

Inflammation, Biomarkers and Personalized Therapies in Pancreatic Cancer

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Abstract (188 words)

It is estimated that pancreatic cancer will be the 2nd leading cause of cancer-related deaths globally by 2030, highlighting the ongoing lack of effective treatment options in this devastating condition. There is a lack of reliable prognostic or predictive markers in pancreatic cancer to guide management decisions, whether for systemic chemotherapy, molecularly targeted therapies, or immunotherapies. To date, the results for targeted agents and immunotherapies in unselected populations of chemorefractory pancreatic cancer have not met expectations. The reasons for this lack of efficacy of immunotherapy in pancreatic cancer are incompletely understood. The challenges in pancreatic cancer include the physical barrier created by the dense desmoplastic stroma surrounding the tumor, chemokine-mediated exclusion of T cells, poor antigenicity, paucity of infiltrating T cells within the tumor, ultimately leading to an immunosuppressive microenvironment. A better understanding of the role of inflammation in pancreatic cancer, its tumor microenvironment and individualized patient-related features, be they molecular, clinical or histopathological would enable a more effective tailored approach to the management of pancreatic cancer. In this review, the role of inflammation, the immune tumor microenvironment and potential immune biomarkers in pancreatic cancer are explored.

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Introduction

Pancreatic cancer currently has the lowest survival rate of all cancers. Only 3 out of every 100 patients diagnosed with pancreatic cancer survives beyond 5 years (1). Recent published data looking at outcomes from 2005-2015 demonstrate no improvements in survival outcomes in the last 10 years (2). Pancreatic cancer is currently the 5th most common cause of cancer related death in the United States, but is projected by 2030, to become the 2nd leading cause of cancer related death globally (3). These statistics highlight the need for not only better therapies but also more durable treatment responses.

The most common form of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC). PDACs are stromal-rich tumours that develop within the exocrine compartment of the pancreatic gland. PDAC accounts for more than 85% of all pancreatic malignancies (4). Early stage or resectable disease occurs in just 20% of cases, 35-40% have locally advanced unresectable disease and 45-55% have metastatic disease at presentation (3). Factors contributing to more advanced stage at diagnosis include the non-specific nature of the presenting symptoms, the lack of good biomarkers to identify patients at risk of the disease, and the retroperitoneal location of the pancreas which limits routine tissue sampling and radiographic screening.

A unifying feature of all pancreatic tumours is the presence of immune and fibroblast cells, suggesting a critical role for inflammation in tumour progression. The impermeable desmoplastic stroma found around the pancreatic tumour is hypothesised to prevent immune cell infiltration and hinder effective delivery of chemotherapy (5, 6). It has also been suggested that abnormal stromal activation contributes to tumorigenesis, and further immunosuppression, hypoxia and an anti-angiogenic tumour microenvironment (5). One proposed model suggests that PDAC evolution occurs from PanIN 1 through to PanIN3 and then to invasive PDAC with progressive accumulation of desmoplastic stroma and modulation of the character of leucocyte infiltration altering through this process (7). Tumour associated macrophages (TAM) are reported to increase early in PDAC tumorigenesis, with reduction or varying levels of neutrophils and T cells present in PanIN and PDAC (8).

Genomic alterations are also implicated in the development of PDAC and may play a role in the immunogenicity of individual tumours. The most commonly occurring mutations in PDAC include KRAS in 90%; TP53 in 50-80%, and CDKN2A in 5-10% of PDAC tumours (8). PDAC typically has a relatively low mutation burden at 3-4/MB which may in-part account for the poor antigenicity and general lack of response to immunotherapy (9, 10). Six immune subtypes have been identified across a range of

solid tumours – immunologically quiet, lymphocyte deplete, inflammatory, wound healing, IFN γ dominant, and TGF β dominant (11). These subtypes appear to be governed by variations in immune cells –namely macrophages and lymphocytes signatures, Th1:Th2 cell ratio, tumour heterogeneity and genomic based differences of aneuploidy, neo-antigen load, cell proliferation, and expression of immunomodulatory genes (11, 12). A correlation between specific mutations and leucocyte levels has also been described across a range of tumour types, such that mutations in BRAF, TP53 or CASP8 relate to increased leucocyte levels while mutations in CTNNB1, NRAS and IDH1 are associated with decreased leucocyte levels in some tumours (11).

Other contributing factors that influence tumour-immune interactions in this complex intracellular and extracellular messaging network include differences in copy number, epigenetic changes, microRNAs and transcription (11, 12). More specifically for PDAC tumours, genomic immune analysis has identified a predominantly inflammatory immune signature that is defined by raised levels of TH17 and Th1 genes, as well as a low to moderate tumour cell proliferation (11). Not surprisingly, a low leucocyte proportion of tumour stromal fraction of <10% was reported.

Current treatment Paradigm

Pancreatectomy remains the single most effective treatment modality for the management of early stage disease, and the only potential for cure (13). However, even with “curative” resection and adjuvant therapy, the 5-year survival rate of early stage resected PDAC remains limited to 20-25% (14). For patients with advanced disease at presentation, the outlook is even more dismal with systemic therapy offering only a modest benefit (15). The use of single agent gemcitabine chemotherapy achieves survival rates of only 27-23% at 1 year and 2% at 5 years (15). The median survival with locally advanced disease ranges from 9 to 15 months, whilst for those with metastatic disease is as low as 3-6 months (3, 4).

In recent years, the use of two combination chemotherapy regimens have demonstrated superior efficacy over single agent gemcitabine. The use of FOLFIRINOX (a combination of oxaliplatin, irinotecan and 5 fluorouracil) in the advanced PDAC setting extended the median OS to 11 months, whilst the combination of albumin bound paclitaxel (Abraxane) with gemcitabine extended the median OS to 8 months (16, 17). Both these chemotherapy combinations are accepted as standard of care first-line palliative options in the advanced PDAC disease setting, but only offer limited benefit. Regardless of these treatments and the choice of first-line chemotherapy, the overall 5-year survival

rate for pancreatic cancer is less than 5% (4). Sadly, disease progression for pancreatic cancer patients with locally advanced or metastatic disease is inevitable.

There is a clear need to step outside the current treatment paradigm and investigate new diagnostic, monitoring and therapeutic opportunities to improve outcomes for this devastating disease, in both early and late stage pancreatic cancer. The results to date in early phase clinical trials of novel targeted agents in unselected chemo-refractory pancreatic cancer have fallen short of the anticipated efficacy, highlighting the lack of a reliable validated predictive or prognostic biomarker in pancreatic cancer that can inform and guide treatment decisions. A more selective approach by identifying and utilizing biomarkers that characterize patients could enable greater benefit from current and novel therapies.

Immune biomarkers in PDAC

Characterization of immune cells within the tumor microenvironment was first described by Virchow in 1863, since then work to immune-phenotype the TME of solid tumors has progressed using immune cell specific antibodies, multiplex immunohistochemistry and most recently spatial transcriptomics. There are limited published reports on the presence of tumor infiltrating lymphocytes (TILs) assessed on H&E sections in PDAC. One study by Hart et al examined H&E sections from 63 PDAC patients, and reported no difference in survival outcomes between patients with a high versus a low intra-tumoral lymphocyte score(18)]. In comparison, the presence of intra-tumoural tertiary lymphoid organs/follicles is associated with longer DFS and OS (19).

A range of immune cell markers have been investigated in PDAC with good prognostic associations found for higher expression of CD3, CD4, CD8, and CD20 (20, 21). In comparison, higher expression of FOXP3, CD66b, CD68, CD163, and CD204 are associated with worse outcomes (20). Myeloid cell infiltrates appear to dominate the immune reaction in PDAC (20, 22). Elevated levels of circulating myeloid-derived suppressor cells (MDSC) and regulatory T cells (Tregs) have been reported in PDAC patients, and are associated with worse prognosis and OS (22).

The mere presence of T cells within the PDAC tumor may have limited clinical correlation. The expansion of tumor-infiltrating CD3+ T cells isolated from resected PDAC specimens confirmed that a subset of T cells is present within the tumor and have tumor reactivity; however, some of these T cells have lost CD3zeta expression required for T cell receptor signaling and activation or lack the T cell activation gene signature. These T cells are there considered to be hypofunctioning (23). Thus, characterizing the type of immune cells, density and location of immune cells in the PDAC is crucial to

filling the gaps in our current knowledge and determining the therapeutic targeting and potential of novel immunotherapies (24)

Of interest are immune biomarkers that could potential be used to guide treatment choices with novel immunotherapies, as they can be monitored easily, and cheaply, by blood-based screening. To date the administration of immunotherapy in clinical trials in PDAC has yet to demonstrate substantial benefit in pancreatic cancer (25-27). The reasons for this are incompletely understood but likely to be multifactorial. Proposed mechanisms leading to immunotherapy resistance in PDAC include a markedly immunosuppressive and “immune-privileged” tumor microenvironment (20). As previously mentioned, contributing factors to this immune-privileged TME include the paucity of T cell infiltration into the tumor, the low mutational burden in PDAC that produces few immunogenic antigens, (9, 28) and the physical barrier created by the dense desmoplastic stroma that hinders access of therapeutic monoclonal antibodies, promotes chemokine-mediated exclusion of T cells within the tumor milieu, and reduces T cell expression of checkpoint receptors (29). Ultimately, an individual’s responses are dependent on a combination of factors shaping PDAC tumor growth, systemic immunity (innate and adaptive) and the individualized tumor genomic alterations (Figure 1).

Figure 1. Understanding systemic immunity in the context of the tumor microenvironment and immune-privilege in pancreatic cancer.

To date, only a small subset of PDAC patients with particular characteristics including high burden of microsatellite instability (MSI-high) and/or tumors with higher effector T cell infiltration have demonstrated a sustained response to PD-1 blockade with improvement in progression free and overall survival. [(20, 27, 30). These responses indicate the potential for effective treatment with immunotherapies in PDAC. Preclinical and early phase clinical trials are now underway to investigate an array of novel immunotherapy targets as well as rational combinations with existing therapeutic modalities (31, 32) Alongside this, the ability to provide a companion immune biomarker may enable these novel immune targets and combination treatments to unlock the full potential of immunotherapy for PDAC patients.

Current checkpoint inhibitors in routine clinical use in solid tumors

The introduction of the immune checkpoint inhibitors, cytotoxic T lymphocyte protein 4 (CTLA4) and programmed cell death protein-1 (PD1), to cancer management has resulted in a paradigm shift in many solid tumors. Anti-CTLA-4 antibodies (ipilimumab and tremelimumab); anti-PD1 antibodies (nivolumab and pembrolizumab) and anti-PDL1 antibodies (atezolizumab and durvalumab) are in routine clinical use for melanoma and lung cancer, with increasing evidence of benefit in other solid tumors as well. These new immune based drugs are enabling long-term disease control lasting years beyond what would have been expected prior to the era of immunotherapy (30).

CTLA-4/CD80 Pathway in Pancreatic Cancer

Checkpoint blockade molecules are present on T cells and serve an inhibitory function. CTLA-4, is homologous to the CD28 receptor. CTLA-4 prevents differentiation and activation of naïve T cells (33). The blockade of CTLA-4 by anti-CTLA-4 antibodies results in unrestricted T cell activation thus enhancing the T cell response to the presence of cancer cells (34). The use of single agent anti-CTLA-4 antibodies in the management of advanced PDAC has shown limited clinical benefit in the majority of pancreatic cancer patients (26, 35, 36). Alternative combination therapy approaches are being investigated that can increase T cell trafficking and infiltration into the tumor microenvironment, thereby sensitizing the PDAC to checkpoint blockade (37).

PDL1/PD1 interaction in Pancreatic Cancer

Programmed cell death protein-1 ligand (PDL1) and its receptor, PD1, interact to promote immune tolerance (Figure 3). The blockade of the PD1/PDL1 interaction acts to increase anti-tumor immunity. Results from trials investigating the use of PD1 therapy alone in advanced PDAC have been disappointing (38, 39). However, results from the phase 2 clinical trial led by Le and colleagues, to evaluate PD1 checkpoint blockade with Pembrolizumab in treatment refractory solid tumors (colorectal and non-colorectal cancers) with MSI-H tumors demonstrated that in this subset of pancreatic cancer patients, the administration of PD1 therapy resulted in durable responses in patients with 24-month progression free survival (PFS) rate of 53% and 24- month overall survival (OS) rate of 64% (30). This study exemplifies that biomarkers for immune response in PDAC exist and are vitally informative. These findings provide a glimmer of hope that other novel immunotherapies perhaps in combination with different treatment modalities may be able to overcome resistant mechanisms to immune response in PDAC. To this end, the identification of other immune biomarkers could determine the appropriate immune-based combination therapy and increase the efficacy of both targeted and novel immune therapies at an individualized level to improve therapeutic efficacy and ultimately long-term outcomes.

Stimulating the immune response in Pancreatic Cancer

Therapeutic vaccine trials involving an array of recombinant constructs, peptides, proteins and whole tumor cells, all designed to prime circulating tumor specific T cells and attack PDAC, so far have reported negative outcomes (39). Despite this lack of efficacy, these studies have provided important insight into the immune response in PDAC, confirming that T cell immunity to tumor associated self-antigens can be generated and break immune tolerance in PDAC. Furthermore, studies like the phase 2b GVAX pancreas ECLIPSE study demonstrated that it was possible to induce tertiary lymphoid structures and T cell infiltration in patients (40). It has been hypothesized that dysfunctional T cells and suboptimal antigen selection, resulted in the overall lack of long-term survival in the therapeutic vaccine trials, despite clear evidence of an immunological response in some patients (41, 42).

Approaches that combine therapeutic vaccines with checkpoint blockade are being undertaken to investigate whether checkpoint blockade can re-invigorate vaccine-primed T cells to overcome the immunosuppressive inhibition generated by the PDAC tumor microenvironment (43).

Novel Immune targets of interest in Pancreatic Cancer

Work by Balli et al into immune cytolytic activity stratified PDAC patients into molecular subsets. Here they identified groups of PDAC patients that despite evidence of activated T cells and detectable neoepitope burden, had a complete lack of benefit from anti-PD1 therapies. Thus, demonstrating that the PDL1/PD1 axis is only one component of the T cell response mechanism in PDAC (44). Alternative inhibitory pathways that are highly expressed in PDAC are being investigated in early phase clinical trials. These include the extracellular enzyme, CD73, that stimulates the release of adenosine, a pro-metastatic and immunosuppressive molecule; inhibitory receptors on T cells analogous to PD1 such as TIM3, TIGIT, and LAG3; and the inhibitory ligand on present on myeloid cells, VISTA, that is analogous in function to PD-L1.

Alternative orthogonal immunotherapeutic approaches of interest in PDAC involve the use of agonistic immunotherapies which include CD40, OX40 agonists, toll like receptors (TLR) agonists and stimulator of interferon genes (STING) agonists; and myeloid-based immunotherapies.

Agonistic immunotherapies

CD40 is expressed by antigen presenting cells including dendritic cells, monocytes, B cells, endothelium, platelets and some tumor cells. CD40 agonists in combination with gemcitabine has been shown to induce anti-tumor T cell response and transform macrophages in PDAC from a pro-

inflammatory status to a tumoricidal phenotype (46). Combining CD40 agonists with nab-paclitaxel and gemcitabine further enhanced the T cell mediated tumor destruction and generated immune memory, not seen with the gemcitabine combination alone (47). Preclinical models indicate that the combination of CD40 agonist with gemcitabine and nab-paclitaxel can sensitize PDAC to immune checkpoint blockade (48).

Conclusion

The relationship between inflammation and PDAC is only just beginning to be appreciated. Our knowledge of the components of the tumor microenvironment and their relationship to patient prognosis in PDAC is in its infancy compared to other cancers [8-9]. There are many inherent challenges in applying immunotherapy in PDAC based on the immunosuppressive and immune-privilege nature of the PDAC microenvironment, the tumor heterogeneity and the quality of the immune response. Nevertheless, strategies are being developed to promote T cell immunity and draw in T cells into the PDAC tumor as well as enhance the efficacy of cytotoxic therapies. Combination strategies with different modalities of treatment, and novel immunotherapy targets may hold the key to unlocking the potential of immunotherapy in PDAC. A comprehensive characterization of the relationship between genetic sub-type, immune cell infiltration, and patient related features could be leveraged for functional studies in animal models as well as used to develop a blood-based stratification biomarker. Identification of protein-based markers linked to treatment and immunotherapy outcome may also enable closer monitoring of a patient response to chemotherapy and immunotherapy, and prediction of clinical outcome.

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Figure 1. Understanding systemic immunity in the context of the tumor microenvironment and immune-privilege in pancreatic cancer.

