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 ductal 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 druggable, and data supporting combination drug approaches that modulate multiple PDAC signals, which should lead to improved clinical outcomes. *Clin Cancer Res; 23(7); 1656–69. ©2017 AACR.*

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Introduction

Current estimates predict pancreatic ductal adenocarcinoma (PDAC) to overtake breast cancer and become the third most common cause of cancer-related death in the United States (1, 2). Only 20% to 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 compared with 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 nonimmune responsive (11). However, clinical trials using single-agent checkpoint immunotherapy in PDAC have been unsuccessful thus far. This may be explained by increasing evidence that 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 (Fig. 1). In this review, we discuss potential approaches to increasing immunogenicity, or immune responsiveness, to PDAC. Specifically, we (i) examine the challenges in developing successful immunotherapies for PDAC; (ii) 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 (iii) discuss novel multiagent 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 and colleagues in this *CCR Focus* (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. Although treatment with single-agent immune checkpoint inhibitors targeting cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) causes 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–programmed death-ligand 1 (PD-L1) therapy (22). Recently completed and planned immunotherapy clinical

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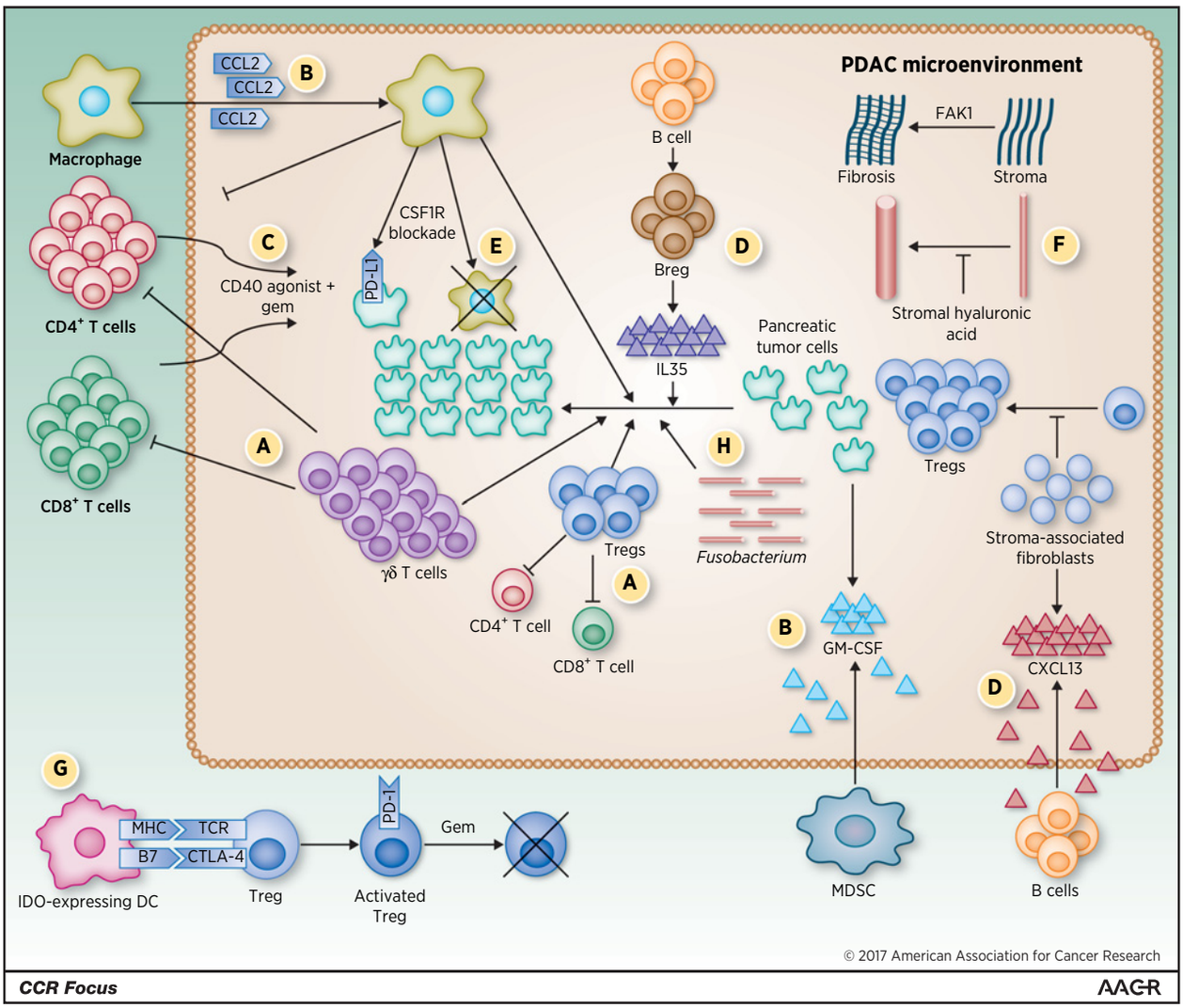

Figure 1.

Mechanisms within the PDAC TME drive resistance to therapies. PDAC comprises complex interactions between T cells, B cells, antigen-presenting cells (APC), pancreatic tumor cells, and stromal elements. These interactions result in a profoundly immunosuppressive TME, and, consequently, single-agent immunotherapy has been largely ineffective. However, emerging preclinical data have suggested that combination therapy may dramatically affect OS. Current trial design is being driven largely by these data. This 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 indoleamine 2,3 dioxygenase (IDO)-activated regulatory T cells (Treg) in tumor-draining lymph nodes (TDLN) from a melanoma model (40), this figure represents data known exclusively from PDAC models. **A**, Tregs and γδ T cells block effector T cell (Teff) division and drive PDAC growth, whereas γδ T cells block T-cell infiltration (47). **B**, Myeloid-derived suppressor cells (MDSC) and macrophages are mobilized into the TME by PDAC-derived granulocyte macrophage-colony stimulating factor (GM-CSF) and chemokine (C-C motif) ligand 2 (CCL2), respectively (145, 182, 183). **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 chemokine (C-X-C motif) ligand 13 (CXCL13), which recruits regulatory B cells (Breg) into the TME. These Bregs produce IL35, which drives PDAC progression (136, 184). These Bregs may be inhibited by Bruton tyrosine kinase (BTK) inhibitors, such as ibrutinib (137). **E**, Tumor-infiltrating macrophages stimulate PDAC progression. Blockade of the colony-stimulating factor-1 receptor (CSF1R) expressed by macrophages can lead to macrophage depletion, cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) upregulation on $CD8^+$ T cells, and programmed death-ligand 1 (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 has achieved encouraging results. Stromal hyaluronic acid deposition results in decreased vascular patency (72, 73), and focal adhesion kinase-1 (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 dendritic cells (DC) by tumors activates Tregs via major histocompatibility complex (MHC) and CTLA-4 pathways (40, 131). In phase II studies, gemcitabine-based therapy synergizes with IDO inhibition (via indoximod) 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). Gem, gemcitabine; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TCR, T-cell receptor.

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* (28), performing RNA sequencing to determine which immune escape mechanisms are upregulated [e.g., PD-1 and indoleamine 2,3 dioxygenase (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 TME 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 multiagent 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 (Fig. 1). Accumulation of effector $CD4^+$ and $CD8^+$ T cells in human PDAC are associated with improved OS (30–32). As pancreatic lesions progress, tumor-infiltrating $CD8^+$ effector T cells decrease while suppressive regulatory T cells (Treg) comprise a higher percentage of the $CD4^+$ T-cell compartment (33), leading to a low number of tumor-infiltrating lymphocytes (TIL) 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 TME, 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 Fig. 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 (ref. 35; Fig. 2). One likely reason why vaccines have not

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 (43, 161).	Responses were observed in a subset of patients with MMR-deficient pancreatic cancer (56), and additional trials in MMR-deficient disease are ongoing (NCT01876511 and 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, and 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 and NCT01896869).
IDO1 Indoximod	IDO1 mediates tumor immunosuppression in preclinical models (non-PDAC), and PDAC frequently overexpresses IDO as a mechanism of immune escape (132, 162, 163).	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 and NCT02436668).
CD40 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-γ) in the TME, and synergize with chemotherapy (145, 164).	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 and NCT02829099).
CCR2 CCX872 PF04136309	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 and NCT02732938).
CSF1R 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, and NCT02713529).
CXCR4 LY2510924	CXCR4 blockade abrogated metastasis in preclinical 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).

Abbreviations: BTK, Bruton tyrosine kinase; CCL2, chemokine (C-C motif) ligand 2; CCR2, C-C chemokine receptor type 2; CSF1R, colony-stimulating factor-1 receptor; CXCR4, C-X-C chemokine receptor type 4; DC, dendritic cell; MMR, mismatch repair.

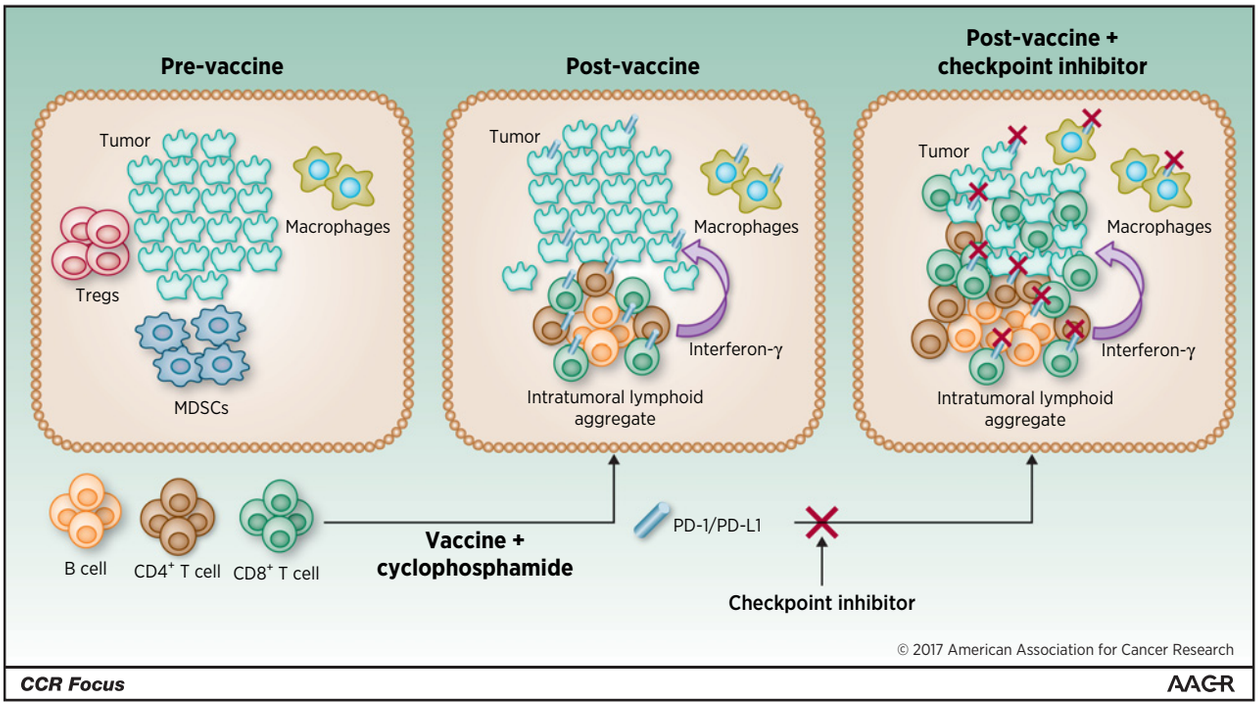

Figure 2.

Therapeutic vaccine immunotherapy for PDAC requires multiple steps to overcome immunosuppression. PDAC and other poorly immune-responsive cancers are characterized by low numbers of TILs, low levels of PD-L1 expression, and high numbers of immunosuppressive cells, such as Tregs and myeloid-derived suppressor cells (MDSC), at baseline (left; ref. 13). Using a vaccine approach will require at least two immunotherapeutics to achieve an immune response. In step 1 (center), a therapeutic vaccine is used to induce accumulation of lymphoid aggregates (35). These lymphocytes secrete interferon-γ and other soluble factors that induce high levels of PD-L1/PD-1 expression on epithelial tumor cells and on immune cells (185). Vaccines can also be combined with other therapies, such as cyclophosphamide, to deplete immunosuppressive cells in the TME (29). In step 2 (right), 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.

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, that 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 suggests that 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 and immune 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. Although melanoma, lung carcinoma, and renal cell carcinoma respond to blockade of one checkpoint pathway (i.e., PD-1/PD-L1 or CTLA-4; refs. 11, 17–20), most cancers will likely require combination therapy to fully activate T-cell responses. Figure 1 depicts a nonexhaustive description of the 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; ref. 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; ref. 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 ($\leq$5% of advanced disease; ref. 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; refs. 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 coinjected with PDAC cells, identifying the stroma as a potential target for therapeutic intervention (61). However, in preclinical models of PDAC, simple depletion of fibroblasts led to increased 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, although leading to disease stabilization in some preclinical studies, ultimately failed in other preclinical models and clinical trials (63–65). These 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 TME activation. 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 improves 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, and NCT02546531). Additionally, targeting hyaluronic acid (HA) restored vascular patency in a preclinical model, and improved OS in patients with high HA content (72–74). Ongoing trials are examining HA depletion with pegylated recombinant human hyaluronidase (PEGPH20) plus standard-of-care chemotherapy for PDAC (NCT02715804, NCT02487277, and NCT01959139).

The stroma also produces factors, such as the proinflammatory cytokine IL6, 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 IL6 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 IL6, 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 IL6 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 IL6 is known to promote chronic inflammation (79), targeting other mechanisms driving chronic inflammation, such as IL17, 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 affect 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 to the development of periodontal disease (89), and RNA sequencing from prediagnostic oral washings have demonstrated that the presence of these two bacteria is also significantly associated with developing PDAC (90). In contrast, oral bacteria of the genus *Leptotrichia* have been associated with decreased PDAC risk. Notably, *Porphyromonas 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 bacterium, *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 have 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 IL17 production (94), and *Porphyromonas* has been implicated in carcinogenesis of oral squamous cell carcinoma in preclinical models (95). Although IL17 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 IL17R-overexpressing PDAC, which has been associated with poorer prognosis (80), to see if the microbiome is altered in these patients versus non–IL17R-overexpressing patients, and then colonize 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 affects PDAC development and progression could inform the development of novel immunotherapies.

Vaccine Immunotherapy Strategies for PDAC Treatment

As PDAC is 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 (Fig. 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 antitumor 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: (i) tumor-associated antigens (TAA), which are found mostly on tumor but have limited expression on normal cells, and (ii) tumor-specific antigens (TSA), 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, similar to 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 suggest 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

Table 2. A nonexhaustive 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 (165) DMOT4039A (Genentech); antibody–drug conjugate (166) Anetumab ravtansine (BAY 94-9343, Bayer); antibody–drug conjugate (167) Anti-mesothelin CAR T cells (University of Pennsylvania, National Cancer Institute; ref. 118)
CEA	Glycosylated homotypic/heterotypic cell-surface intracellular adhesion molecule, overexpressed in 56%–98% of pancreatic cancers and also expressed on oncofetal tissues (168).	CEA peptide vaccine (CAP1-6D) emulsified in montanide and GM-CSF (169) TRICOM-CEA(6D); poxvirus-based vaccine expressing costimulatory molecules and CEA (170, 171) AVX701 (AlphaVax); poxvirus-based vaccine expressing costimulatory molecules and CEA (170, 172) GI-6207 (GlobeImmune/Celgene); recombinant yeast-CEA vaccine (173)
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 TSA (174).	MUC1-peptide–pulsed dendritic cells (175) Autologous dendritic cell vaccine (176) MUC1-peptide vaccine with SB-AS2 adjuvant (177) Adoptive transfer with MUC1 peptide–pulsed DCs and activated T lymphocytes (178)
HER/EGFR/ERBB family proteins (e.g., 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 (179, 180) MM-141 (Merrimack Pharmaceuticals); bispecific antibody targeting IGF-1R and ErbB3 (HER3; ref. 181)
Mutated KRAS	Intracellular GTPase important for cell growth and survival, mutated in up to 90% of pancreatic cancers (16, 106). Mutated KRAS is a TSA.	GI-4000 (GlobeImmune); attenuated yeast-expressing mutated RAS proteins (107) TG01 (Targovax); mutated RAS peptide vaccine coadministered with GM-CSF as an adjuvant (108)

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; ref. 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.

Although 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 two 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. Although the combination of GVAX plus CRS-207 showed encouraging results in early phase II studies (121), unfortunately an interim analysis of phase IIb 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 suggests that whole-cell vaccines should be combined with other immune therapies to maximize antitumor 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 and NCT02648282). Additionally, GVAX and CRS-207 are now in clinical development in combination with the PD-1 inhibitor nivolumab in a phase II 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 one another 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* (130). Immunotherapies in clinical development for PDAC in combination with standard chemotherapy include the IDO inhibitor indoximod, the Bruton tyrosine kinase (BTK) inhibitor ibrutinib, CD40 agonists, and C-C chemokine receptor type 2 (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 with 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). Although 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 and 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 and NCT02829099) and CCR2 blockade (NCT02732938) are currently being tested in clinical trials in multiple settings (141, 142).

The presence of tumor-infiltrating macrophages (TIM) is 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 TIMs 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 and NCT02777710).

The C–X–C chemokine receptor type 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, and NCT02826486).

Due to the suppressive nature of the PDAC TME, it is likely that multiple suppressive cell types will need to be targeted in order to improve clinical outcomes. The Treg, the APC, 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 an 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 suggests 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.

Disclosure of Potential Conflicts of Interest

E. M. Jaffee reports receiving commercial research grants from Aduro Biotech and Bristol-Myers Squibb, is a consultant/advisory board member for Adaptive Biotech, and has the potential to receive royalties from GVAX as a result of a licensing agreement with Aduro Biotech and Johns Hopkins University. No potential conflicts of interest were disclosed by the other authors.

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