Pancreatic cancer: optimizing treatment options, new, and emerging targeted therapies

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Abstract: Pancreatic cancer is the fourth leading cause of cancer death in the US and is expected to become the second leading cause of cancer-related deaths in the next decade. Despite 5-fluorouracil/leucovorin with irinotecan and oxaliplatin (FOLFIRINOX) and gemcitabine/nab-paclitaxel significantly improving outcomes for metastatic cancer, refractory disease still poses significant challenges. Difficulties with early detection and the inherent chemoresistant nature of this malignancy led to attempts to define the sequential biology of pancreatic cancer in order to improve survival outcomes. Pancreatic adenocarcinoma is characterized by several germline or acquired genetic mutations, the most common being KRAS (90%), CDK2NA (90%), TP53 (75%-90%), DPC4/SMAD4 (50%). In addition, the tumor microenvironment, chemoresistant cancer stem cells, and the desmoplastic stroma have been the target of some promising clinical investigations. Among the core pathways reproducibly shown to lead the development and progression of this disease, DNA repair, apoptosis, G1/S cell cycle transition, KRAS, Wnt, Notch, Hedgehog, TGF-beta, and other cell invasion pathways, have been the target of “precision therapeutics”. No single molecularly targeted therapeutic though has been uniformly successful, probably due to the tumor heterogeneity, but biomarker research is evolving and it hopes to select more patients likely to benefit. Recent reports note activity with immunotherapies such as CD40 agonists, CCR2 inhibitors, cancer vaccines, and novel combinations against the immunosuppressive tumor milieu are ongoing. While many obstacles still exist, clearly we are making progress in deciphering the heterogeneity within pancreatic cancers. Integrating conventional and immunological targeting will be the key to effective treatment of this deadly disease.

Keywords: pancreatic cancer, immunotherapies, signaling pathway inhibitors, targeted therapies

Introduction

Pancreatic cancer accounts for 277,000 new cases diagnosed each year in the world,1 among which approximately 49,000 each occur in the US2 and Europe.3 Despite modest improvements in detection, which may have contributed to its rise in incidence, the 5-year overall survival (OS) rate only increased from 5% to 6% in the past three decades.3,4 While currently pancreatic cancer represents the fourth leading cause of cancer death in the US (approximately 40,000 deaths annually), it is expected to become the second cause of cancer-related deaths in the US in the next decade.1 Pancreatic ductal adenocarcinoma is the most common histologic type of pancreatic cancer and will be reviewed in this paper.

Among the known risk factors for pancreatic cancer, smoking and overweight/obesity are major contributors. The significant decrease in tobacco smoking prevalence since the 1990s has been expected to reduce the incidence of pancreatic cancer, but it may have been overcome by the increased prevalence in overweight/obesity and type II...
diabetes with untoward effects on risk and mortality from pancreatic cancer. The high mortality rate of pancreatic cancer is attributed to the lack of reliable screening methodologies for early detection of precancerous neoplasia and early-stage invasive pancreatic cancers, as well as the relative poor efficacy of systemic chemotherapy treatments. More than 50% of pancreatic cancers are identified in metastatic stage where survival rates range from 7–11 months, and in 30%–40% of patients the disease is localized but not surgically resectable, with OS of 11–18 months. Patients with resected pancreatic cancer have poorer outcomes compared to other resected solid tumors, and the median survival after surgery and adjuvant therapy averages 24 months.

While gemcitabine is still used as the adjuvant therapy of choice for resected pancreatic cancer, since 2011 two combination regimens for metastatic disease have become gold standard: 5-fluouracil (5-FU)/leucovorin with irinotecan and oxaliplatin (FOLFIRINOX), and nab-paclitaxel with gemcitabine. With these approaches, response rates range between 23% and 31%, progression-free survival (PFS) rates are 5.5–6.6 months, and OS is between 8.5 and 11 months. Given the success with FOLFIRINOX and gemcitabine/nab-paclitaxel in metastatic disease, these regimens are being studied in several adjuvant and neoadjuvant trials for resectable and borderline resectable disease, as well as for the treatment of locally advanced but inoperable pancreatic cancers. Preliminary data with combination chemotherapy indicate superior responses and survival rates vs historical reports with gemcitabine alone. It is now evident that these more aggressive chemotherapy backbones can be used to build upon for combinations with novel targeted therapies.

Pancreatic adenocarcinoma is characterized by several germline or acquired genetic mutations, the most common being KRAS (90%), CDK2NA (90%), TP53 (75%–90%), SMAD4/DPC4 (50%), as well as genomic and epigenetic alterations, which can guide personalized cancer therapy. In addition, the tumor microenvironment, the chemoresistant cancer stem cells, and the desmoplastic stroma have been the target of recent clinical investigations.

### Current treatment options and limitations

In the past three decades, the standard chemotherapies for pancreatic cancer consisted of fluoropyrimidines like 5-FU, and the antimetabolite drug gemcitabine, which were mostly equivalent in randomized clinical trials, contributing 0%–10% each to tumor response, and with PFS and OS rates of 4–6 months. Several combinations with other chemotherapies and/or biologically targeted agents were studied with almost invariably negative results (Table 1). In 2005, the

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Abbreviations: FDR, fixed dose rate; FOLFIRINOX, 5-fluorouracil/leucovorin with irinotecan and oxaliplatin; mos, months; n/a, not available; OS, overall survival; PFS, progression-free survival.
first statistically significant survival improvement with a gemcitabine combination was noted with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, erlotinib (6.2 vs 5.9 months, hazard ratio [HR] 0.82, \( P=0.004 \)) in the NCIC Phase III study.\(^\text{24}\) Nevertheless, the benefit was small, and these results were not practice changing. Furthermore, no biomarkers have been identified to predict benefit from erlotinib.\(^\text{25}\) Due to grade ≥2 rash from erlotinib being associated with increased OS (10.5 months) in the NCIC study, Van Cutsem et al tested gemcitabine plus erlotinib dose escalated to rash, in the Phase II randomized RACHEL study.\(^\text{26}\) While more patients in the escalation group developed rash (41%) vs 9% in the standard group, the PFS and OS were not superior (3.5 vs 4.5 months, and 7 vs 8.4 months, respectively).

The excessive chemoresistance which characterizes pancreatic cancer has been studied in both xenograft and genetically engineered \(Kras^{\text{SLG}-G12D/\text{+}},\ Trp53^{\text{LSL-R172H/\text{+}},\ Cre}\) mouse models (KPC GEMM).\(^\text{27}\) These preclinical models, as well as several human clinical studies noted that the dense desmoplastic tumor stroma, mostly devoid of functional vasculature and infiltrated by an immunosuppressive environment, contributes to poor access by therapeutics, and confers chemo- and radiotherapy resistance.\(^\text{28,29}\) Targeting the tumor stroma has become an area of intense research.\(^\text{30–33}\)

### Nab-paclitaxel

Taxanes are important components in the systemic treatment of many cancers including breast, ovarian, lung, and gastro-esophageal. Paclitaxel and docetaxel have been studied in the treatment of pancreatic adenocarcinoma with few noticeable results, until recently with the new generation nanoparticle albumin-bound paclitaxel (nab-paclitaxel). Taxanes stabilize microtubules by increasing their polymerization, and induce cell cycle arrest at the G2/M phase, resulting in cell death.\(^\text{34}\) Nab-paclitaxel, a water soluble compound has enhanced distribution properties within the tumor microenvironment compared to paclitaxel, and increases intratumoral gemcitabine levels in mouse models.\(^\text{35}\) A putative mechanism for the intratumoral accumulation of nab-paclitaxel is the presence of albumin-binding proteins such as gp60 and secreted protein acidic and rich in cysteine (SPARC/osteonecine) in the tumor microenvironment.\(^\text{36,37}\) While SPARC expression in peritumoral stroma seems to correlate with worse outcomes in early stage pancreatic cancer,\(^\text{38,39}\) it is debatable whether it correlates to improved efficacy from nab-paclitaxel-based therapy.\(^\text{40–43}\)

Targeting the tumor stroma has been one of the proposed mechanisms of action for nab-paclitaxel. The stromal disrupting effects of nab-paclitaxel have also been noted in a small neoadjuvant study for resectable pancreatic cancer patients. Among the ten patients who underwent surgical resection, treatment with two 4-week cycles of nab-paclitaxel and gemcitabine prior to surgery did not induce objective radiological responses, but caused increased “tumor softness” by endoscopic ultrasound-based elastography, and histopathologically resulted in one complete pathological response, and six major pathological responses (a few isolated malignant cells left).\(^\text{44}\) Compared to untreated controls, nab-paclitaxel with gemcitabine was associated with less abundant collagen matrix infiltration around tumor glands, and a decreased number of cancer-associated fibroblasts. A more recently described mechanism of nab-paclitaxel synergism with gemcitabine involves the inactivation by nab-paclitaxel of the gemcitabine catabolizing enzyme cytidine deaminase, via production of destabilizing reactive oxygen species.\(^\text{45}\)

The most significant benefit to date with nab-paclitaxel has been reported in the setting of metastatic pancreatic cancer. The phase III international MPACT study among 861 randomized patients, showed a significant response (23% vs 7%, \( P<0.001 \)), PFS (5.5 vs 3.7 months, \( P<0.001 \)), and OS rate (8.5 vs 6.7 months, \( P<0.001 \)) benefit with nab-paclitaxel plus gemcitabine compared to gemcitabine alone.\(^\text{46}\) Overall, the combination treatment was well tolerated, and the most significant grade ≥3 toxicities were neutropenia (38%), fatigue, and neuropathy (17% each), and thrombocytopenia (13%). These results led to the approval by the United States Food and Drug Administration (FDA) of nab-paclitaxel on September 6, 2013 for metastatic pancreatic cancer. In this trial, archival tumor biopsies have been collected to analyze the role of stromal and tumor SPARC expression, both as prognostic and predictive markers of benefit. While earlier studies proposed SPARC as a prognostic marker for worse outcomes in early stage pancreatic cancer,\(^\text{38}\) in MPACT high SPARC levels did not correlate with worse survival or with superior benefit from nab-paclitaxel therapy.\(^\text{47}\) This is consistent with recent preclinical data in pancreatic cancer mouse models indicating SPARC-independent desmoplasia, and intratumoral paclitaxel levels no different among SPARC\(^{\text{+}}\)/ (positive) and SPARC\(^{\text{-}}\)/ (negative) tumors.\(^\text{48}\) The role of circulating SPARC is controversial, but it has been proposed that it may be linked to higher intravascular nab-paclitaxel levels.\(^\text{49}\)

Given potential synergy seen in preclinical models, nab-paclitaxel/gemcitabine was combined with capecitabine, but likely due to lower administered doses (gemcitabine 750 mg/m\(^2\), nab-paclitaxel 100 mg/m\(^2\) both on day 4; and capecitabine 750 mg/m\(^2\) twice daily, days 1 to 7, every 14 days), results were modest: response rates 14% and median survival 7.5 months.\(^\text{49}\) Nab-paclitaxel has been
tested as monotherapy or in combination with gemcitabine in the second-line treatment of advanced pancreatic cancer. In a Phase II trial, single agent nab-paclitaxel showed low response rates (5%) and PFS of 1.7 months, but the OS of 7.3 months suggests benefit from additional lines of therapy. Another retrospective analysis of nab-paclitaxel showed median PFS and OS of 2 and 3 months, respectively. Similarly, gemcitabine/nab-paclitaxel showed little benefit in the salvage setting, with median PFS of 3 months.

Small clinical trials with nab-paclitaxel-based chemotherapy in the first or second line setting added biologically targeted agents such as the EGFR-tyrosine kinase inhibitor erlotinib, or the VEGFR-2 (vascular endothelial growth factor and receptor), EGFR, RET kinase inhibitor vandetanib, but the results are not clearly superior to chemotherapy alone (median PFS 5.3 months, and OS 8–9 months).

Given high response rates in advanced disease, several small clinical trials noted activity of nab-paclitaxel with gemcitabine in the preoperative setting. Radiological responses, likely an inadequate surrogate, with neoadjuvant therapy were only 0%–16%, but higher pathological response rates were noted (30%–70%); long-term survival data are not yet available. A Phase III trial with nab-paclitaxel and gemcitabine (APACT, NCT01964430) is ongoing in the United States for patients with resected pancreatic adenocarcinoma, and neoadjuvant studies integrating chemoradiotherapy with gemcitabine/nab-paclitaxel are planned or ongoing. In Europe, the NEONAX trial (NCT02047513) will test neoadjuvant plus adjuvant vs only adjuvant gemcitabine/nab-paclitaxel for resectable pancreatic cancer patients.

**FOLFIRINOX**

The combination of 5-FU, irinotecan, and oxaliplatin (FOLFIRINOX) first demonstrated activity in pancreatic cancer in a Phase I solid tumors trial published in 2003, which was based on the independent clinical activity previously noted with its constituent agents: 5-FU, irinotecan, and 5-FU/oxaliplatin. A subsequent Phase II study among 47 patients with pancreatic cancer demonstrated response rates of 26%, time to progression of 8.2 months, and the median OS was 10.2 months. These data led to the conduct of the Phase II–III PRODIGE4/ACCORD 11 study of 342 patients selected for excellent performance status (ECOG PS 0 or 1), randomized to FOLFIRINOX vs gemcitabine alone. The toxicity profile with FOLFIRINOX demonstrated an increased risk of grade 3/4 myelosuppression (46% neutropenia, 5.4% febrile neutropenia), fatigue (24%), vomiting (15%), diarrhea (13%), and sensory neuropathy (9%). The efficacy results showed remarkable responses of 31% (vs 9% with gemcitabine), PFS of 6.4 months (vs 3.3 months, HR =0.47, P<0.001), and median OS of 11.1 months (vs 6.8 months, HR 0.57, P<0.001), clearly setting FOLFIRINOX as one of the most effective first-line treatment options for good performance status patients younger than 76 years old. Nevertheless tolerability remains a big issue, and as many as a third of patients discontinue treatment early for toxicity reasons. FOLFIRINOX modifications have been implemented clinically in most practices, often with the omission of the 5-FU bolus and/or dose reductions for irinotecan, as well as growth factor support. Prospective studies with modified FOLFIRINOX will be able to define its efficacy, but interim reports do not seem to indicate detrimental effects compared to the standard regimen.

The activity of FOLFIRINOX in advanced disease led to its use as a means of cancer downstaging to increase resectability rates for locally advanced pancreatic cancer (LAPC) and borderline resectable disease. Local institutional studies, mostly retrospective, report that FOLFIRINOX confers response rates of 27% or higher, PFS of 11–12 months, and resectability rates of approximately 10%–20% for patients with LAPC, but longer term survival data is not available.

For patients with resected pancreatic cancer, the UNICANCER group in France will test adjuvant FOLFIRINOX vs gemcitabine, and the study is planned to complete a 490-patient accrual in 2018.

**Radiotherapy**

The role of chemoradiotherapy is still debated for both resectable and LAPC pancreatic adenocarcinomas. While it is clear that pancreatic cancer is an inherently systemic disease, up to 30% of patients die with local progression. In the past two decades, two randomized European studies (EORTC and ESPAC-1) concluded that radiation therapy offers no benefit after pancreatic cancer surgery. Currently the RTOG 0848 trial (NCT01013649) is studying the role of adjuvant radiotherapy in addition to gemcitabine vs gemcitabine alone after resection. For LAPC disease, similar controversy exists. The LAP-07 trial (gemcitabine with or without capecitabine-based chemoradiotherapy and with or without erlotinib), did not show a survival advantage when radiotherapy was added after a 4 months chemotherapy induction period. Nevertheless, contemporary studies are testing the addition of chemoradiotherapy to the more effective combination backbones of FOLFIRINOX.
(CONKO-7 trial in Germany due to complete 2018), and gemcitabine/nab-paclitaxel (LAPACT, NCT02301143). DPC4/SMAD4 has been identified as a putative genetic biomarker predictive of local progression when intact, or metastatic dissemination when mutated.\textsuperscript{60} The RTOG1201 Phase III randomized study (NCT01921751) will prospectively test whether the DPC4 gene status will identify LAPC patients more likely to benefit from chemoradiotherapy in addition to gemcitabine/nab-paclitaxel vs gemcitabine/nab-paclitaxel chemotherapy alone.

### New and emerging treatments

Pancreatic cancer tumor behavior is influenced by both the malignant potential of the cancer cells as well as by the host microenvironment defined by fibroblasts, myofibroblasts, pancreatic stellate cells, vascular cells, immune cells, and the extracellular matrix with associated cytokines.\textsuperscript{63}  

#### Immunotherapies

One of the most frequently invoked reasons for the aggressiveness and chemoresistance associated with pancreatic cancer has been its immunosuppressive tumor microenvironment. Preclinical data suggest that the pancreatic cancer mass is represented roughly by 50% immune and inflammatory cells, with an abundance of regulatory T cells (CD4\textsuperscript{+}FoxP3\textsuperscript{*}) and myeloid-derived suppressor cells (MDSC), and macrophages, but a relative lack of cytotoxic T cells.\textsuperscript{64,65} Pancreatic cancer prognosis has been directly associated with the induction of a humoral response to the cancer antigens MUC-1 and mesothelin, as well as with the presence of intratumoral cytotoxic T lymphocytes and helper T cells.\textsuperscript{66} Furthermore, the tumor expression level of the negative immune checkpoint regulator programmed death-1 ligand,\textsuperscript{70} the intratumoral accumulation of Treg,\textsuperscript{71} and the ratio of Treg relative to CD4\textsuperscript{+} lymphocytes\textsuperscript{72} may inversely correlate with prognosis. On these premises, several approaches have been taken to augment antitumor immune recognition of pancreatic cancer (Table 2).

#### Macrophage-targeted therapies

CD40, a tumor necrosis factor receptor superfamily member regulates activation of T-cells and regulates cancer associated inflammation and fibrosis. CD40 activation has been shown preclinically to induce anti-tumor T cell responses. In a clinical trial in metastatic pancreatic cancer with anti-CD40 agonist antibody CP-870,893 with gemcitabine, responses were noted to be associated with tumor macrophage, but not lymphocyte, infiltration.\textsuperscript{73,74} Biologically, the activation of tumor-associated macrophages (TAM) with the induction of a cytotoxic macrophage phenotype was implicated in tumor stroma depletion.\textsuperscript{73} In other preclinical models, suppression of inhibitory TAM with chemokine (C-C motif) receptor 2 or colony-stimulating factor-1 receptor antagonists improved chemotherapy

### Table 2 Targeting the immune system, microenvironment, and tumor stroma

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**Notes:** *Statistically significant; Study in borderline/unresectable disease; *study in resectable disease; **14.1 represents median DFS.

**Abbreviations:** CCR2, chemokine (C-C motif) receptor 2; CTLA 4, cytotoxic T-lymphocyte-associated protein 4; DFS, disease-free survival; FOLFIRINOX, folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin; GVAX, granulocyte-macrophage colony stimulating factor vaccine; hTERT, human telomerase reverse transcriptase; mos, months; n/a, not available; NR, not reached; OS, overall survival; PEGPH20, preclinically pegylated hyaluronidase; PFS, progression-free survival.
response and increased anti-tumor T cell responses. Given that TAM may manifest both pro- and anti-tumor effects, a productive macrophage-directed immunotherapy needs to induce anti-stroma and anticancer activity. Wang-Gillam et al reported on PF-04136309, a novel chemokine (C-C motif) receptor 2 inhibitor capable of depleting inflammatory immunosuppressive TAM, in combination with FOLFIRINOX in borderline and LAPCs. The combination therapy was safe and among 23 evaluable patients, response rates were 52%, but long-term outcomes were not available. These preliminary data about targeting the TAM component of the tumor microenvironment have promising results and should be further explored.

Cytotoxic T-lymphocytes-targeted therapies
Inducing an immunoreactive environment may be accomplished by stimulating endogenous CD8 T cells via adoptive T cell therapies, cancer vaccines, or interferons (IFNs), targeted depletion of inhibitory MDSC, checkpoint blockade inhibitors against the programmed cell death 1, the programmed cell death ligand-1, against indoleamine 2,3-dioxygenase, and against the cytotoxic T-lymphocyte-associated protein 4.

Immune checkpoint inhibitors
Possibly because of a reduced pancreatic cancer neo-antigenic potential, to date clinical trials with checkpoint blockade inhibitors alone (programmed cell death ligand-1 and cytotoxic T-lymphocyte-associated protein 4) have not demonstrated significant activity in pancreatic cancer. Nevertheless, combinatorial approaches of checkpoint blockade inhibitors plus vaccines (NCT02243371), with agents that target immunosuppressive cell populations (Treg, MDSC), and with chemotherapy (NCT02309177, NCT01313416) or radiotherapy (NCT02305186) are actively being pursued.

Adoptive T cell therapy
Adoptive immunotherapy utilizing patient’s own T cells expanded and stimulated ex vivo against various tumor antigens is currently being tested. In refractory pancreatic cancer patients, pilot data with cytokine-induced T cells showed safety and PFS rates of 11 weeks and OS of 27 weeks, comparable with second-line chemotherapy regimens. Genetic engineering of T cells to enhance the expression and affinity of tumor-antigen specific receptors or to render them refractory to inhibitory signals may bring promise to the immunotherapy field in pancreatic cancer.

Cancer vaccines
Cancer vaccines can stimulate and mature dendritic cells for specific tumor antigen presentation with the ultimate goal for activating the adaptive immune response with effective cytotoxic T lymphocytes. Several types of cancer vaccines have been tested in pancreatic cancer: 1) whole-cell vaccines engineered to overexpress a certain epitope; 2) peptide vaccines; and 3) DNA vaccines, in which DNA coding a target antigen is inserted into a vector which is taken up by tumor cells.

Several whole cell vaccines have reported results in pancreatic cancer. Algenpantucel-L is an irradiated combination of two human allogeneic pancreatic cancer cell lines HAPa1 and HAPa2 genetically engineered to express the murine enzyme α-1,3-galactosyl transferase (αGT). Humans naturally do not express αGal epitopes but possess large amounts of anti-αGal antibodies; therefore, vaccination with algenpantucel-L results in “hyperacute rejection” based on complement-mediated lysis and antibody dependent cell mediated cytotoxicity (ADCC) against the allogeneic vaccine cells. Exposure to the entire vaccine cells antigenic repertoire results in the activation of the immune system, similar to that observed with transplant rejection. Hardacre et al reported on a Phase II study (NCT00569387) of adjuvant algenpantucel-L with gemcitabine and 5-FU based chemoradiotherapy after surgery for pancreatic cancer patients. The 1-year survival was 86%, which compared favorably with the adjuvant RTOG-9704 study (1-year survival 69%), the 1-year disease-free survival was 62%, and the treatment was well tolerated. Higher vaccine doses (300 million cells/dose) resulted in superior results (96% 1-year survival). Biomarker studies noted that patients who mounted an anti-membrane-bound recombinant mesothelin response (31% of patients) or anti-calreticulin antibody response (48% of patients) had improved OS (42 months vs 20 months, P=0.027, and 35.8 months vs 19.2 months, P=0.03, respectively, A Phase III study (NCT01072981) with algenpantucel-L and chemoradiotherapy for patients with resected pancreatic cancer completed accrual, and results are eagerly awaited. Currently, New Link Genetics is conducting a Phase III study of algenpantucel-L with FOLFIRINOX or gemcitabine/nab-paclitaxel plus chemoradiotherapy in borderline and LAPC (NCT01836432).

The GVAX pancreas vaccine is another allogeneic whole cell vaccine transfected with granulocyte-macrophage colony-stimulating factor which acts as a maturation factor for the antigen presenting cells/dendritic cells. GVAX vaccine has been studied with chemoradiotherapy after resection...
of pancreatic cancer, and showed modest median disease-free and OS rates of 17.3 and 24.8 months, respectively.\textsuperscript{86} For patients with metastatic pancreatic cancer, a randomized Phase II study tested the GVAX vaccine combined with a boost live-attenuated \textit{Listeria monocytogenes} (Lm) vaccine modified to deliver the pancreatic tumor antigen mesothelin (CRS-207), vs GVAX alone.\textsuperscript{87} Among 90 patients, 50\% previously treated with 2+ lines of chemotherapy, median OS was 6.1 vs 3.9 months for the GVAX +/- CRS-207 vaccine therapy, and toxicity was manageable. A larger Phase IIb randomized multicenter 3-arm trial of GVAX plus CRS-207, vs CRS-207 alone vs chemotherapy is ongoing in patients with refractory metastatic pancreatic adenocarcinoma (ECLIPSE, NCT02004262). GVAX pancreas has also been combined with ipilimumab (cytotoxic T-lymphocyte-associated protein 4 antibody) in a randomized study vs ipilimumab alone, for patients with previously treated locally advanced or metastatic pancreatic cancer.\textsuperscript{88} OS rates were 5.7 months with the combination, and 3.6 months with ipilimumab alone. In both of the GVAX pancreas vaccine studies referenced above, an immunological T cell response to mesothelin has been associated with increased OS.\textsuperscript{87,88} Building upon the results with ipilimumab combined with GVAX OS in refractory pancreatic cancer, the Johns Hopkins’ group is leading a randomized Phase II trial of FOLFIRINOX for 8–12 doses induction chemotherapy followed by GVAX vaccine plus ipilimumab every 3 weeks $\times$4, then every 8 weeks, vs continuing FOLFIRINOX until progression or toxicity (NCT01896869).

Dalglish et al reported on IMM-101, a heat-killed whole cell vaccine of \textit{Mycobacterium obuense} able to induce a systemic immune response, combined with gemcitabine among 110 untreated patients with advanced pancreatic cancer, and observed OS of 7.2 months vs 5.6 months with gemcitabine alone ($P=0.022$, HR $=0.60$), and a similar improvement of PFS 4.4 vs 2.3 months ($P<0.001$, HR $=0.40$).\textsuperscript{95} While statistically significant, these results need to be reproduced in combination with contemporary chemotherapy regimens in larger randomized studies.

Peptide vaccines are based on cancer-specific peptides capable of binding human leukocyte antigen class molecules and activating a CD4/CD8 immune response. Mutant KRAS peptide vaccines have been evaluated after surgical resection of pancreatic cancer and conferred average survival rates of 27 months, and 5-year survival rates of 20\%, comparable with historical adjuvant chemotherapy data.\textsuperscript{96,97} Telomerase is a ribonucleotide enzyme which maintains telomeres and confers cancer cells immortality. The telomerase peptide vaccine GV1001 while promising in a Phase I/II study,\textsuperscript{92} failed to improve survival (median OS 7–8 months in all arms) when combined sequentially or concurrently with gemcitabine/capecitabine chemotherapy vs chemotherapy alone in the Phase III randomized TeloVac trial.\textsuperscript{93} To date, the overall benefit conferred by pancreatic cancer vaccines is still under investigation, as no large study has shown convincing results. Nevertheless, rational synergistic immuno-therapeutic approaches are ongoing, and results are eagerly awaited.

\textbf{Interferon}

IFNs have been studied since 1990s in various immunoresponsive malignancies such as malignant melanoma and renal cell carcinomas, but also in pancreatic adenocarcinomas based on preclinical evidence of immunostimulatory properties, and chemosensitization.\textsuperscript{94} Type I IFN, both $\alpha$ and $\beta$ demonstrated inhibition of pancreatic cancer cells growth by induction of apoptosis and cell cycle arrest.\textsuperscript{95,96} Another mechanism invoked in IFN-mediated suppression of pancreatic tumor growth and metastasis has been the modulation of the host tumor response through increased nitric oxide production.\textsuperscript{95} Clinically, the role of IFN$\alpha$ has been debated, between reports of increased clinical activity in institutional series, and detrimental toxicity without statistical survival benefit in the larger Phase II and III studies. Picozzi et al initially reported on 43 patients treated with adjuvant 5-FU, cisplatin, and IFN$\alpha$-2bplus radiotherapy followed by adjuvant 5-FU in patients with resected pancreatic adenocarcinoma, and 5-year OS rates were 55\%.\textsuperscript{98} Updated data after a median follow up of 45 months showed median disease-free survival of 22 months, median OS of 42 months, with 5- and 10-year OS rates of 42\% and 28\%, respectively.\textsuperscript{99} Efficacy results with this regimen were not reproduced by the multicenter ACOSOG Z05031 study, which noted median disease-free survival and OS of 14.1 and 25.4 months, respectively, but all-cause grade $\geq$3 toxicity was 95\% resulting in patient accrual stopping early.\textsuperscript{100} Furthermore, the CapRI randomized Phase III study comparing adjuvant chemoimmunoradiotherapy vs 5-FU/leucovorin noted no significant survival difference (median OS 32 vs 28 months, and 2-year OS 62\% vs 52\%, $P=0.49$), but significantly higher toxicities.\textsuperscript{101}

\textbf{Stromal disruption}

Tumor stroma is an active component in the biology of pancreatic cancer. The quest of therapies capable of inducing stromal collapse and easing chemotherapy access inside fibrotic pancreatic tumors has shown mixed results to date.
Targeting matrix metalloproteinases has been ineffective and inhibiting stromal related angiogenic factors such as the VEGF and receptor VEGFR or the platelet-derived growth factor/receptor demonstrated no activity when combined with gemcitabine. It is unclear whether in combination with other chemotherapy backbones like FOLFIRINOX or nab-paclitaxel anti-angiogenic agents may prove more successful.

One of the most exciting and anticipated stroma-directed approaches in pancreatic cancer has been the targeting of hyaluronic acid (HA). HA poses a physical barrier within the pancreatic tumor extracellular matrix by creating very high interstitial pressure, and therefore vascular compression and hypoperfusion. Preclinically pegylated hyaluronidase (PEGPH20), an enzyme capable of depleting HA, reduced the interstitial pressure, leading to increased activity of gemcitabine in the KPC GEMM model. Similar results were observed in a Phase I/II clinical trial when PEGPH20 was combined with gemcitabine as first-line treatment among 28 patients with metastatic pancreatic cancer. The toxicity profile included muscle spasms (54%), myalgias (39%), and arthralgias (29%), among the most common toxicities, but most were grade 1–2. Overall, while median PFS and OS were 154 and 219 days, in an exploratory analysis patients with high intratumoral HA content (HA(high)) showed median PFS of 219 days (95% CI: 159–276) and OS of 395 days (95% CI: 210–578). PEGPH20 is currently being tested in two national randomized Phase II clinical trials in combination with gemcitabine plus nab-paclitaxel (NCT01839487), and with FOLFIRINOX (SWOG1313, NCT01959139).

Other components of the tumor stroma, such as the connective tissue growth factor which is overexpressed in pancreatic cancer, are being targeted. FG-3019, a monoclonal antibody against connective tissue growth factor, has been studied with gemcitabine/erlotinib in 75 patients with metastatic pancreatic cancer. The toxicity profile included muscle spasms (54%), myalgias (39%), and arthralgias (29%), among the most common toxicities, but most were grade 1–2. Overall, while median PFS and OS were 154 and 219 days, in an exploratory analysis patients with high intratumoral HA content (HA(high)) showed median PFS of 219 days (95% CI: 159–276) and OS of 395 days (95% CI: 210–578). PEGPH20 is currently being tested in two national randomized Phase II clinical trials in combination with gemcitabine plus nab-paclitaxel (NCT01839487), and with FOLFIRINOX (SWOG1313, NCT01959139).

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This agent is currently being tested as neoadjuvant therapy with gemcitabine/nab-paclitaxel for LAPC in an attempt to reduce the fibrotic stroma and enhance the chemotherapy efficacy (NCT02210559). The tumor growth factor beta (TGF-β) activates pancreatic stellate cells to become activated myofibroblasts, responsible for the formation of the extracellular matrix, a component of the pancreatic cancer stroma, and also acts as an immunosuppressor. Targeting the TGF-β pathway is undergoing clinical trials in solid tumors including pancreatic cancer.

**Targeting core pathways**

Several critical signaling pathways have been identified spanning the pancreatic cancer genomic map. Jones et al, Biaunik et al, and others characterized key genetic mutations and RNA transcripts which occur with most propensity in pancreatic tumors. Among the 12 key pathways encompassing an average of 63 genetic mutations per tumor, genetic alterations have been found in the DNA repair, apoptosis, G1/S cell cycle transition, KRAS, Wnt, Notch, and Hedgehog (Hh) signaling, TGF-beta, chromatin remodeling (ARID1A and MLL3 genes) and other cell invasion pathways, which are the target of “precision therapeutics”.

To date, despite several targeting attempts with farnesyltransferase inhibitors no single agent has successfully targeted mutated KRAS, possibly because of its complex crystallographic nature. The KRAS effector pathways, PI3K (phosphatidylinositol 3-kinase)/AKT (alpha serine/threonine-protein kinase)/mTOR (mammalian target of rapamycin) and RAF (rapidly accelerated fibrosarcoma)/MEK/MAPK (mitogen-activated protein kinase) have been targeted in several clinical trials in patients with metastatic pancreatic cancer, and as single agents they do not have significant activity (PFS 2 months and OS 5 months in second line setting). Preclinical data suggest that MEK inhibitors may be active only in certain KRAS mutational subsets (KRAS V12 mutation and KRAS copy number variation—gains or loss, are resistant to MEK inhibitors). Others suggest that MEK inhibitors alone or in combination with EGFR pathway inhibition may be effective in epithelial but not mesenchymal subtypes of pancreatic cancer. Furthermore, MEK inhibitors alone result in feedback activation of the EGFR and PI3K pathways, thus combination strategies are expected to be more effective. Indeed, MEK inhibitors in combination with gemcitabine have not demonstrated superior results vs gemcitabine alone in clinical trials, with PFS of 4–6 months and OS rates of 6–9 months, but a subgroup of KRAS wild-type patients demonstrated higher than expected PFS and OS (9 and 18 months respectively). Dual MEK and EGFR inhibition showed median PFS and OS of 2.6 and 7.5 months, respectively in the second line treatment of metastatic pancreatic cancer. The combination of PI3K/mTOR and MEK inhibitors is synergistic preclinically, but clinical data to date show only modest activity when these pathways are concomitantly blocked (disease stabilization in 30%–50% of patients as best response, PFS and OS of 2 months and 5 months
Chronic inflammation contributes to pancreatic cancer carcinogenesis. Deregulated cytokines like interleukin (IL)-6, and respectively). Results of the SWOG S1115 randomized Phase II trial (NCT01658943) with MEK plus AKT inhibition (MEK inhibitor selumetinib AZD6244, and AKT inhibitor MK2206) vs folinic acid, 5-FU, and oxaliplatin (FOLFOX) in second line treatment for metastatic pancreatic cancer will be presented at ASCO 2015. mTOR inhibitors alone or in combination with EGFR blockade similarly have not demonstrated notable activity in pancreatic cancer. Despite the general lack of meaningful activity even in combination with chemotherapy, there has been interest in PI3K/mTOR blockade for select patients who manifest an upregulation of this pathway, such as patients with TSC (tuberous sclerosis complex) gene mutations or with Peutz–Jeghers syndrome, where STK11/LKB1 gene mutations can lead to inactivation of the LKB1/AMPK/TSC signaling with subsequent mTORC1 activation. With several mechanisms involved in resistance to PI3K/AKT/mTOR and MEK inhibitors, including feedback loop activation of parallel pathways, and EGFR or HER3 upregulation, it seems that best targeted treatment strategies with these agents are yet to be identified for patients with epithelial vs mesenchymal subtypes, and for those with various types of KRAS mutations. While targeting the EGFR with erlotinib provided marginal benefit in combination with gemcitabine in the NCIC Phase III trial, cetuximab did not increase survival (6.3 vs 5.9 months, P=0.23) when added to gemcitabine in an unselected population. Several other trials reported on erlotinib in combination with other chemotherapy backbones and/or biologics such as insulin growth factor receptor 1 inhibitors or VEGFR inhibitors, and had modest results (Table 3). Another member of the EGFR pathway, the HER2 (neu/ERBB2) gene rarely exhibits amplification in pancreatic cancer (2%), but those cases seem to be associated with lung and brain metastases. Several trials tested trastuzumab or lapatinib with chemotherapy in HER2 overexpressing tumors, and none showed improved survival rates (OS 4–7 months). It is possible that better patient selection using genomic profiling such as the one used in the IMPaCT trial (Australasian Gastrointestinal Trials Group ACTRN12612000777897), may show improved results.

Another stumbling block showing discrepant results from tumor models occurred when Hh pathway was targeted with smootherned inhibitors. Despite promising evidence of Hh blockade causing depletion of cancer associated fibroblasts and improving perfusion in preclinical models, clinical trials with Hh inhibitors and chemotherapy did not demonstrate improved results compared to chemotherapy alone – vismodegib (GDC0449) plus gemcitabine showed PFS of 2.8–3.7 months and OS of 5.3–6.3 months (vs PFS 2.4 months and OS 5.4 months for gemcitabine alone), and in a recent trial in combination with gemcitabine/nab-paclitaxel PFS and OS rates were 5.5 and 10 months, respectively. The randomized Phase II trial of saridegib (IPI-926) with gemcitabine was terminated early due to lack of efficacy (OS >6 months in the gemcitabine plus placebo arm, and OS <6 months in gemcitabine plus IPI-926 arm) (NCT01130142), and a Phase I trial of saridegib with FOLFIRINOX (NCT01383538) is awaiting final results. One possible reason for the lack of activity of Hh inhibitors in pancreatic cancer could be the increased metastatic potential of cancer cells in the absence of a contained fibroelastic lattice, and possibly the increased perfusion within the tumor bed. The depletion of cancer associated fibroblasts has been recently associated with worse cancer aggressiveness, epithelial–mesenchymal transition (EMT), increased cancer stem cells, and reduced survival in pancreatic cancer mouse models. It is now apparent that cancer stroma can both restrain and enhance tumor growth, and future targeting needs to carefully elucidate its various components contribution to cancer progression.

The Delta–Notch pathway has been recognized as an important member of tumor angiogenesis and a contributor to cellular growth, differentiation, and stem cells self-renewal, including in pancreatic cancer. Preclinically, Notch/Dll4 inhibition has antitumor activity, and preliminary efficacy from Phase Ib studies with tarextumab (OMP-59R5) and MK-0752 show early signs of activity when combined with chemotherapy. A Phase II study of single agent gamma-secretase inhibitor RO4929097 in previously treated advanced pancreatic cancer had poor results, with PFS and OS of 1.5 and 4.1 months, respectively, and the study accrual stopped early (NCT01232829). The novel Dll4 inhibitor demcizumab (OMP-21M18) in combination with gemcitabine and nab-paclitaxel showed encouraging PFS of 5.9 months in a Phase Ib study, and it is currently being tested in a larger randomized Phase II study (Onconova, NCT0289898). The emerging cardiovascular toxicity (such as pulmonary hypertension) seen with Dll4 inhibitors may be addressed by limiting treatment exposure to less than 100 days.

The Wnt-β-catenin developmental pathway has been implicated in pancreatic cancer progression, and cancer stem cells maintenance. PRI-724, a cAMP-response element-binding protein/β-catenin modulator which induces stem cells differentiation, is currently being tested in combination with gemcitabine in the second line treatment of metastatic pancreatic cancer (NCT01764477). Chronic inflammation contributes to pancreatic cancer carcinogenesis. Deregulated cytokines like interleukin (IL)-6, and
### Table 3 Targeted therapies to signaling pathways

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<td>Cook et al(^{57})</td>
<td>29</td>
<td>Gemcitabine + MK-0752</td>
<td>5.0</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Cubilo Gracian et al(^{58})</td>
<td>24</td>
<td>Gemcitabine/nab-paclitaxel + demcitumab</td>
<td>25.0</td>
<td>5.9</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>JAK/STAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurwitz et al(^{127})</td>
<td>127</td>
<td>Capecitabine + ruxolitinib</td>
<td>8.0</td>
<td>1.7</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Capecitabine + placebo</td>
<td>1.6</td>
<td>1.6</td>
<td>4.3</td>
</tr>
</tbody>
</table>

**Notes:** *Statistically significant; 13-month PFS.*

**Abbreviations:** EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor; IGF-1, insulin-like growth factor; JAK, Janus kinase; MEK, mitogen-activated protein kinase; mos, months; mTOR, mammalian target of rapamycin; n/a, not available; OS, overall survival; PFS, progression-free survival; PI3K, phosphatidylinositol 3-kinase; STAT, signal transducer and activator of transcription; VEGFR, vascular endothelial growth factor receptor.
IL-1α with activation of downstream effectors signal transducer and activator of transcription (STAT)-3 and NF-kB lead to recruitment of proinflammatory cells from bone marrow to the pancreatic tumor stroma. Inflammatory signaling through STAT3, NFκB, and cyclooxygenase-2 (COX-2) also leads to pancreatic stellate cells proliferation, induces desmoplasia and promotes cancer stem cells.\textsuperscript{160–162} While COX-2 is frequently upregulated in pancreatic adenocarcinoma (50%–60%), clinically COX-2 inhibition did not provide additive benefits to chemotherapy.\textsuperscript{164,165} Another strategy targeting the inflammatory environment is by blocking the STAT and Janus kinase (JAK) pathways which have lead roles in cancer inflammation and immunity.\textsuperscript{160,161,166} Ruxolitinib, an oral inhibitor of JAK1 and JAK2 signaling, has been evaluated in combination with capecitabine in the randomized Phase II RECAP trial for second line treatment of pancreatic cancer.\textsuperscript{167} The addition of ruxolitinib conferred a slightly improved survival vs capecitabine/placebo (median OS 137 vs 130 days, HR = 0.79, \(P=0.25\)), but this effect was limited to a subset of patients with higher C-reactive protein levels (\(>13\) mg/L): median OS 83 vs 55 days, and 6-months OS 42% vs 11% (HR 0.47, \(P=0.01\)) (NCT01423604). The Phase III randomized Janus 1 study with this combination including only patients with CRP levels >10 mg/L is ongoing (NCT02117479).

A summary of targeted therapies against various signaling pathways is provided in Table 3.

Pancreatic cancer is characterized by genomic instability,\textsuperscript{110,111} and several DNA repair defects occur through mutations in DNA mismatch repair gene MLH1 (MutL homolog 1, 3%–15% incidence), tumor suppressor genes TP53 (50% incidence), and the Fanconi anemia pathway genes BRCA1/2 (breast cancer genes 1,2) and PALB2 (partner and localizer of BRCA2, 7% incidence in sporadic, and up to 17% in familial cases),\textsuperscript{168–171} as well as FANCC and FANCQ (5%–10%),\textsuperscript{172–174} Other key factors and pathways in DNA repair including ATM/Chk2, ATR/Chk1, Rad51, ERCC1, and PTEN, can be mutated or inactivated in pancreatic cancer.\textsuperscript{175–177} Impaired DNA damage response pathways in pancreatic cancer create vulnerabilities that can be exploited therapeutically, and DNA cross-linking agents such as platinum and mitomycin C, topoisomerase inhibitors like irinotecan, and poly ADP-ribose polymerase (PARP) inhibitors are of particular interest.\textsuperscript{178} To date PARP inhibitors have demonstrated clinical activity as single agents in pancreatic cancers harboring BRCA1/2 or PALB2 mutations: olaparib demonstrated 22% response rates and median PFS and OS rates of 4.6 and 9.8 months, respectively in patients who received at least two prior lines of therapy, while veliparib showed 31% 4 months + stable disease rate, and a median PFS of approximately 2 months in refractory disease.\textsuperscript{179–181} Veliparib in combination with FOLFOX,\textsuperscript{182} gemcitabine/cisplatin\textsuperscript{183} or folinic acid, 5-FU, and irinotecan (FOLFIRI)\textsuperscript{184} chemotherapy in first or second line setting demonstrated sustained responses in BRCA mutated or non-mutated patients, and response rates were 14%–56%, and OS rates were up to 7.7 months. Clearly the DNA-repair pathway should be further explored and genomic characterization beyond BRCA1/2/PALB2 status should be determined for a “personalized” approach in a larger patient population.

Another hallmark of cancer is altered gene expression by variations that are independent from changes in DNA sequence.\textsuperscript{185–187} In pancreatic cancer, epigenetic dysregulation of tumor-associated genes, both tumor suppressors and oncoproteins, can occur via DNA methylation, histone acetylation or microRNA (miRNA) expression. Epigenetic alterations have been implicated both in pancreatic cancer development and progression.\textsuperscript{188} Some the most common DNA methylation changes involve the promoter hypermethylation and silencing of the mismatch repair gene MLH1 (linked to the medullary subtype of pancreatic cancer),\textsuperscript{190} or of the CDKN2A/p16 gene,\textsuperscript{190} but several other methylation aberrations have been already reported.\textsuperscript{191} Histone acetylation can upregulate genes such as c-MYC,\textsuperscript{192} while deacetylation (with histone deacetylases – HDAC1 and HDAC2) results in transcriptional repression. HDAC1 can accelerate EMT and pancreatic cancer metastasis,\textsuperscript{193} and HDAC2 has been responsible for resistance to DNA damage.\textsuperscript{194} HDAC inhibitors were noted to have preclinical activity in pancreatic cancer mouse models by reinducing gene expression.\textsuperscript{195} Several miRNAs such as miRNA34a, a transcriptional target of p53, have been implicated in pancreatic tumor invasiveness, EMT, and stem cell maintenance.\textsuperscript{196,197} In pancreatic cancer cell lines re-expression of miRNA-34a after 5-aza-2′-deoxycytidine (Aza-dC) inhibited cell proliferation, cell cycle progression, EMT, and invasion.\textsuperscript{196} As many as 64 different miRNAs have been reported to be deregulated in pancreatic cancer, and many were associated with increased cancer risk or poor outcomes, thus could serve as future biomarkers.\textsuperscript{198} Epigenetically targeted therapeutics, including hypomethylating agents (eg, azacytidine, decitabine), and HDAC inhibitors (eg, vorinostat, belinostat, entinostat, panobinostat) have been explored in solid tumors clinical trials including pancreatic cancer patients, but miRNA-targeted therapeutics are not yet available.\textsuperscript{199,200} To date, despite promising preclinical data, HDAC inhibitors have not manifested meaningful efficacy in pancreatic cancer.\textsuperscript{201–204} CC-486 (oral azacytidine) is currently being tested in the adjuvant setting among high-risk-node-positive patients after pancreatic cancer resection and completion of adjuvant gemcitabine (NCT01845805).
Novel chemotherapies/formulations

MM-398 (Merrimack Pharmaceuticals) is a nanoparticle liposomal irinotecan formulation with improved biodistribution and pharmacokinetics which had been studied as monotherapy in second line treatment for pancreatic cancer, and showed moderate efficacy with PFS and OS rates of 2.4 and 5.2 months, respectively. The international Phase III NAPOLI 1 trial in second line metastatic pancreatic cancer tested MM-398 (80 mg/m² iv over 90 minutes) with 5-FU every 2 weeks, vs 5-FU alone, vs MM-398 (120 mg/m² iv over 90 minutes) every 3 weeks. MM-398 alone was not active, but in combination with 5-FU showed 16% response rates and it increased PFS and OS compared to 5-FU alone (PFS 3.1 vs 1.5 months, P=0.0001, and OS 6.1 vs 4.2 months, HR 0.57, P=0.0009).

Another novel chemotherapy, TH-302 is a hypoxia activated cytotoxic DNA cross-linking agent which has been tested with gemcitabine in a randomized Phase II study, and showed interesting activity at the 340 mg/m² weekly dose x3/4 weeks: response rates of 26%, median PFS 6 months and OS 9.2 months, compared to gemcitabine: 12% response, PFS and OS 3.6 and 6.9 months, respectively. Based on these encouraging results, the global Phase III MAESTRO trial (NCT01746979) of gemcitabine with and without TH-302 is currently ongoing, and due to preclinical synergy, a Phase Ib/II study of TH-302 with gemcitabine/nab-paclitaxel (NCT02047500) is also recruiting patients.

Conclusion

There currently are several promising therapies in pancreatic adenocarcinomas, between novel chemotherapeutics, stroma- and immune-targeted agents. While many obstacles still exist, such as defining biomarkers of benefit from signaling pathways inhibitors, and fostering an optimal immunological response in the context of an immunosuppressive tumor environment, clearly we are making progress in deciphering the heterogeneity within pancreatic cancers. Integrating conventional and immunological targeting will be the key to effective treatment of this deadly disease.

Disclosure

The authors have declared no conflict of interest.

References


