing the utilization of neoadjuvant treatment (12). Thus, we have gradually seen less patients with chemo-naive PDAC at our institution. The classification and characterization of treated tumor is of importance. It is anticipated that following treatment there will be changes to the transcriptome in both the tumor and its interacting tumor microenvironment. The immune cell compartment in particular can see significant changes following treatment. Previous studies have suggested that cyclophosphamide may enhance immune responses by depleting regulatory T cells resulting in higher avidity of effector T cells specific for tumor antigen while Gemcitabine has been found to increase *de novo* T cell activation in treated PDAC patients (13-15). Thus, the molecular profile categories may be altered with different prognostic significance in the treated PDAC patient. Nevertheless, these defined molecular profiles may offer guidance if obtainable from pretreatment biopsy tissue with different implications and altered treatment response patterns. As this study was performed solely from FFPE tissue, this is a feasible direction for this current era with growing use of neoadjuvant treatment for PDAC.

Gemcitabine appears to be the only adjuvant agent described in this study and was not an independent predictor of patient prognosis (9). It would be interesting to see if molecular subtypes had effects on the responsiveness to adjuvant therapy. Moreover, correlation with changes in biomarkers such as circulating tumor cells or circulating tumor DNA and repeat characterization after treatment and/or metastatic progression would help progress the understanding of how different molecular subtypes predict treatment responses or evolve over the course of therapy. This study has shown viability with many potentially targetable agents such as CXCR4 or FAK, however caution is imperative when interpreting expression profiles. Significant maturity of clinical trial data is necessary in order to truly know what markers can guide therapy. Nevertheless, this study certainly provides important headway to the PDAC precision medicine initiative.

The immune environment was investigated and included in profile definitions, which is an important stride to accurate characterization that previous molecular classifications have failed to comprehensively address. However, in this study the infiltrating immune environment in many cases was combined dichotomously as present or not. Although PDAC is frequently immune excluded, those infiltrating immune cells are not equal in the tumor microenvironment with different roles and effects on patient prognosis. For every anti-tumor CD8+ T cell infiltration, there is an inhibitory immune component such as myeloid derived suppressor cells, regulatory T cells and a tumor-promoting subset of Th17 cells (13,16-18). Thus, mixing these immune cells in the same class broadly as "immune" is a limitation of this study. This limitation is understandable as the signaling web associated with PDAC is incredibly dense and difficult to tease out. As our understanding of the immune cell contribution to PDAC improves, further sub-classifications based on different immune component contributions may be established.

The stroma can serve as an impenetrable physical barrier limiting therapeutic access as well as forming a complex signaling axis between neoplastic and stromal cells. This stromal network can manipulate immune surveillance, regulate cancer growth and limit efficacy of therapeutics. Its consideration and incorporation by the authors in their molecular profiling should be applauded. Previously, Moffitt *et al.* used a "virtual microdissection" approach to separate PDAC tumor from stromal components allowing the identification of two stroma-specific gene expression signatures leading to different prognostic implications (7). Targeting the stroma has been considered a promising approach to enhance therapeutic response. However, this strategy remains controversial as some studies have

actually reported an acceleration of cancer progression following depletion of cancer associated fibroblasts (19,20). Interestingly, this work notes that the impact of stromal signals can be unique based upon the subtype of coexisting neoplastic cells: dense stroma in combination with a classical compartment is associated with inferior survival and may thus be a more appropriate subgroup to target with stromal agents than the basal-like neoplastic compartment counterparts, as stroma appeared to improve prognosis in these individuals potentially restraining progression (9). The findings of stromal FAK expression by the authors highlight the challenges of stromal therapeutic efforts: patients with high FAK expression had improved survival in this present study, which is opposite of the trend previously reported with data surrounding the encouraging FAK inhibitor therapy (9,21).

In conclusion, Puelo *et al.* utilize RNA sequencing of prospectively collected untreated resected PDAC FFPE specimen to identify molecular profiles. Five subtypes were classified while importantly also considering stroma and immune cell compartments. These subtypes were associated with prognosis and thus may potentially be used to help guide decision making regarding treatment for PDAC. This is an important initial step towards a precision medicine approach for PDAC treatment, however these gene expression classifications must now be translated into clinical practice with prospective biopsy based clinical trials.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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