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Strategies for Increasing Pancreatic Tumor Immunogenicity

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Abstract

Immunotherapy has changed the standard of care for multiple deadly cancers including lung, head and neck, gastric, and some colorectal cancers. However, single agent immunotherapy has had little effect in pancreatic adenocarcinoma (PDAC). Increasing evidence suggests that the PDAC microenvironment is comprised of an intricate network of signals between immune cells, PDAC cells, and stroma, resulting in an immunosuppressive environment resistant to single agent immunotherapies. In this review, we discuss differences between immunotherapy sensitive cancers and PDAC, the complex interactions between PDAC stroma and suppressive tumor infiltrating cells that facilitate PDAC development and progression, the immunologic targets within these complex networks that are drugable, and data supporting combination drug approaches that modulate multiple PDAC signals, which should lead to improved clinical outcomes.

Keywords

pancreatic cancer; immunotherapy; tumor microenvironment

Introduction

Current estimates predict PDAC to overtake breast cancer and become the third most common cause of cancer-related death in the United States (1,2). Only 20–30% of patients with PDAC have resectable disease at diagnosis, and the majority of patients who undergo surgical resection subsequently relapse (3–7). Most patients present with metastatic disease at diagnosis and have only a 2% five-year survival (2). To date, the rate of successful clinical trials in pancreatic cancer remains low (8). Of the many therapies investigated in large clinical trials over the past two decades, only two systemic therapies have demonstrated a statistically significant and clinically meaningful improvement in overall survival (OS) as

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compared to gemcitabine alone (9,10). As a result, the five year survival rate for PDAC has improved only marginally since the 1970s, from 3% to 7% (2). This highlights the continued need for new and effective therapies in PDAC.

Immune checkpoint immunotherapies have produced unprecedented clinical benefits in a variety of different cancers, including lung cancer, which was previously thought to be non-immune responsive (11). However clinical trials using single agent checkpoint immunotherapy in PDAC have been unsuccessful thus far. This may be explained by increasing evidence which suggests that PDAC creates a potently immunosuppressive microenvironment via activation of multiple regulatory mechanisms (12,13), whereby interactions between the tumor, stroma, and immune cells in the pancreatic tumor microenvironment (TME) result in cancer progression (Figure 1). In this review, we discuss potential approaches to increasing immunogenicity, or immune responsiveness, to PDAC. Specifically, we will (1) examine the challenges in developing successful immunotherapies for PDAC, (2) describe the complex immune components of the TME and discuss how the immune system, pancreatic tumor cells, microbiome, and stromal signals suppress immune-mediated attack, and (3) discuss novel multi-agent therapeutic strategies to target signals within this integrated immunosuppressive network that are under development in clinical trials. Current standard of care therapy and clinical trials in progress are also reviewed by Manji et al. in this CCR Focus issue (14).

Clinical Challenges in Developing Immunotherapies for PDAC

There is mounting evidence that immune mediated inflammation is an integral component of the environment that supports PDAC development and progression (15). Genomic analyses show that PDAC frequently upregulates multiple pathways involved in acquired immune suppression, and upregulation of these pathways is associated with poor survival (16). This may explain why early human clinical studies involving immunotherapy monotherapy in PDAC have been discouraging. While treatment with single agent immune checkpoint inhibitors targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) cause meaningful objective responses in many tumor types (11,17–20), only 1 of 27 patients with PDAC responded to the CTLA-4 inhibitor ipilimumab (21), and 0 of 14 patients with PDAC had an objective response to anti-PD-L1 therapy (22). Recently completed and planned immunotherapy clinical trials for patients with PDAC have been reviewed in detail elsewhere (23–27). Although single-agent immunotherapies have failed to show benefit in PDAC, increasing data support the testing of combinatorial approaches that target multiple suppressive mechanisms. In addition to examining genetic mutations in PDAC tumor samples, which is reviewed by Dreyer and colleagues in this CCR Focus Issue (28), performing RNA sequencing to determine which immune escape mechanisms are *upregulated* (e.g. PD-1, IDO) may allow us to further personalize therapy for patients by combining immunotherapy agents with chemotherapy to reset the immune system (29). This may be critical specifically in patients with PDAC as the failure of single agent checkpoint therapy indicates that the PDAC tumor microenvironment is more complicated and suppressive than in other more immunogenic cancers. This would also have the advantage of being able to determine a tumor’s immunogenicity upfront before initiating treatment. As we better understand the role of the multiple immunologic contributors to

PDAC growth, it should be possible to design multi-agent immunotherapies that target multiple pathways, leading to increased antitumor immunity.

The multiple immunosuppressive components of the PDAC TME collectively suppress effector T cells (cells that recognize and kill tumor cells), preventing immune mediated destruction (Figure 1). Accumulation of effector $CD4^+$ and $CD8^+$ T cells in human PDAC are associated with improved overall survival (30–32). As pancreatic lesions progress, tumor infiltrating $CD8^+$ effector T cells decrease while suppressive regulatory T cells (Tregs) comprise a higher percentage of the $CD4^+$ T cell compartment (33), leading to a low number of tumor infiltrating effector lymphocytes (TILs) and a high number of immunosuppressive cells (13). Thus PDAC is considered to be a poorly immune responsive cancer. By contrast, highly immune responsive solid tumors are characterized by a high number of TILs at baseline and a high response rate to immune checkpoint inhibitors (34). Although PDAC is poorly immunogenic, that is likely due to having a more complex and suppressive tumor microenvironment, not because the immune system does not recognize the tumor. Discovery of the complex immune pathways involved in PDAC progression and immune escape (summarized in Figure 1) has led to additional novel PDAC immunotherapy targets (Table 1). Increasing data suggest that poorly immune responsive cancers like PDAC require multiagent therapy to elicit an immune response. One multipronged approach involves vaccines, which stimulate accumulation of lymphoid aggregates in PDAC (35) (Figure 2). One likely reason why vaccines have not stimulated effective antitumor responses, despite inducing lymphoid infiltration, is that vaccines also upregulate T cell inhibitory pathways such as the PD-1/PD-L1 pathway (36). Although vaccine therapy has thus far been unsuccessful, we believe that these lymphoid infiltrates represent increased immunogenicity, and speculatively, patients with vaccine-induced infiltration of lymphoid aggregates may benefit from a combination approach involving vaccine plus costimulatory blockade. Also, upregulation of immune checkpoint pathways after vaccine therapy may be a biomarker of increased immunogenicity and suggest these patients may also respond to checkpoint blockade. It is also possible that vaccines upregulate multiple immune escape mechanisms, and elucidation of these would be necessary to ensure vaccine efficacy. As chemotherapy transiently depletes suppressive Tregs in PDAC patients (37–39), chemotherapy should be considered in addition to administration of an immunomodulatory agent to attempt to overcome the potent immunosuppressive TME.

The TME’s Role in PDAC Development and Progression

Immune Checkpoints andImmune Checkpoint Inhibitors

There are many immune signaling pathways that regulate antitumor immunity, which involve costimulatory and inhibitory receptors (immune checkpoints) on T cells. Most studies of immunomodulatory agents in PDAC have examined the role of the inhibitory costimulatory receptors CTLA-4 and PD-1. Both receptors are critical in activation and suppressive activity of Tregs (40), and exist primarily to prevent autoimmunity and excessive immune responses to infection (41). However, tumors also induce Treg activation and suppression via these pathways, leading to dampened antitumor immune responses (40). This concept is critical, because it suggests that the immune system is not ignorant of PDAC;

rather, the immune system detects PDAC, but is instructed by the tumor not to attack it (42). Thus inhibition of immunosuppression, rather than immune activation alone, is critical to achieve durable clinical responses. Consistent with this, CTLA-4 and PD-pathway expression are upregulated in PDAC (43–45), and both are associated with worse survival (44,46). Furthermore, PD-1 is expressed on multiple PDAC infiltrating T cell subsets, including Tregs, and $CD4^+$ and $CD8^+$ effector T cells (37). Additionally, PDAC-infiltrating γδ T cells were recently identified, which represent a subset of suppressive T cells that express PD-L1 and suppress effector T cell activation (47). Collectively these studies indicate that immune checkpoint inhibition may be a target for PDAC related immunotherapy.

A number of principles have emerged that characterize immune checkpoint pathways. First, these pathways develop in response to the genetic changes that occur within developing tumors and are shaped by the evolving inflammatory response to these genetic changes. Second, there are many inhibitory and activating signaling pathways (48,49), but much still needs to be learned about their role in different cancer types. While melanoma, lung carcinoma, and renal cell carcinoma respond to blockade of one checkpoint pathway (i.e. PD-1/PD-L1 or CTLA-4) (11,17–20), most cancers will likely require combination therapy to fully activate T cell responses. Figure 1 depicts a non-exhaustive description ofthe broad range of suppressive mechanisms in PDAC, which account for single agent immunotherapy having limited clinical activity. Increasing preclinical evidence (see below) suggests that combining checkpoint inhibition with other targeted therapy may improve clinical efficacy. Third, additional studies are needed to understand primary (patients who do not respond) and secondary (patients who initially respond but then recur) resistance to these agents.

Although immune checkpoint inhibitors have thus far failed as single agents to demonstrate convincing clinical activity in PDAC, there may be subgroups of PDAC that are more likely to respond to these agents as monotherapy. Predictive biomarkers have now been used in multiple cancer types to identify patients who may be more likely to respond to immune checkpoint inhibitors. For example, expression of PD-L1 is used to identify patients who should receive frontline PD-1 inhibitor immunotherapy instead of chemotherapy in non-small cell lung cancer (NSCLC) (50). In gastrointestinal malignancies including PDAC, one emerging biomarker of response to immune checkpoint inhibitors is mismatch repair deficiency (MMR-d), which results in a failure to repair errors in base pair mismatches in tumor DNA (e.g. C-T instead of C-G), leading to microsatellite instability (MSI) (51). In unselected populations of colorectal cancer, little to no clinical activity was reported in the initial clinical trials of immune checkpoint inhibitors. However, the PD-1 inhibitor pembrolizumab demonstrated significant clinical activity in the small subset of colorectal cancers (≤5% of advanced disease, (52)) with MMR-d (53). This activity is likely due to the high baseline immunogenicity of the MMR-d cancer subtype, as evidenced by the increased lymphoid infiltration in MMR-d colorectal carcinomas at baseline, as well as the high expression of multiple immune checkpoints, including PD-L1 (54,55).

Mismatch repair status is not routinely checked in PDAC, and we are aware of only four reported cases of MMR-d pancreatic cancer treated with a PD-1 inhibitor. Of these four cases, one patient had a partial response to pembrolizumab, and the other three achieved

stable disease (56). Additional basket trials of single-agent PD-1 inhibition in MMR-d cancers (including PDAC) are ongoing. Although MMR-d PDAC is a small subset of all PDAC, (13–17.4% in prior studies (57–59)), these preliminary data suggest that single agent immune checkpoint inhibitors may have meaningful clinical activity in such cases. These studies also suggest that it is important to perform genetic sequencing studies on all patient tumors to better define each cancer's biology and to identify potential therapeutic options that may otherwise be missed.

Stroma

The dense stroma surrounding pancreatic cancers creates a hypovascular environment that can block the penetration of chemotherapeutics and facilitate immune escape. T cells were first demonstrated in the late 1990s to form aggregates in the fibrotic tissue of pancreatic cancer samples (60), leading to the current hypothesis that interactions between stroma, lymphocytes, and antigen presenting cells (APC) create a complex TME that makes overcoming immunosuppression difficult. Initial studies demonstrated that tumor incidence and metastasis increased when an increased proportion of pancreatic stellate cells were co-injected with PDAC cells, identifying the stroma as a potential target for therapeutic intervention (61). However, in preclinical models of PDAC, simple depletion of fibroblasts lead to increased regulatory T cell (Treg) accumulation and decreased survival, suggesting that the relationship between PDAC and stroma may be more complex than previously appreciated (62). This may explain why depletion of fibroblasts via inhibition of Hedgehog signaling, while leading to disease stabilization in some preclinical studies, ultimately failed in other preclinical models and clinical trials (63–65). This conflicting data are described in more detail elsewhere (66), and may reflect heterogeneity between fibroblasts (67) or different systems used.

However, targeting other factors that drive stromal fibrosis have elicited encouraging preclinical data in PDAC that may overcome the limitations of targeting fibroblasts alone and also facilitate effector T cell access and activation the TME. As one example, inhibition of focal adhesion kinase-1 (FAK1), a tyrosine kinase expressed on PDAC cells and stroma that drives stromal fibrosis, with the selective inhibitor VS-4718, can improve responses to chemotherapy and immunotherapy in a preclinical model of PDAC (68). Unfortunately, three previous clinical trials studying single agent FAK inhibition in patients with solid tumors, including PDAC, demonstrated no objective responses (69–71). However, several trials of combination FAK inhibition with gemcitabine and/or PD-1 blockade are now ongoing (NCT02758587, NCT02651727, NCT02546531). Additionally, targeting hyaluronic acid (HA) restored vascular patency in a preclinical model, and improved overall survival in patients with high HA content (72–74). Ongoing trials are examining PEGPH20 plus standard of care chemotherapy for PDAC (NCT02715804, NCT02487277, NCT01959139).

The stroma also produces factors, such as the proinflammatory cytokine IL-6, which are associated with poorer survival when expressed in peripheral blood of patients with PDAC (46,75). Unfortunately no objective responses were noted in any patients who received single agent IL-6 blockade in a phase I trial of patients with solid tumors, including nine

patients with PDAC (76). More recently, Lesinski and colleagues demonstrated that blockade of IL-6, which upregulates PD-L1 in viral models (77), synergized with PD-L1 inhibition to increase lymphocyte infiltration and improve $CD8^+$ T cell dependent antitumor immunity (78). This suggests that in addition to the stroma functioning as a physical barrier for immune infiltration, the stroma actively suppresses T cell infiltration via production of soluble factors, and blocking IL-6 may increase PDAC immunogenicity via upregulation of PD-L1. Speculatively, instead of complete stromal depletion, targeting the soluble factors produced may lead to improved outcomes. As IL-6 is known to promote chronic inflammation (79), targeting other mechanisms driving chronic inflammation, such as IL-17, may also be relevant (80,81). Overall the stroma is complex and requires further study to determine which components support and which suppress antitumor immune responses.

The Microbiome

Systemic factors also appear to impact the development and progression of PDAC, and several reviews have examined the relationship between the oral microbiome and PDAC (82,83). Multiple studies have found a possible relationship between tooth loss, self-reported periodontal disease, or clinically documented periodontitis (respectively) and PDAC or PDAC-associated mortality (84–86). This association between periodontitis and PDAC appears to remain even after controlling for multiple risk factors (87,88). Certain bacteria, such as *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*, are frequently linked with the development of periodontal disease (89), and RNA sequencing from pre-diagnostic oral washings have demonstrated that the presence of these two bacteria are also significantly associated with developing PDAC (90). In contrast, oral bacteria of the genus *Leptotrichia* has been associated with decreased PDAC risk. Notably, *P. gingivalis* and *Leptotrichia* levels collected more than 2 years prior to PDAC diagnosis retained their respective positive and negative associations with PDAC, suggesting that the altered oral microbiome may have been present prior to PDAC carcinogenesis (90). Another bacteria, *Fusobacterium*, was associated with decreased risk of acquiring PDAC when it was found in the oral cavity (90), but was associated with decreased survival when it was found in human PDAC tissues (91). *Fusobacterium* may therefore have differential effects pre- and post-diagnosis, or its carcinogenic effects may be dependent on its location.

Several explanations have been proposed to explain why certain oral microorganisms correlate with PDAC development. The altered oral microbiota may simply be a consequence of systemic inflammation, as patients with diabetes, a risk factor for PDAC (92), also has a significantly different oral microbiome than normal controls (93). Alternatively, it is biologically plausible that certain microbes may directly facilitate PDAC carcinogenesis. Consistent with this notion, the colonic bacterium Enterotoxigenic *Bacteroides fragilis* has been implicated in causing colon cancer via IL-17 production (94), and *Porphyromonas* has been implicated in carcinogenesis of oral squamous cell carcinoma in preclinical models (95). Although IL-17 has been implicated in facilitating PDAC and may emerge as a potential therapeutic target (80,81), further studies are needed to determine whether alterations in the oral microbiome play a role in the development of PDAC, which immune signals are involved,or if these findings are simply correlative. One potential study would be to examine patients with IL-17R overexpressing PDAC, which has been associated

with poorer prognosis (80) to see if the microbiome is altered in these patients versus non-IL-17R overexpressing patients, and then colonization of mice predisposed to obtain PDAC with the microbe in question to see if this accelerates PDAC. If the microbiome is conclusively shown to affect PDAC tumorigenesis or progression, prospective clinical studies of novel therapeutic agents that modify the microbiome as a treatment or prevention of PDAC will be warranted. Additionally, understanding the immune mechanisms through which the microbiome affect PDAC development and progression could inform the development of novel immunotherapies.

Vaccine Immunotherapy Strategies for PDAC Treatment

AsPDAC is a a poorly immunogenic cancer for which single agent vaccines have been ineffective, using a vaccine based approach will require at least one additional immunotherapeutic agent to optimally achieve an antitumor immune response (Figure 2). Optimal vaccine design will require knowledge of immune relevant antigens that are recognized by effector T cells that have the potential to be activated, and identification of vaccine approaches that effectively activate them. The second step is determining which immune escape mechanisms (such as checkpoint pathways) are induced by the vaccine itself. Thus, a baseline biopsy before vaccine therapy will not be the best indicator to determine which immune checkpoints require modulation.

Tumor antigens and antigen delivery systems for generating anti-PDAC T cells

A few PDAC tumor antigens capable of inducing an anti-tumor immune response have been identified. An ideal tumor antigen target should be highly expressed in PDAC cells and minimally expressed in normal tissue. Most PDAC antigens fall into one of two categories: 1) tumor-associated antigens (TAAs), which are found mostly on tumor but have limited expression on normal cells, and 2) tumor-specific antigens (TSAs), also called neoantigens, which are expressed exclusively on malignant cells and not expressed on normal cells (96). TAAs have received the most attention as targets for PDAC immunotherapy because of the potential to treat many patients with the same therapy. Epidermal growth factor receptor (HER/EGFR/ERBB) family proteins (97,98), and mesothelin (99–101) are examples of TAAs that are under clinical investigation as therapeutic targets in PDAC. However, because these antigens are also expressed on normal cells, off-target toxicity remains an important clinical concern (102,103). Due to their tumor-specific expression, TSAs are particularly appealing targets for PDAC immunotherapy. However, most TSAs arise from individual tumor mutations and are not shared between most patients. Therefore, while most (if not all) PDACs have TSAs (104), therapies targeting TSAs may need to be personalized.

A notable exception in PDAC is the driver oncogene KRAS, which is mutated at codon 12 in approximately 90% of PDACs and has been explored as a target for immunotherapy (105–108). KRAS is often described to be an ‘undruggable’ protein because despite several decades of intensive efforts, no pharmacologic inhibitors of KRAS have reached the clinic. However, mutated KRAS, like other tumor antigens, is presented on the cell surface of cells and thus is accessible to the immune system. Recently, the Rosenberg group provided proof of principle for KRAS immune targeting by successfully inducing a durable partial response

in a patient with KRAS-mutant colorectal cancer by infusing an enriched population of CD8+ T cells that reacted to the specific KRAS mutation expressed by the colorectal cancer (109). Although additional studies are still needed to determine which type of antigen induce the T cells best equipped to eradicate PDAC, increasing data suggests that immune suppressive mechanisms may be more complex and harder to bypass in the case of TAAs and mutated driver gene antigens such as mutated Kras because of the extensive length of time that they are expressed within the TME, which suggest these antigens have undergone immunoediting and subsequent immune escape (96).

Many different platforms are available for inducing TAA and TSA specific T cells, including various vaccine and adoptive T cell strategies. Notable antigen targets in PDAC and the therapies targeting these antigens are reviewed in Table 2. A number of vaccine delivery systems under development include plasmid DNA, polypeptide, and modified viral and bacterial approaches. In addition, new adjuvants under clinical development activate specific innate immune responses, via Toll Like Receptors and STING pathways (110,111). Chimeric antigen receptor (CAR) T cells, which are genetically engineered to express an antigen receptor specific for a malignancy-related target, are a platform for targeted immunotherapy that has shown promise in treating hematologic malignancies (112–114) and is now under clinical investigation in PDAC. Recently CAR T cells have been developed that target MUC1, a cell membrane protein that is overexpressed in PDAC and other cancers (115,116). In preclinical studies, mice harboring pancreatic cancer xenografts had increased OS when they received MUC1-specific CAR T cell therapy (116). CAR T cells targeting MUC1 are currently in clinical trials for solid tumors, including metastatic PDAC (NCT02587689). CAR T cells targeting mesothelin, a glycoprotein overexpressed in PDAC (100), are also being explored in human clinical trials for PDAC (NCT01583686) (117). However, no objective radiographic responses were reported in the initial PDAC clinical trial results for this agent (118). Although additional single-agent studies of these novel targeted immunotherapies are ongoing, it is likely that these targeted approaches will need to be combined with other therapies to overcome the immunosuppressive signals within the TME.

While most therapeutic cancer vaccines are categorized by their antigen target, whole cell vaccines deliver many tumor antigens without the need for specific knowledge of the relevant target. Autologous vaccines use the patient’s own tumor as an antigen source, whereas allogeneic vaccines are derived from another patient’s tumor. Allogeneic vaccines are more convenient and pragmatic because a single vaccine can be used to treat many patients, by presenting many relevant PDAC TAAs (119,120), whereas autologous vaccines must be personalized from each patient’s individual tumor. It is usually not feasible to utilize autologous tumor cells due to the lack of adequate tumor specimen.

The most studied whole cell vaccine platform in human PDAC trials is composed of 2 allogeneic granulocyte macrophage-colony stimulating factor (GM-CSF) secreting pancreatic tumor cell lines (GVAX). The PDAC GVAX has been combined with CRS-207, an attenuated *Listeria monocytogenes*-based vaccine targeting mesothelin. While the combination of GVAX plus CRS-207 showed encouraging results in early phase II studies (121), unfortunately an interim analysis of Phase 2b data failed to demonstrate improved OS compared with chemotherapy alone. A different whole-cell vaccine, algenpantucel-L, also

recently failed to produce a clinical benefit in a recent phase III study, despite promising phase II data (122). These mixed clinical results suggest that although whole cell vaccination monotherapy induces TAA specific T cells, it is likely not enough to overcome the potently immunosuppressive TME of PDAC (35,123–125).

Despite these recent clinical setbacks, whole cell vaccines may be an important component of combination strategies for PDAC immunotherapy. We and others have shown that GVAX and other vaccines may prime the TME for treatment with an immune checkpoint inhibitor by inducing high levels of PD-L1 expression on epithelial tumor cells and intratumoral lymphoid aggregates (35). The upregulation of immunosuppressive regulatory mechanisms by PDAC suggest that whole cell vaccines should be combined with other immune therapies to maximize anti-tumor efficacy. Combination therapy with GVAX and PD-1 blockade improves survival in tumor-bearing mice (36). This hypothesis that whole-cell vaccine therapy can convert an immunosuppressive tumor into a tumor responsive to immune checkpoint blockade is currently being tested with combination PD-1 inhibitor and GVAX in patients with surgically resectable and borderline resectable PDAC (NCT02451982, NCT02648282). Additionally, GVAX and CRS-207 are now in clinical development in combination with the PD-1 inhibitor nivolumab in a phase 2 trial (STELLAR, NCT02243371).

Treating PDAC via combination therapy

Other combination approaches are actively being tested in patients with PDAC. These approaches include combining immunomodulatory agents with each other or with chemotherapy. (Table 1). Gemcitabine-based chemotherapy is often used as the chemotherapy backbone in these combination immunotherapy trials because it has been shown to increase tumor antigen availability, and transiently deplete immunosuppressive Tregs and myeloid derived suppressor cells (MDSC) in the PDAC TME (37–39,126). Lower numbers of intratumoral Tregs are associated with increased disease free survival after pancreatectomy (30), suggesting that Treg accumulation is an important determinant of survival in patients with PDAC. We and others have demonstrated that low dose cyclophosphamide can also deplete Tregs, modulate the TME and maximize clinical responses to immunotherapy (123,127,128). Another approach is combination therapy with epigenetic modulators, as epigenetic therapy appears to be immunomodulatory (129), and epigenetic therapy in PDAC is reviewed by Evan and colleagues in this CCR focus issue (130). Immunotherapies in clinical development for PDAC in combination with standard chemotherapy include the indoleamine 2,3 dioxygenase (IDO) inhibitor indoximod, the bruton tyrosine kinase (BTK) inhibitor ibrutinib, CD-40 agonists, and CCR2 inhibitors (Table 1). IDO is a tryptophan-catabolizing enzyme that, when activated via tumors or another inflammatory stimulus, activates suppressive activity in dendritic cells (DC) and leads to Treg activation (40,131,132). In a phase II study of untreated metastatic PDAC, the combination of indoximod plus gemcitabine/nab-paclitaxel demonstrated a response rate of 45% (133). This appears favorable compared to the 23% historical response rate of patients treated with gemcitabine/nab-paclitaxel alone in phase III studies (10), but must be tempered with phase II data demonstrating a 48% overall response rate with this chemotherapy combination (134). Another suppressive cell involved in Treg generation is the regulatory B

cell (Breg), which has been implicated in converting resting $CD4^+$ T cells to Tregs in a breast cancer model (135), and promotes tumorigenesis in PDAC (136). While identifying a specific Breg inhibitor is an area of active study, targeting BTK, which is expressed by tumor infiltrating B cells and myeloid cells, with ibrutinib synergizes with gemcitabine to inhibit murine PDAC growth (137). Ibrutinib is currently in clinical trials in combination with gemcitabine and nab-paclitaxel in the first line setting for metastatic PDAC (NCT02562898, NCT02436668).

CD40 is a TNF receptor superfamily member that is expressed by many cells, including B cells, DCs, monocytes, endothelial cells, and fibroblasts (138). CD40 agonists have been shown to activate APCs and promote tumor regression (139), and synergize with gemcitabine in mice to increase intratumoral effector T cell infiltration and induce T cell dependent PDAC tumor regression (140). CD40 agonists (NCT02588443, NCT02829099) and CCR2 blockade (NCT02732938) are currently being tested in clinical trials in multiple settings (141,142).

The presence of tumor infiltrating macrophages (TIMs) are associated with poorer outcomes in patients with resected PDAC (143,144). CCR2 is a chemokine receptor involved in the recruitment of immunosuppressive macrophages; CCR2 inhibition depletes CCR2 expressing tumor infiltrating macrophages and improves survival in mouse models (145). CCR2 blockade (NCT02732938) is currently being tested in combination with gemcitabine / nab-paclitaxel in a phase Ib/II study (142).

Another receptor whose inhibition facilitates depletion of TIMs in preclinical models is the colony-stimulating factor-1 receptor (CSF1R), which synergized with gemcitabine to increase effector T cell infiltration and slow pancreatic tumor growth (146). CSF1R inhibition also increased expression of checkpoint molecules on PDAC tumor cells and T cells, and when combined with checkpoint blockade and gemcitabine, further slowed murine PDAC growth (147). Multiple human trials are examining whether targeting CSF1R synergizes with PD-pathway blockade in solid tumors, including PDAC (NCT02526017, NCT02777710).

The C-X-C chemokine receptor 4 (CXCR4) is a chemokine receptor whose expression in human pancreatic tissues is associated with a poorer prognosis (148–150). CXCR4 blockade abrogated invasion and metastasis (151–153), and transfection of CXCR4 into pancreatic tumor cells increased their metastatic potential (154). Gemcitabine upregulates CXCR4 expression in human pancreatic cancer cells (155), which may be a mechanism of acquired resistance to gemcitabine (155–157). Inhibiting CXCR4 synergized with anti-PD-L1 blockade to decrease tumor size in a mouse PDAC model (158). Based on this encouraging preclinical data, clinical trials are examining the combination of CXCR4 inhibition with PD-pathway blockade in advanced solid tumors, including PDAC (NCT02737072, NCT02472977, NCT02826486).

Due to the suppressive nature of the PDAC tumor microenvironment, it is likely that multiple suppressive cell types will need to be targeted in order to improve clinical outcomes. The Treg, antigen presenting cell, and speculatively, the Breg are the three cell

subtypes that appear to most potently suppress immune responses in PDAC. Chemotherapy should be the backbone of most trials in metastatic PDAC due to its immunomodulatory effect and already proven (although modest) survival benefit. Targeting Treg suppression via the PD-pathway is reasonable if done with chemotherapy (to transiently eliminate already established Tregs to “reset” the immune system) and in combination with at least one other immunomodulatory agent that affects another immune cell type, preferably either suppressive APCs or Bregs. Targeting the IDO pathway is attractive due to its induction of tolerogenic DCs and Treg activation and the encouraging phase II results in PDAC. Synergy with IDO inhibition and PD-pathway inhibitors or chemotherapy in early studies with other tumor types suggests that combination therapy with IDO inhibitor, PD-pathway, and chemotherapy may be efficacious if not overly toxic (159,160). Similarly, promising data in early studies with combination macrophage targeting (via CCR2 inhibition) and FOLFIRINOX in patients with borderline resectable or locally advanced PDAC make CCR2 an appealing target (142).

Future Directions

The failure of single agent immunotherapy in PDAC (21,22) at first glance suggests that immunotherapy may not have a role in future management of PDAC. However, the documented involvement of an integrated suppressive network of immune cells and stroma in PDAC development and progression suggest that a combination approach involving chemotherapy, immunotherapy, targeted therapy against stromal elements, and other modalities will be necessary in order to improve survival. Combination therapy, including strategies to boost adaptive immunity, break systemic tolerance, and increase tumor immunogenicity, has the potential to revolutionize PDAC treatment. Increasing our understanding of the PDAC TME, and how therapies affect the suppressive milieu, will help identify the best potential targets for therapeutic development and testing in clinical trials.

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Abbreviations

PDAC	pancreatic ductal adenocarcinoma
PanIN	pancreatic intraepithelial neoplasia
Treg	regulatory T cell

PD-1	programmed death-1
PD-L1	programmed death ligand 1
TIM	tumor infiltrating macrophage
MDSC	myeloid derived suppressor cell
BTK	Bruton's tyrosine kinase
γδ T cells	gamma delta T cells
CTLA-4	cytotoxic T lymphocyte associated protein 4
CSF1R	colony stimulating factor 1 receptor
GM-CSF	granulocyte macrophage colony stimulating factor
DC	dendritic cell
FAK1	focal adhesion kinase 1
TAA	tumor associated antigen
TSA	tumor specific antigen
MMR	mismatch repair
GVAX	granulocyte macrophage colony stimulating factor secreting pancreatic tumor cell vaccine
CAR T cells	chimeric antigen receptor T cells

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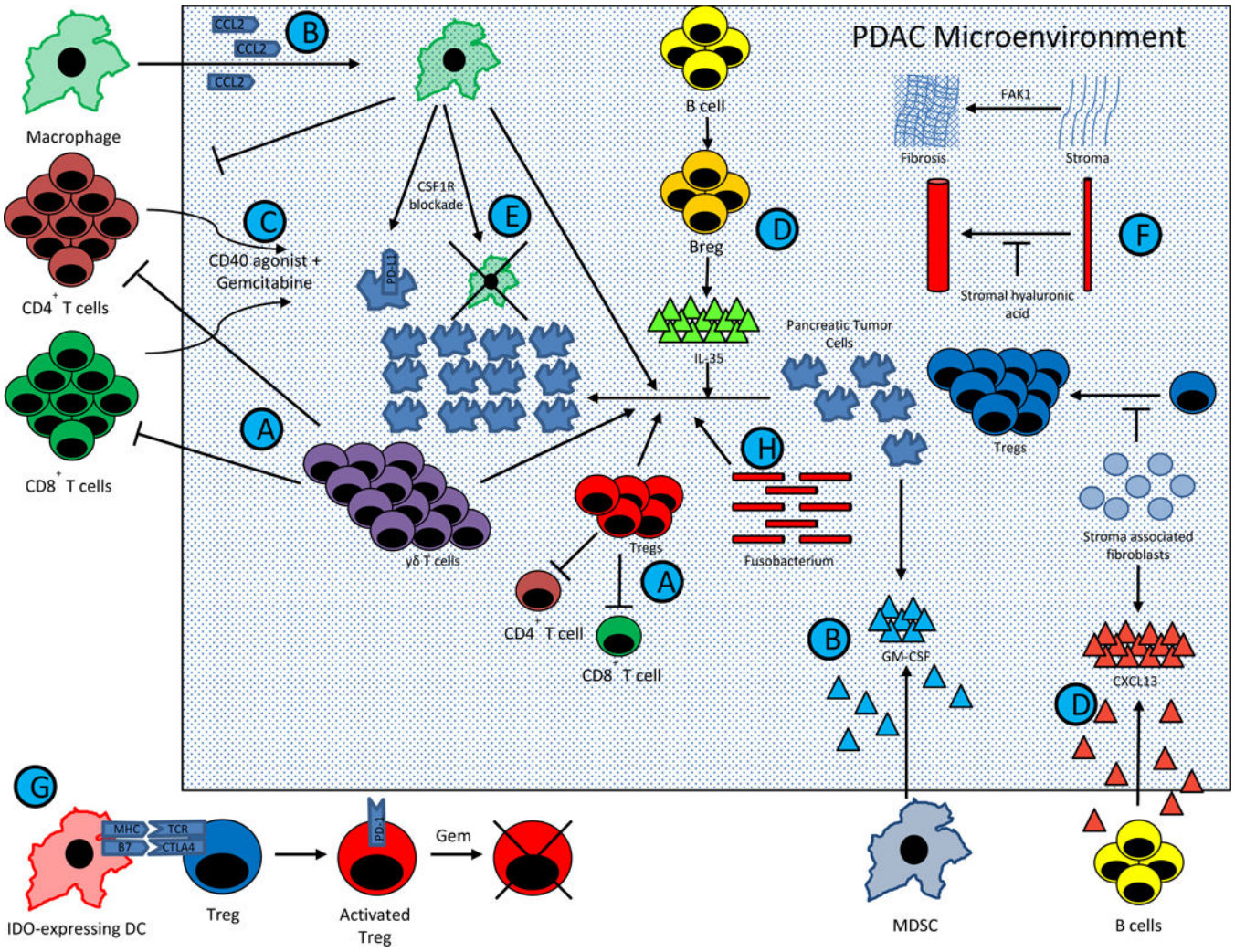

Figure 1. Mechanisms within the PDAC TME drives resistance to therapies

PDAC comprises of complex interactions between T cells, B cells, APCs, pancreatic tumor cells, and stromal elements. These interactions result in a profoundly immunosuppressive tumor microenvironment, and consequently single agent immunotherapy has been largely ineffective. However, emerging preclinical data has suggested that combination therapy may dramatically affect overall survival. Current trial design is being driven largely by this data. The figure summarizes major pathways in PDAC tumorigenesis that are being manipulated in clinical trials for patients with metastatic PDAC. Except for (G.), which represents in part IDO activated Tregs in TDLNs from a melanoma model (40), this figure represents data known exclusively from PDAC models.

(A.) Tregs and γδ T cells block Teff division and drive PDAC growth, while γδ T cells block T cell infiltration (47).

(B.) MDSCs and macrophages are mobilized into the TME by PDAC derived GM-CSF and CCL2, respectively. (145,183,184).

(C.) Macrophages block $CD4^+$ T cell entry into the PDAC microenvironment. CD40 is expressed on these $CD4^+$ T cells, and activation of the CD40 pathway concurrently with gemcitabine can drive T cell infiltration (140).

(D.) Stromal associated fibroblasts produce CXCL13, which recruits regulatory B cells into the TME. These regulatory B cells produce IL-35, which drives PDAC progression (136,185). These Bregs may be inhibited by BTK inhibitors, such as ibrutinib (137).
(E.) Tumor infiltrating macrophages stimulate PDAC progression. Blockade of the CSF1 receptor expressed by macrophages can lead to macrophage depletion, CTLA-4 upregulation on $CD8^+$ T cells, and PD-L1 upregulation on pancreatic tumor cells (146,147).
(F.) Stromal elements create a physical barrier to immune infiltration and therapeutic agents. Stromal fibroblasts block Treg accumulation and PDAC progression (62), but targeting other stromal elements have achieved encouraging results. Stromal hyaluronic acid deposition results in decreased vascular patency (72,73), and FAK1 drives stromal fibrosis (68). Inhibition of either target has led to decreased PDAC progression when combined with chemotherapy in preclinical models.
(G.) IDO induction in DCs by tumors activate Tregs via MHC and CTLA4 pathways (40,131). In phase II studies, gemcitabine based therapy synergizes with IDO inhibition to improve response rates in PDAC (133), possibly via transient depletion of Tregs (39). This provides an immune system reset, allowing for chemotherapy-mediated elimination of previously activated Tregs, followed by indoximod mediated inhibition of subsequent Treg activation.
(H.) Recent evidence suggests the *Fusobacterium* found within the PDAC microenvironment drives PDAC progression, but the mechanism of this is unknown (91).

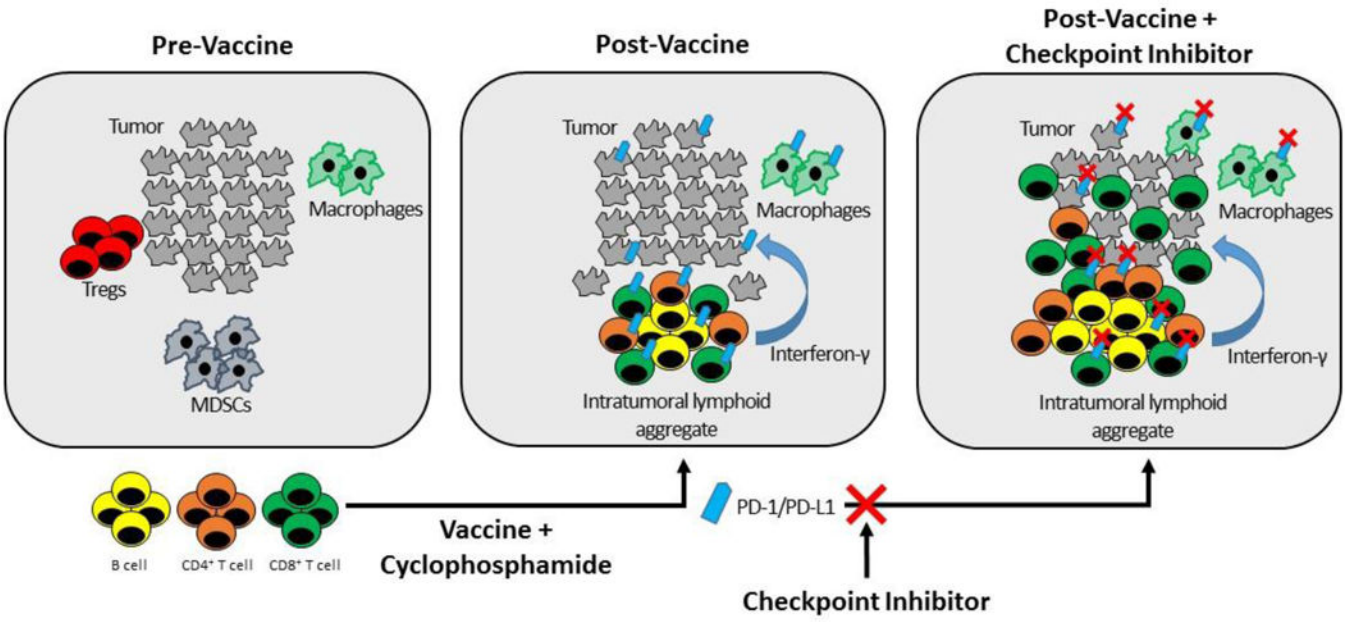

Figure 2. Therapeutic vaccine immunotherapy for PDAC requires multiple steps to overcome immunosuppression

PDAC and other poorly immune responsive cancers are characterized by low numbers of tumor infiltrating lymphocytes (TILs), low levels of PD-L1 expression, and high numbers of immunosuppressive cells such as Tregs and MDSCs at baseline (left panel) (13). Using a vaccine approach will require at least two immunotherapeutics to achieve an immune response. In Step 1 (center panel), a therapeutic vaccine is used to induce accumulation of lymphoid aggregates (35). These lymphocytes secrete interferon gamma and other soluble factors that induce high levels of PD-L1/PD-1 expression on epithelial tumor cells and on immune cells (186). Vaccines can also be combined with other therapies such as cyclophosphamide, to deplete immunosuppressive cells in the TME (29). In Step 2 (right panel), the addition of a PD-pathway inhibitor to a vaccine-primed tumor inhibits PD-L1/PD-1 signaling to increase lymphocyte proliferation and activation and promote tumor eradication (36). The hypothesis that vaccine therapy can synergize with immune checkpoint inhibition is currently under clinical investigation in multiple trials in PDAC.

Table 1

A list of notable immunotherapies in clinical development for PDAC.

Therapeutic target and agents under investigation for PDAC	Preclinical rationale	Clinical evidence and ongoing trials
PD-1/PD-L1 nivolumab pembrolizumab durvalumab	PD-1/PD-L1 inhibition has activity in a wide number of tumors. PD-L1 expression is upregulated in a subset of PDAC, and is associated with shortened survival (161,162).	Responses were observed in a subset of patients with MMR-deficient pancreatic cancer (≤ 5% of PDAC) (56), and additional trials in MMR-d disease are ongoing NCT01876511, NCT02465060). None of 14 pancreatic patients responded in a study of single-agent nivolumab (22). Multiple combination immunotherapy trials are ongoing (NCT02558894, NCT02268825, NCT02472977, NCT02243371, NCT02777710).
CTLA-4 ipilimumab tremelimumab	Anti-CTLA-4 therapy may reduce intratumoral Tregs and shift the threshold needed for T cell activation. A trial of ipilimumab failed to show convincing clinical activity, but a possible delayed response was observed in one patient (21).	Multiple combination trials are ongoing, including combinations with PD-1 inhibition and/or therapeutic vaccines (NCT02558894, NCT01896869).
IDO1 indoximod	IDO1 mediates tumor immunosuppression in preclinical models (non-PDAC), and PDAC frequently overexpresses IDO as a mechanism of immune escape (132,163,164).	Evidence of clinical activity was observed in combination with chemotherapy (133). A clinical trial is ongoing in combination with gemcitabine-based chemotherapy (NCT02077881).
BTK Ibrutinib	BTK is involved with B cell receptor signaling and is also expressed by macrophages. In preclinical models ibrutinib synergizes with gemcitabine to increase antitumor immunity (137).	Clinical trials are ongoing in combination with gemcitabine-based chemotherapy in PDAC (NCT02562898, NCT02436668)
CD-40 RO7009789 (CP-870,893) JNJ-64457107	CD40 is expressed on B cells, DCs, and other cell types. CD40 agonists inhibit PDAC stroma, increase CCL2 levels and interferon gamma (IFN-g) in the TME, and synergize with chemotherapy (145,165).	Evidence of clinical activity was observed in an early stage clinical trial in PDAC (141). Additional trials of monotherapy or combination with gemcitabine-based chemotherapy are ongoing (NCT02588443, NCT02829099).
CCR2 CCX872 PF-04136309	CCR2 recruits suppressive macrophages to the immunosuppressive TME in PDAC, and CCR2 inhibition depletes tumor infiltrating macrophages and improves survival in a preclinical model (145).	CCR2 inhibition has shown safety and possible evidence of clinical activity in combination with chemotherapy. Clinical trials in combination with chemotherapy in PDAC are ongoing (NCT02345408, NCT02732938)
CSF-1R Cabiralizumab (FPA008) Pexidartinib (PLX3397) BLZ945 AMG 820	CSF1R inhibition reprograms tumor-associated macrophages and upregulates immune checkpoints. Synergistic activity has been observed with immune checkpoint inhibitors in preclinical models of PDAC (146,147).	Multiple agents are in clinical trials in metastatic PDAC in combination with PD-1 inhibitors (NCT02526017, NCT02777710, NCT02829723, NCT02713529)
CXCR4 LY2510924	CXCR4 blockade abrogated metastasis in prelclinical models (151), and synergized with PD-L1 therapy to increase antitumor immunity (158)	CXCR4 inhibitor is in clinical trial in combination with PD-L1 blockade to treat advanced solid tumors, including PDAC (NCT27037072).

Table 2

A non-exhaustive list of antigen targets for pancreatic cancer immunotherapies, and notable therapies targeting these antigens.

Target	Expression	Notable immunotherapies against antigen target
Mesothelin	Highly overexpressed in virtually all pancreatic cancers and also expressed at lower levels in pleura, peritoneum, and pericardium (100).	CRS-207 (Aduro Biotech); live-attenuated listeria monocytogenes engineered to secrete mesothelin (99,121) Amatuximab (MORAb-009, Morphotek); monoclonal antibody (166) DMOT4039A (Genentech); antibody-drug conjugate (167) Anetumab ravtansine, (BAY 94-9343, Bayer); antibody-drug conjugate (168) Anti-mesothelin CAR-T cells (UPENN, NCI) (118)
CEA	Glycosylated homotypic/heterotypic cell surface intracellular adhesion molecule, overexpressed in 56–98% of pancreatic cancers and also expressed on oncofetal tissues (169).	CEA peptide vaccine (CAP1-6D) emulsified in montanide and GM-CSF (170) TRICOM-CEA(6D); poxvirus-based vaccine expressing costimulatory molecules and CEA (171,172) AVX701 (Alphavax); poxvirus-based vaccine expressing costimulatory molecules and CEA (171,173) GI-6207 (GlobeImmune/Celgene); recombinant yeast-CEA vaccine (174)
MUC1	Transmembrane glycoprotein, overexpressed in ~90% of pancreatic tumors. Also low levels of expression on ductal and glandular epithelial cells. However, cancer-associated MUC1 is structurally different from normal MUC1 (hypoglycosylated) and may function as a tumor-specific antigen (175).	MUC1-peptide pulsed dendritic cells (176) Autologous dendritic cell vaccine (177) MUC1-peptide vaccine with SB-AS2 adjuvant (178) Adoptive transfer with MUC1 peptide-pulsed dendritic cells and activated T lymphocytes (179)
HER/EGFR/ ERBB family proteins (eg HER1, HER2, HER3)	Cell-surface receptors implicated in tumor growth. HER2/neu is overexpressed in approximately 50% and EGFR in approximately 70% of pancreatic cancers and expression correlates with poor survival (97,98). These proteins are also expressed at lower levels in normal tissues.	Cetuximab (Erbitux, Lilly); EGFR antibody previously failed in clinical trials alone or in combination with cytotoxic agents, but may induce innate and adaptive immune responses that could synergize with novel immunotherapies (180,181). MM-141 (Merrimack Pharmaceuticals); bispecific antibody targeting IGF-1R and ErbB3 (HER3) (182)
Mutated KRAS	Intracellular GTPase important for cell growth and survival, mutated in up to 90% of pancreatic cancers (16,106). Mutated KRAS is a tumor-specific antigen.	GI-4000 (GlobeImmune); attenuated yeast expressing mutated RAS proteins (107) TG01 (Targovax); mutated RAS peptide vaccine co-administered with GM-CSF as an adjuvant (108)