

Immunotherapy in pancreatic adenocarcinoma – overcoming barriers to response

Ari Rosenberg, Devalingam Mahalingam

Department of Medicine, Northwestern University, Chicago, IL, USA

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Correspondence to: Devalingam Mahalingam, MBBChBAO, Department of Medicine, Northwestern University, Chicago, IL, USA.

Email: Mahalingam@nm.org.

Abstract: Pancreatic adenocarcinoma (PAC) remains one of the leading causes of cancer-related death. Despite multiple advances in targeted and immune therapies, the 5-year survival in advanced PAC remains poor. In this review, we discuss some of the unique aspects of the tumor microenvironment (TME) in PAC that may contribute to its resistance to immune therapies, as well as opportunities to potentially overcome some of these inherent barriers. Furthermore, we discuss strategies to enable immune therapies in PAC such as cytotoxic chemotherapy and radiation therapy, cancer vaccines, cytokine based therapy, oncolytic viruses, and adoptive T-cell therapy. Finally, we address a variety of targeted therapies as a strategy to further amplify immune responses in PAC.

Keywords: Pancreatic cancer; immunotherapy; tumor microenvironment (TME); targeted therapies

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Introduction

Pancreatic adenocarcinoma (PAC) remains one of the most lethal of human malignancies. It is the fourth leading cause of cancer-related death in the United States (1). Unfortunately, the only curative option is surgical resection. The majority of patients are diagnosed with advanced stage disease, and as such, for the large majority of patients, curative surgery is not an option. Despite significant advances in improvements in cancer therapy, mortality for pancreatic cancer has remained relatively unchanged (2). The mainstay of treatment for patients with advanced PAC remains systemic combination chemotherapy (3). Patient outcomes remain poor, with 5-year survival of less than 10%. Development of new therapies for PAC is greatly needed.

Recently, immunotherapies that boost T-cells to destroy cancer cells have generated much excitement in cancer therapy. In particular, inhibition of programmed death 1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CTLA-4),

has demonstrated clinical benefit in a number of malignancies (4) such as melanoma (5,6), lung cancer (7), bladder cancer (8), kidney cancer (9), head and neck cancer (10), hepatocellular cancer (HCC) (11), as well as Hodgkin lymphoma (12). This has generated hope and enthusiasm for a potential effective therapy in pancreatic cancer, however results of early clinical studies utilizing single-agent immune checkpoint inhibition in PAC have been disappointing (13,14). PAC has a unique tumor microenvironment (TME) that promotes immune evasion, and has demonstrated remarkable resistance to immune therapies (15). In this review, we discuss some of the strategies of overcoming barriers to response to immune therapies in PAC, as well as ongoing strategies currently being evaluated in the clinical trial setting.

The TME in PAC

The TME in PAC, which consists of complex and

heterogeneous stroma, has been identified as a major contributor to resistance to systemic therapies (16). The stroma in PAC is very dense, fibrotic, and heterogeneous, and consists of fibroblasts, stellate cells, immune cells, and extracellular matrix (16). Furthermore, the immune infiltrate in PAC is unique in that it consists predominantly of macrophages and other myeloid cells, which interestingly are associated with inflammation in pancreatitis which in of itself is a risk factor for PAC (17). Macrophages are an important component of the innate immunity, and higher ratios of M1 macrophages (classically activated macrophages by Th1 cytokines) is associated with longer survival in PAC (17). In contrast, myeloid derived suppressor cells (MDSCs) are immature myeloid cells that suppress T-cell responses (17,18).

The T-cell infiltrate in PAC is unique in both its location and function within the TME, which may inform our strategies with regards to immune therapies in PAC. The impact of T-cell infiltration on prognosis has demonstrated inconsistent results (19,20). Some studies have shown that increased intratumoral CD3+ T-cells are associated with improved OS (19,20). Other reports have not shown an association between T-cell density and patient survival (21,22). There is a unique distribution of T-cell infiltrates in PAC. In PAC, T-cell infiltrates are found more commonly at the invasive front of the tumor mass, suggesting malignant cell exclusion from the center of the tumor mass (17,22). Additionally, T-cells appear to be trapped within peritumoral tissues, with limited direct contact with tumor cells (22,23). These findings suggest that the TME may limit T-cell interaction with malignant cells.

However, further characterization of T-cells in PAC does seem to indicate that PAC may indeed be immunogenic to some extent. In contrast to immune infiltrate in chronic pancreatitis, in PAC the T-cell infiltrate consists of decreased CD4+ and CD8+ T-cells and increased regulatory T-cells (Tregs) (20), suggesting that immune suppression may play a role in malignant transformation. T-cell clonal expansion and proliferation has been identified in PAC with a T-cell receptor (TCR) repertoire similar to melanoma (24). PAC vaccine therapy can induce the formation of intratumoral tertiary lymphoid aggregates in PAC, in which suppressed Tregs was associated with improved prognosis (25). Another study that characterized the T-cell infiltrate of PAC has shown that increased Tregs are associated with worse prognosis, while higher levels of tumor infiltrating CD4+ and CD8+ T-cells are associated with improved prognosis (17).

Taken together, while the T-cell infiltrate of PAC may have immunogenic properties, the TME in PAC seems to limit the immunogenic potential of T-cells in PAC and may act as a barrier to the effectiveness of immunotherapeutic strategies (26).

The question then becomes, how can we overcome the intrinsic resistance presented by PAC and its associated TME to enable T-cell mediated immune attack?

Immunomodulation following chemotherapy and radiation therapy

Chemotherapy and radiotherapy in PAC

In recent years, a number of chemotherapeutic agents have demonstrated efficacy in PAC, which has provided some improvement in patient prognosis in advanced disease (3). Gemcitabine monotherapy demonstrated efficacy in a landmark paper published in 1997 with a clinical benefit response in 23.8% of patients, and a median OS of 5.65 months (27). Subsequent work evaluating fluoropyrimidine based combinations demonstrated the clinical benefit of FOLFIRINOX (infusional FU/leucovorin, oxaliplatin, and irinotecan) in patients with advanced PAC (28). In this trial, FOLFIRINOX was directly compared to gemcitabine monotherapy demonstrating an improvement in median OS of 11.1 versus 6.8 months, with an improvement in ORR of 31.6% versus 9.4% respectively (28). Additional strides were achieved when gemcitabine was combined with nanoparticle albumin-bound paclitaxel (nab-paclitaxel) in which the combination of gemcitabine with nab-paclitaxel compared with gemcitabine alone demonstrated an improvement in median OS of 8.7 versus 6.6 months (29), and an ORR of 23% *vs.* 7%. With these therapeutic advances, systemic chemotherapy has become the mainstay treatment for metastatic PAC.

Given the technical challenges, limitations, and morbidity of surgery for loco-regional treatment of PAC, radiation with or without systemic chemotherapy has been incorporated as an effective tool for local control of PAC. Radiotherapy has a role with or without chemotherapy in a number of clinical settings in PAC, such as in the neoadjuvant setting for borderline resectable disease, for locally advanced unresectable disease, in the adjuvant setting for resectable disease, and in the palliative or recurrent settings (3). The current use of systemic chemotherapy with or without radiotherapy in the management of advanced PAC has led to great interest in the immunomodulatory

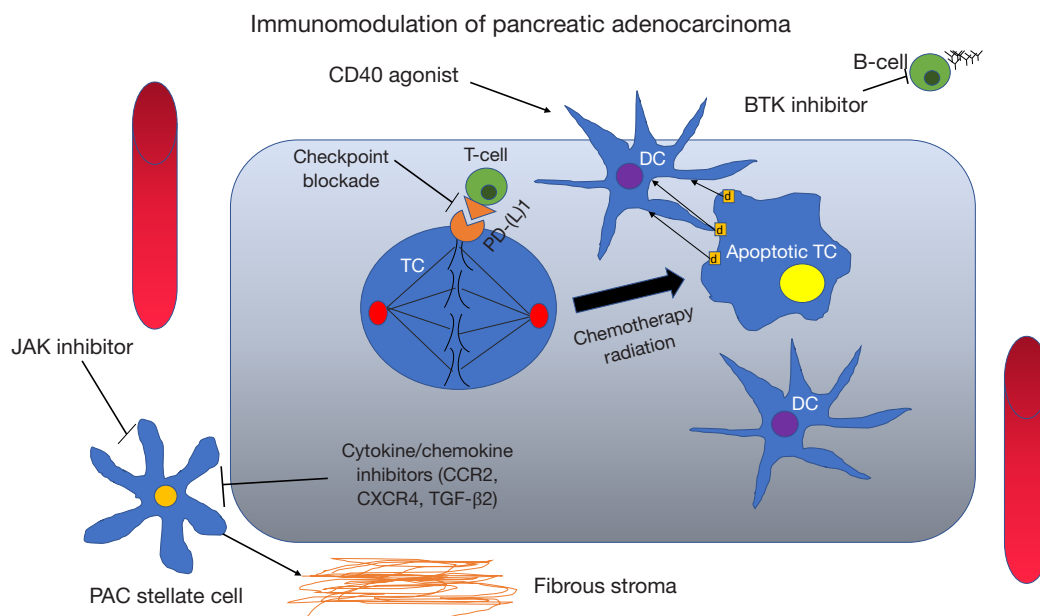


Figure 1 Immunomodulation of PAC: TC (tumor cell), PD-(L)1 [programmed death (ligand) 1], d (DAMP, damage associated membrane protein), BTK (bruton's tyrosine kinase), JAK (janus associated kinase), DC (dendritic cell), PD-(L)1 (programmed death ligand 1). Cytotoxic chemotherapy and radiotherapy kill malignant cells by immunogenic cell death which results in exposure of DAMPs, which are subsequently taken up by DCs which subsequently prime T-cells to trigger a tumor-specific immune response. Immune checkpoint blockade can inhibit the inhibitory PD-1/PD-L1 interaction between TCs and T-cells that enhance anti-tumor immunity. CD40 agonists mediate T-cell dependent and independent mechanisms of tumor regression through enhancing antigen presentation by DCs and other antigen presenting cells (APCs). JAK inhibitors may inhibit pancreatic stellate cell activation and may reduce fibrotic extracellular matrix to enhance TC and immune cell interface. BTK inhibitors suppresses B-cell and macrophage mediated suppression of T-cells. Inhibition of immunosuppressive cytokines such as CCR2, CXCR4, and TGF- β , may enhance anti-tumor immune response through reduction of immunosuppressive monocytes, and interactions between pancreatic stellate cells and cancer cells.

effects of these modalities.

Effect of cytotoxic chemotherapy and radiotherapy on immune microenvironment

Although cytotoxic chemotherapy previously had been regarded as immunosuppressive with regards to its effects on anti-tumor immunity, more recently it has been suggested that it may actually increase tumor immunogenicity (30). Chemotherapy can kill malignant cells by immunogenic cell death (*Figure 1*) (31). It has been suggested that dying cells undergoing immunogenic cell death expose proteins on its surface (DAMPs; damage-associated molecular patterns) to facilitate uptake of dying cells by dendritic cells (DC), which can subsequently prime CD4+ and CD8+ T-cells to trigger an immunogenic, tumor-specific, immune response (32). Furthermore, cytotoxic chemotherapy can also modify host immunity via modulation of a variety of immunoregulatory

cells (30). This can occur through decreasing the immunosuppressive effects of Tregs, decreasing MDSCs which inhibit T-cell mediated responses, enhancement of DC responses, and promotion of T-cell lymphocyte proliferation of anti-tumor immune response (30).

Chemotherapy and radiotherapy and immune modulation in solid tumors

The role of cytotoxic chemotherapy and radiotherapy as immune modulators have been explored in a number of solid tumors. Initial combinations of cytotoxic chemotherapy with immune therapies suggested that the addition of chemotherapy does not reduce anti-tumor immunity. In a phase I/II trial in patients with metastatic colorectal cancer (mCRC) and elevated carcinoembryonic antigen (CEA), patients received concurrent chemotherapy with 5-fluorouracil/leucovorin and irinotecan (FOLFIRI)

with a CEA derived peptide (CAP-1) vaccine therapy (31). Of seventeen patients, 8 (47%) demonstrated increased CAP-1 specific cytotoxic T-cells. A similar trial randomized 118 patients with mCRC to vaccine therapy before and concomitant with chemotherapy, vaccine therapy before and concomitant with chemotherapy with tetanus toxoid added, and chemotherapy followed by vaccine therapy (33). CEA-specific T-cells were increased in 50%, 37%, and 30% respectively with no statistical differences between the groups, suggesting that combining vaccine therapy with chemotherapy was able to elicit an anti-tumor immune response. In metastatic androgen independent prostate cancer, a study comparing a PSA-based vaccine therapy alone or in combination with docetaxel demonstrated similar increases in T-cell precursors to PSA (3.33-fold increase) in both groups (34), again suggesting that chemotherapy does not inhibit anti-tumor immune responses.

Subsequent clinical trials combining immune therapies with chemotherapy confirm this observation with checkpoint inhibitor therapy. In patients with metastatic melanoma, dacarbazine in combination with ipilimumab demonstrated improved overall survival compared with dacarbazine alone with median OS of 11.2 and 9.1 months respectively (35). Subsequently, a randomized phase II trial in patients with advanced stage non-small cell lung cancer (NSCLC) and extensive stage small cell lung cancer (ES-SCLC) compared a concurrent combination with ipilimumab with carboplatin and paclitaxel for four cycles followed by carboplatin and paclitaxel alone for two cycles, versus a phased combination in which carboplatin and paclitaxel for two cycles followed by ipilimumab with carboplatin and paclitaxel for four cycles, versus carboplatin and paclitaxel alone for six cycles. The phased strategy demonstrated improvement in immune-related PFS (irPFS) in both groups, whereas the concurrent strategy did not demonstrate a statistical improvement in irPFS (36,37). Another example is a large phase II trial in advanced NSCLC comparing pembrolizumab with carboplatin and pemetrexed versus carboplatin and pemetrexed alone, demonstrating an improved PFS of 19.0 versus 8.9 months respectively (38).

Radiation therapy also seems to have immunomodulatory effects that has been explored in solid tumors. It has been previously recognized that ionizing radiation can lead to an abscopal effect, or off-target responses, thought to be mediated by anti-tumor T-cell responses induced by immunogenic cell death (39,40). In a proof-of-principle clinical trial of patients with metastatic solid tumors treated

with radiation to 1 of 3 or more metastatic sites with concurrent granulocyte-macrophage colony stimulating factor (GM-CSF), abscopal responses occurred in 11 of 41 patients (41). This suggests that local radiation may induce anti-tumor immunity which leads to off-target efficacy.

Immunomodulatory effects of chemotherapy and radiotherapy in PAC

This has led to the hypothesis that in pancreatic cancer, a strategy of combining cytotoxic chemotherapy and radiotherapy with immune therapy may increase tumor immunogenicity and sensitize pancreatic tumors to immune therapy. Evaluation of the local immune environment in resected PAC treated with neoadjuvant chemoradiotherapy (chemoXRT) suggests that these modalities may enhance the immunogenicity of this disease. In one study, 52 patients who underwent surgical resection for PAC with 22 having received neoadjuvant chemoXRT, demonstrated that there were increased numbers of CD4+ and CD8+ T-lymphocytes in those patients treated with chemoXRT compared with patients resected without neoadjuvant therapy, and that high accumulation of CD8+ cells was associated with improved OS (42). Another study in which 7 of 17 patients received neoadjuvant chemoXRT prior to resection demonstrated no difference in the number of CD4+ and CD8+ T-cell infiltration, but the number of Tregs was significantly lower in the neoadjuvant chemoXRT group, suggesting a sensitizing effect to anti-tumor immunity with this strategy (43). Additionally, in PAC increased T-cell infiltrating lymphocytes (TILs) in patients treated with neoadjuvant chemotherapy was associated with improved disease free survival (DFS) (44), supporting the immunomodulatory role of cytotoxic chemotherapy in this disease. Chemotherapy, radiotherapy, and immune therapies have been combined with vaccine therapy (25,45-49), cytokine therapy (50), and checkpoint blockade (51) in the treatment of PAC.

The combination of chemotherapy and radiation therapy with various immune therapies are being explored in both pre-clinical and clinical settings in the treatment of PAC (52). In a mouse model of pancreatic cancer, combining radiation with dual blockade of PD-(L)1 and CTLA-4 resulted in improved survival and tumor responses than dual blockade without radiation or radiation alone (53). A phase Ib/II trial of neoadjuvant chemoradiotherapy in combination with pembrolizumab is currently ongoing and appears to be safe, yet efficacy data has not yet been reported (54). Interestingly,

Table 1 Clinical trials involving checkpoint inhibitor therapy in PAC

Setting	N	Intervention	Response rate	Reference
Advanced or metastatic	14	Anti-PD-L1	0	Brahmer, Tykodi <i>et al.</i> 2012
Metastatic/locally advanced	20/7	Ipilimumab	0	Royal, Levy <i>et al.</i> 2010
Metastatic, first line	19 evaluable	Gemcitabine	2/19 (10.5%)	Aglietta, Barone <i>et al.</i> 2014
		Tremelimumab		
Advanced or metastatic	16	Gemcitabine	2/16 (12.5%)	Kalyan, Kircher <i>et al.</i> 2016
		Ipilimumab		
Advanced or metastatic	11	Pembrolizumab	NR	Weiss, Waypa <i>et al.</i> 2017
		Chemotherapy		
Metastatic	17	Gemcitabine	5/17 (29.4%)	Wainberg, Hochster <i>et al.</i> 2017
		Abraxane		
		Nivolumab		

PAC, pancreatic adenocarcinoma.

while some data suggests that radiation may enhance anti-tumor T-cell responses, other pre-clinical data has suggested an immunosuppressive T-cell effect as well. In mouse models, radiation exposure also induced a macrophage immunosuppressive phenotype, as well as a reduction in CD8+ T-cells with increased Tregs (55), suggesting that immune responses to ionizing radiation may be mixed. It has also been suggested that inhibition of macrophage signaling may enhance anti-tumor responses (56), and that this may be a reasonable strategic approach in combination with radiotherapy (26). A number of clinical trials are currently ongoing investigating the role of radiation in combination with immune therapies. A phase II trial evaluating stereotactic body radiation therapy (SBRT) in combination with pembrolizumab and GVAX (GM-CSF secreting allogeneic pancreatic cancer vaccine) is currently ongoing (NCT02648282). Similarly, a pilot study evaluating SBRT in combination with tremelimumab (CTLA-4 monoclonal antibody) and/or MEDI4736 (PD-L1 monoclonal antibody) is also ongoing (NCT02311361). An open label phase II study in metastatic PAC is currently combining radiation with nivolumab with or without ipilimumab (NCT02866383). The true impact of radiation on anti-tumor immunity in-vivo has yet to be determined, as we wait with anticipation for the results of these clinical trials.

At this time, chemotherapy and radiotherapy are being combined with a number of immunomodulatory strategies in order to overcome these challenging barriers to the efficacy in immune therapies in PAC.

Immunomodulatory strategies in pancreatic cancer

Checkpoint inhibitors

Unfortunately, as of yet, the combination of cytotoxic chemotherapy with an immune therapy has not resulted in an overwhelming improvement in effectiveness of immune therapies (summarized in *Table 1*). Gemcitabine has been combined with CTLA4 blockade in several early phase clinical trials. In a phase I trial combining gemcitabine with tremelimumab, of 28 evaluable patients, two patients received a partial response (PR), and seven patients had stable disease (SD) for >10 weeks (57). In a phase Ib trial combining gemcitabine and ipilimumab, of sixteen evaluable patients, two patients had a PR and five patients had SD (58). This combination was well tolerated, yet objective response rate did not seem to be significantly improved over gemcitabine alone (59,60).

Gemcitabine in combination with programmed death (ligand) 1 [PD-(L)1] blockade, is also being evaluated. In murine models, gemcitabine and PD-(L)1 blockade demonstrate synergy and resulted in some complete responses (CR) (61). In a phase Ib trial evaluating pembrolizumab in combination with chemotherapy in advanced solid tumors, there were ten evaluable patients who received gemcitabine in combination with pembrolizumab. Of these, two patients had a PR, and six patients had SD (62). Recently, a phase I trial combining nab-paclitaxel with or without gemcitabine with nivolumab

reported results (51). The combination was overall well tolerated, with disease control (SD or PR) in 12 of 17 patients with locally advanced or metastatic PAC. Responses were observed in both the second line and upfront setting. This compares favorably with a historical control of chemotherapy alone, in which gemcitabine plus nab-paclitaxel reported a disease control rate was 48% (63). This provides at least a signal regarding combining single-agent checkpoint blockade with chemotherapy. However, larger clinical trials need to be completed to demonstrate a clinical benefit in this setting.

Cancer vaccines

In addition to checkpoint inhibition, cancer vaccine therapy has also been developed in hopes of inducing an anti-tumor immune response in PAC. In addition to evaluating advanced stage disease, a number of vaccine-based studies have also been evaluated in the adjuvant setting, as the low disease burden post-resection may suggest a role for a consolidative anti-tumor immune response (26,64). The most extensively evaluated anti-tumor vaccine is GVAX, an irradiated allogeneic whole tumor cell vaccine that expresses granulocyte-macrophage colony-stimulating factor (GM-CSF) (15). In early phase clinical trials, GVAX demonstrated anti-tumor delayed hypersensitivity responses in PAC (65). A phase II trial of 60 patients evaluating GVAX in combination with chemoradiotherapy in the adjuvant setting for resected PAC demonstrated 17.3 months DFS and 24.8 months OS, was well tolerated, and demonstrated mesothelin-specific CD8+ T-cells which correlated with DFS (25). Mesothelin had been previously demonstrated to be a tumor-associated antigen overexpressed in PAC (66). Subsequently, a GVAX immunization strategy was modified by combining with low dose cyclophosphamide with the goal of inhibiting Tregs, with increased anti-mesothelin CD8+ T-cell responses (67). GVAX was subsequently combined with CRS-207, a recombinant live-attenuated, double-deleted *Listeria Monocytogenes*, engineered to secrete mesothelin into antigen presenting cells in order to enhance mesothelin-specific CD8+ T-cell activity. A phase II trial was conducted in which patients with metastatic PAC were randomized in a 2:1 fashion to GVAX with cyclophosphamide (Cy/GVAX) followed by CRS-207 or Cy/GVAX alone. The Cy/GVAX plus CRS-207 arm demonstrated an OS benefit of 6.1 months versus 3.9 months in the Cy/GVAX alone arm, and mesothelin-specific CD8+ T-cell responses were associated with

longer OS (46).

Unfortunately, several other attempts at vaccine therapy for PAC have not similarly demonstrated improvements in patient outcomes, despite eliciting anti-tumor T-cell immunity. A large, randomized, phase III trial combining chemotherapy with a telomerase vaccine did not demonstrate improvement in patient survival (49). Algenpantucel-L, irradiated allogeneic pancreatic cancer cells transfected to express alpha-1,3-galactosyltransferase, has been evaluated in a phase II adjuvant trial in combination chemoradiotherapy demonstrated a 12-month DFS of 62% and an OS of 86% (45), however a large phase III randomized trial comparing chemoradiotherapy plus algenpantucel-L versus chemoradiotherapy alone in the adjuvant setting demonstrating no difference in OS (68). A phase I/II trial consisting of twenty-three patients who were treated with a mutant RAS peptide vaccine, demonstrated some long-term T-cell immune responses, with four of twenty evaluable patients demonstrating 10-year survival (69). Peptide vaccines consisting of VEGFR1 and VEGFR2 (antiangiogenic) and KIF20A have been evaluated in a phase 2 setting of locally advanced and metastatic PAC in combination with chemotherapy, suggesting that patients who mount a cytotoxic T-cell immune response seemed to have improved OS (47). Gemcitabine was combined with elpamotide, a VEGFR2 vaccine, in a randomized phase II/III trial in advanced and metastatic PAC with no difference in OS (48). Other peptide vaccines have been attempted including personalized peptide vaccines (70), KIF20A-66 (member of kinesin super family protein 20A that is transactivated in PAC) peptide vaccine (70), and RAS peptide vaccine (71-73), demonstrating that these peptides can illicit variable immune responses.

Chemotherapy in combination with immune vaccines has been evaluated without much success. An investigation pancreatic cancer vaccine, algenpantucel-L, was evaluated in a phase III trial with standard of care chemotherapy, demonstrating an overall survival of 27.3 months versus 30.4 months with chemotherapy alone (45). An additional phase III trial investigating chemotherapy (gemcitabine and capecitabine) with either sequential or concomitant telomerase cancer vaccination also demonstrated no difference in overall survival (49). Gemcitabine was combined with IMM-101, a systemic immune modulator containing heat-killed *Mycobacterium obuense*, in a phase II randomized trial which demonstrated a non-significant improvement in OS of 6.7 vs. 5.6 months (P=0.074) when IMM-101 was combined with gemcitabine compared with

Table 2 Cancer vaccine therapy trials in PAC

Setting	N	Intervention	Results	Reference
Adjuvant	14	GM-CSF secreting vaccine	3/14 DFS \geq 25 m	Jaffee, Hruban <i>et al.</i> 2001
Adjuvant	60	GM-CSF secreting vaccine	DFS 17.3 m, mOS 24.8 m	Lutz, Yeo <i>et al.</i> 2011
Advanced	60	GM-CSF secreting vaccine +/- Cy	mOS 2.3 m (no Cy); mOS 4.3 m (with Cy)	Laheru, Lutz <i>et al.</i> 2008
Metastatic	90	Cy/GVAX +/- CRS-207	mOS 6.1 m (with CRS-207); mOS CyGVAX (no CRS-207)	Le, Wang-Gillam <i>et al.</i> 2015
Locally advanced or metastatic	1,062	Chemotherapy +/- GV1001 (sequential or concurrent)	mOS 6.9 m (chemo); mOS 7.9 m (sequential); 8.4 m (concurrent)	Middleton, Silcocks <i>et al.</i> 2014
Adjuvant	70	Algenpantucel-L + chemotherapy or chemoXRT	12 m DFS 62%, mOS 86%	Hardacre, Mulcahy <i>et al.</i> 2013
Adjuvant	20	K-ras vaccine	mOS 27.5 m; 10 y survival 4/20 (20%)	Wedén, Klemp <i>et al.</i> 2011
Advanced	68	KIF-20A + VEGFR1/2 peptide	ORR 8/66 (12%)	Suzuki, Hazama <i>et al.</i> 2017
Locally advanced or metastatic	153	Chemotherapy +/- VEGFR2 peptide	mOS 8.54 m	Yamaue, Tsunoda <i>et al.</i> 2015
Metastatic, second line	29	KIF20A-66 peptide	ORR 8/29 (27.6%)	Asahara, Takeda <i>et al.</i> 2013

PAC, pancreatic adenocarcinoma; GM-CSF, granulocyte-macrophage colony stimulating factor; m, months; ORR, overall response rate; DFS, disease free survival.

gemcitabine alone, and was well tolerated.

Taken together, clinical data suggests that vaccines in PAC can elicit an anti-tumor T-cell response. While some of these trials provide a signal of clinical benefit, others demonstrating no clinically meaningful endpoints, which suggests that additional barriers to effective anti-tumor immune therapy are at play (*Table 2*).

Cytokines

The use of cytokines as an immune therapy has been evaluated in the clinical setting both alone and in combination with other systemic therapies for PAC in advanced and adjuvant settings. In a large phase III trial of resected PAC, chemotherapy (5-fluorouracil; 5-FU) alone was compared with combination chemotherapy (5-FU plus cisplatin) with interferon alfa-2b. The combination was more toxic than 5-FU alone and did not demonstrate an improvement in overall survival (74). An adjuvant trial comparing surgical resection alone with combination chemotherapy with or without IL-2 therapy directly injected into superior mesenteric artery (SMA) demonstrated an improvement in survival (31.0, 25.0, and 18.8 months respectively for

adjuvant chemoimmunotherapy, adjuvant chemotherapy alone, or no adjuvant therapy respectively) (50), however this was a small trial, and the rationale behind injection of adjuvant therapy into the SMA was not adequately explained. In a phase Ib trial, FOLFOX was combined with AM0010, PEGylated human IL-10. Of nineteen evaluable patients, two patients had a CR, 1 patient had a PR, and 11 patients had SD. Treatment resulted in increased serum cytokine levels and expansion of novel T-cell clones (75). Similar to vaccines therapies, while some evidence of eliciting anti-tumor immunity was demonstrated in some studies, significant strides in improving clinical outcomes has not been demonstrated.

Oncolytic viral therapy

Oncolytic viral therapy is a strategy that can induce tumor responses through direct tumor cell lysis by infecting tumor cells, replicating, and eventually lysing the cell. However, cell lysis also releases damage associated molecular pattern molecules (DAMPs) which can trigger innate and adaptive immune responses (76). Of these, adenovirus-based oncolytic viruses have been the most extensively evaluated.

One of these, ONYX-015, is an E1B-55kDa region-deleted adenovirus that selectively replicates in and lyses tumor cells with p53 abnormalities. In a phase I dose escalation study in which ONYX-015 was directly injected into locally advanced unresectable pancreatic cancer, but resulted in no responses, and no evidence of viral replication (77). A phase I/II study of direct tumor injection of ONYX-015 followed by gemcitabine in localized PAC demonstrated PRs in 4 of 11 patients after gemcitabine, but no objective responses after treatment of the oncolytic virus alone (75). Another oncolytic viral therapy has utilized a reovirus that preferentially replicates in cells with activated RAS pathways, reolysin. In a phase II study, of 29 evaluable patients with advanced or metastatic PAC, one patient demonstrated a PR. Interestingly however, there was upregulation of PD-L1 in reolysin treated patients, again suggesting the potential immunomodulatory impact of oncolytic viral therapy in PAC (78). While these strategies seem to be limited by anti-tumor potency and immune neutralization, follow-up clinical studies utilizing reolysin in combination with anti-PD1 therapy (pembrolizumab) are ongoing (NCT02620423) (76).

Adoptive T-cell therapy

Adoptive T-cell therapy involving genetically engineered T-cells expanded ex-vivo and re-infused into patients to target malignancies has been an exciting advancement in cancer therapy and is being explored in PAC. Adoptive T-cells utilizing a chimeric antigen receptor (CAR) has demonstrated impressive results in a number hematologic malignancies such as acute lymphoblastic leukemia (ALL) (79) which eventually has led to its Food and Drug Administration (FDA) approval (80). This has led to investigation and hope for an adoptive T-cell strategy in solid tumor malignancies as well (81). CAR T-cells that target mesothelin have demonstrated an anti-tumor immune response in patients with metastatic PAC (82). Furthermore, in one patient treated with mesothelin-specific CAR T cells with metastatic PAC, there was resolution of an FDG-avid liver metastasis after one month of therapy (83). Despite only small numbers of patients with metastatic PAC that have been treated thus far with adoptive T-cell therapy, this at least suggests a signal of immune-mediated anti-tumor effects, yet with variable effects in metastatic sites versus primary tumor (22). Mixed responses of metastatic and primary lesions have

also been described in the setting of 20 patients treated with MUC-1 specific cytotoxic T-cells with a MUC-1 dendritic cell vaccine therapy, including one patient who experienced resolution of multiple pulmonary metastases (22,84). It therefore begs to reason that perhaps despite antitumor efficacy of adoptive T-cell therapy, selective barriers to efficacy based on primary versus metastatic sites may explain these variable responses. Currently, adoptive T-cell therapies under investigation include an anti-mesothelin CAR-T in the metastatic setting that is currently recruiting patients (NCT01583686), as well as prostate stem cell antigen (PSCA)-specific CAR T-cells (NCT02744287).

Targeted therapies to amplify T-cell mediated immunity

The biology of PAC and genomic makeup demonstrates a number of driver mutations, yet at this time no targeted therapies have made significant inroads into improving patient prognosis with this disease. The current model of oncogenic transformation suggests a stepwise progression from polyp to adenocarcinoma, similar to what is seen in colon cancer, with pancreatic intraepithelial neoplasia (PIN) as the precursor lesion (85). In fact, over 90% of PINs of various grades demonstrate KRAS mutations, while mutations in CDKN2A, p53, and SMAD4 are seen more frequently in higher grade PINs, suggesting that these are cumulative events in the malignant transformation of PAC (85). PACs are heterogeneous with regards to its genomic mutational pattern, which is notable for mutational frequencies of >90% KRAS, 60–70% p53, >50% CDKN2A, ~50% SMAD4, and other less frequent mutations (85). Other emerging drivers include mutations in BRCA1/2, which has a prevalence of 4–5% in unselected patients with PAC, with BRCA2 as the more common variant (86). This is of particular importance in light of mounting evidence that these patients may have enhanced sensitivity to platinum-based chemotherapy as well as poly(ADP-ribose) polymerase (PARP) inhibition (87). Other less common mutations which may have clinical relevance is human epidermal growth factor receptor 2 (Her2), which has a prevalence of approximately 2% in unselected patients with PAC (88). Although as of yet, Her2 targeting in PAC has not yielded significant improvements in clinical outcomes (89,90). Mutational patterns of PAC may also have prognostic

Table 3 Ongoing clinical trials evaluating immunomodulation using targeted therapies

Target	Drug	Clinical trial identifier(s)
MEK/ERK	BVD-523	NCT02608229
FAK, MEK	GSK2256098, Trametinib	NCT02428270
CD40	RO7009789	NCT02588443
BTK	Ibrutinib	NCT02562898, NCT02403271
CSF1R	Pexidartinib	NCT02777710
CXCR4	Plerixafor	NCT03277209, NCT02179970
TGF- β	Galunisertib	NCT02734160
CDK4/6	Palbociclib	NCT03065062
Wnt/ β -catenin	LGK974	NCT01351103

MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase-1; BTK, bruton's tyrosine kinase; CSF1R, colony stimulating factor 1 receptor; TGF- β , transforming growth factor- β ; CDK4/6, cyclin D dependent kinase 4 and 6.

significance, and long-term survivors of resected PAC tend to have lower rates of KRAS, p53, and SMAD4 mutations (91). Despite this work however, targeted therapies in PAC has proved challenging, in large part due to tumor heterogeneity (92). With the exception of erlotinib (93), other attempts at targeted therapies have failed to yield positive results such as bevacizumab (94,95), cetuximab (96), axitinib (97,98), sorafenib (99), and aflibercept (100).

Recently, targeted therapies as a means of modulating and enhancing T-cell immunity, is being explored as a potential partner with immunotherapies such as checkpoint inhibitors. Specifically, research is focusing on targeted strategies that are able to transform "cold" tumors, or noninflamed tumors, into "hot" tumors that demonstrate T-cell infiltration and may enhance the efficacy of checkpoint inhibitor therapies. A number of targeted therapies appear to affect tumor cells directly, as well as modulation of immune cells, such as BRAF and MEK inhibition in melanoma (101). This has sparked interest that combining immunotherapies with targeted therapies may overcome some of the barriers in efficacy found with single-agent checkpoint inhibitor therapies, especially in malignancies such as PAC with poor responses to single-agent checkpoint inhibition.

Targeted therapies that enhance T-cell mediated immunity is a strategy that has been evaluated in PAC as well. Some of these strategies have included blockade of additional immune inhibitory pathways, stimulation of activating pathways, epigenetic modifications, which include not only lymphocytes but also macrophages, natural

killer (NK) cells, and stromal cells (102). Many different targets have been and are currently being evaluated in the immune activation cascade including T-cells, myeloid cells, and stromal tissue in order to both amplify T-cell immune response and sensitize pancreatic tumors to anti-tumor immunity (*Table 3*).

MAP-ERK kinase (MEK) inhibition

Other targets have been suggested as potential mechanisms of synergism with immune therapy include inhibition of MEK pathway and epigenetic targeting (22). PAC is characterized by KRAS mutations present in >90% of cases, which has generated much interest in targeting downstream pathways such as MEK in this disease (103). For example, MEK inhibition has been shown to upregulate MHC I on tumor cells and induce T-cell infiltration into tumors (104), and enhance the activity of PD-(L)1 blockade. Combination MEK inhibition with checkpoint blockade is being evaluated in colorectal cancer and appears to be safe and demonstrates some responses (105). However in PAC, these have not yet reached the clinical trial arena in combination with immune therapy.

CD40 agonist

CD40 is broadly expressed on immune cells such as B-cells, dendritic cells, and monocytes, and CD40 agonists can mediate both T-cell dependent and independent mechanisms of tumor regression in PAC (*Figure 1*) (106).

Of the strategies utilized to activate myeloid cells as a means of enhancing anti-tumor immunity, CD40 are the most extensively evaluated target. CD40 activates APCs and enhances immune responses (107). CD40 agonist monoclonal antibody, has demonstrated some responses in solid tumors (108), leading to interest in investigating this strategy in PAC. Gemcitabine in combination with a CD40 agonist therapy demonstrated that out of 21 evaluable patients with 90% having metastatic PAC, four had a PR, and eleven had SD (109). Additionally, CD40 agonist therapy resulted in cytokine release syndrome (CRS), increased inflammatory cytokines, and an increase in B-cell co-stimulatory molecules (110). Importantly however, extratumoral macrophages regulate infiltration of T-cell into PAC, suggesting that reversal of immune privilege may increase efficacy of T-cell immunotherapy in PAC (22,111).

JAK-STAT pathway inhibition

The PAC TME is composed of fibrotic extracellular matrix, produced by pancreatic stellate cells within the TME (112), which may limit the therapeutic efficacy of immune therapies in PAC (*Figure 1*). The JAK/STAT pathway plays an important role in activation of pancreatic stellate cells (113), and inhibition of this pathway may have a favorable impact on the efficacy of immune therapies. Targeted therapies that target the unique PAC microenvironment have also been investigated in combination with immune therapy (26). In mouse models of PAC, JAK pathway blockade results in immune-mediated inhibition of tumor growth (114). However, in two large phase III trials, ruxolitinib in combination with capecitabine did not improve outcomes of metastatic PAC over capecitabine alone (115). (NCT02117479 and NCT02119663).

Bruton's tyrosine kinase (BTK) inhibition

Another target that is being investigated in PAC is inhibition of BTK. BTK is a Tec family non-receptor tyrosine kinase that is required for B-cell receptor (BCR) signaling, and has been developed primarily for B-cell malignancies such as chronic lymphocytic leukemia (CLL), mantle cell lymphoma, and Waldenstrom's macroglobulinemia (116). In PAC, BTK regulates B-cell and macrophage mediated suppression of T-cells (*Figure 1*) (117). This has led to the evaluation of ibrutinib as an immunomodulatory agent in PAC. In pre-clinical

models of PAC, ibrutinib demonstrates reduction in stromal fibrosis and inhibition of tumor progression (118), suggesting that it may have the potential to sensitize tumors to checkpoint blockade. This combination is currently being investigated in a phase Ib/II, multicenter, study in combination with durvalumab (NCT02403271) (119).

Colony stimulating factor 1 receptor (CSF1R)

Myeloid lineage immune cells are also being targeted through inhibition of colony stimulating factor 1 receptor (CSF1R) with a goal of amplifying checkpoint inhibitor efficacy. Tumor associated macrophages exposed to CSF1 enhances a tumor promoting and immune suppressive macrophage phenotype (120), which has led to this as an attractive target for immune modulation in PAC. Preclinical data has demonstrated that inhibiting CSF1R can reprogram macrophages and thereby enhance antigen presentation to increase anti-tumor T-cell responses (56). CSF1R blockade is being evaluated in combination with PD-1 blockade and vaccine therapy (NCT03153410 and NCT03153410).

Cytokine and chemokine pathway inhibition

A number of cytokines and chemokines involved in recruitment of immunosuppressive myeloid cells have also gained interest as immunomodulatory targets in PAC. The CCL2/CCR2 is a chemokine receptor pathway that is involved in recruitment of immunosuppressive monocytes into the TME (*Figure 1*), and has been evaluated as a target for inhibition in combination with chemotherapy, demonstrating some responses (121). Chemokine receptor type 4 (CXCR4) with its chemokine ligand CXCL12 is a major player in the immunosuppressive TME in PAC, and contributes to chemotherapy resistance and poor outcomes in this disease (122). An early phase clinical trial evaluating CXCR4 blockade in combination with PD-1 blockade in patients with metastatic PAC is currently recruiting (NCT02826486). Another immunosuppressive target that has been investigated in PAC is transforming growth factor- β 2 (TGF- β 2). TGF- β 2 plays an important role in both development of pancreatic cancer stem cells as well as mediating the interaction between pancreatic stellate cells and cancer cells (123). Trabedersen, an anti-sense peptide which inhibits biosynthesis of transforming growth factor- β 2 (TGF- β 2) (75). This has been evaluated in a phase I/II trial including patients with advanced PAC (76).

One PAC patient was reported to have had a CR of liver metastasis, but no other efficacy data was reported (76).

Cyclin-dependent kinases 4 and 6 (CDK4/6) inhibition

Cyclin-dependent kinase 4 and 6 are involved in cell cycle progression and are required for malignant transformation in solid tumors, such as breast cancer (124). This has led to the development of CDK4/6 inhibitors that act through inhibition of the phosphorylation of the retinoblastoma (RB) tumor suppressor gene, resulting in cell cycle arrest in tumor cells (125). In breast cancer, inhibitors of CDK4/6 demonstrated efficacy in combination with endocrine therapy for hormone-responsive breast cancer in the metastatic setting (126,127). Recently, work in CDK4/6 inhibition has suggested that in addition to direct tumor cell cytotoxicity, it may also have immunomodulatory properties. Enhanced anti-tumor immunity may be related to tumor cell expression of endogenous retroviral elements leading to increased tumor antigen presentation and suppression of regulatory T-cell proliferation (125).

This data has led to the hypothesis that CDK4/6 inhibition in PAC may have a role, and may enhance antitumor immunity in combination with immunotherapeutic interventions. In PAC, inactivation of CDKN2A is found in approximately 95% of cases, which encodes the tumor suppressor p16^{INK4A} whose role is inhibition cyclin dependent kinases 4 and 6 (128). In previous work evaluating CDK4/6 suppression in patient-derived xenographs of PAC, tumor proliferation was completely suppressed (129). In another study of patient-derived xenograft models of pancreatic cancer, palbociclib as a single-agent demonstrated greater than 50% tumor growth, and the combination with gemcitabine and nab-paclitaxel increased the degree of tumor response as well (130). Currently, CDK4/6 inhibitors are being evaluated in combination with anti-PD-L1 antibody in multiple solid tumors, including PAC (NCT02791334).

Wnt inhibitor therapy

The Wnt/ β -catenin signaling pathway has been implicated in carcinogenesis, including gastrointestinal cancers. In colorectal cancer the loss of the APC gene is an early pathogenic occurrence and is a major driver of Wnt/ β -catenin signaling with accumulation of β -catenin leading to promotion of cellular proliferation, and this seems to

play an important role in tumor maintenance (131). Wnt/ β -catenin pathway activation has also been demonstrated in other upper gastrointestinal cancers such as in gastric cancer, HCC, and cholangiocarcinoma (132-134). In PAC, Wnt/ β -catenin pathway mutations are rare, however nuclear localization of β -catenin can be found (131,135). Furthermore, RNF43 inhibits Wnt/ β -catenin signaling in PAC, and PAC cell lines with RNF43 mutations were sensitive to inhibition of Wnt/ β -catenin signaling (136). Upregulation of the Wnt/ β -catenin signaling pathway may also have immunomodulatory properties through effects on dendritic cells leading to reduced CD8+ T-cell function, interactions with tumor-associated macrophages, and increased Treg survival (137). Given the suggested role of Wnt/ β -catenin in PAC and the immunomodulatory effects, combining Wnt inhibitors with immunotherapeutic interventions may be a rational combination to be explored in the pre-clinical and subsequently clinical setting.

Conclusions

Unfortunately, PAC remains a major cause of cancer-related mortality, with little improvement despite significant strides made in cancer therapy in recent years. Harnessing the immune system to attack cancer is revitalizing progress and hope for a wide range of malignancies, yet these strategies have not yet made significant inroads in the realm of PAC. A number of barriers to immune therapy in PAC include lower levels of neoantigens, the unique immunosuppressive TME, and low levels of intratumoral infiltrating T-lymphocytes. Despite this, a number of pre-clinical and early clinical data suggests that PAC may be more immunogenic than initially thought, however these strategies have yet to make significant strides in terms of clinical benefit. Further investigation into overcoming barriers to immune therapy in PAC must be strategically applied to discover combinations that have the potential to improve outcomes of patients with PAC.

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Footnote

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

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