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**Molecular therapeutics in pancreas cancer**

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**Abstract**

The emergence of the “precision-medicine” paradigm in oncology has ushered in tremendous improvements in patient outcomes in a wide variety of malignancies. However, pancreas ductal adenocarcinoma (PDAC) has remained an obstinate challenge to the oncology community and continues to be associated with a dismal prognosis with 5-year survival rates consistently less than 5%. Cytotoxic chemotherapy with gemcitabine-based regimens has been the cornerstone of treatment in PDAC especially because most patients present with inoperable disease. But in recent years remarkable basic science research has improved our understanding of the molecular and genetic basis of PDAC. Whole genomic analysis has exemplified the genetic heterogeneity of pancreas cancer and has led to ingenious efforts to target oncogenes and their downstream signaling cascades. Novel stromal depletion strategies have been devised based on our enhanced recognition of the complex architecture of the tumor stroma and the various mechanisms in the tumor microenvironment that sustain tumorigenesis. Immunotherapy using vaccines and immune checkpoint inhibitors has also risen to the forefront of therapeutic strategies against PDAC. Furthermore, adoptive T cell transfer and strategies to target epigenetic regulators are being explored with enthusiasm. This review will focus on the recent advances in molecularly targeted therapies in PDAC and offer future perspectives to tackle this lethal disease.

**Key words:** Pancreas neoplasm; Targeted therapy; Immunotherapy; Vaccines; *KRAS*

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**Core tip:** The treatment of pancreas ductal adenocarcinoma is in an exciting phase due to a tremendous surge in knowledge regarding the molecular mechanisms that underlie pancreas cancer that has fueled interest in devising novel strategies to target signal transduction factors downstream to *KRAS*, desmoplastic tumor stroma and cancer stem cells. Furthermore, immunotherapy by utilizing vaccines and immune checkpoint inhibitors is gaining momentum. Alluring results from studies evaluating molecularly targeted therapies have not only proven the feasibility of this approach but are also indicative of a paradigm shift in management of pancreatic cancer in the near future.

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**INTRODUCTION**

Over the past few decades, pancreas ductal adenocarcinoma (PDAC) has claimed notoriety by proving to be one of the most recalcitrant solid-organ malignancies. As a telltale sign of its lethality, PDAC accounts for less than 3% of new cancers diagnosed annually in developed nations and in the United States, yet it is the fourth leading cause of cancer related mortality[1]. Ominously, PDAC is also poised to surpass breast, prostate and colon cancers to become the second leading cancer related cause of death by 2030[2]. Owing to the late stage at presentation, most patients with PDAC are not candidates for surgical resection. Even patients with early-stage disease who undergo surgical resection and adjuvant therapy eventually relapse and succumb to it[3,4].

Patients with advanced disease have a dismal prognosis with 5-year survival rates of less than 5%[5]. Following the initial success of gemcitabine in the metastatic setting[6], oncologists have traditionally relied upon cytotoxic chemotherapy to tackle locally advanced and metastatic disease but with limited success. After nearly two decades of research to identify optimal regimens for metastatic PDAC, the PRODIGE 4/ACCORD 11 and MPACT trials have proven the efficacy of combination chemotherapy with meaningful increase in overall survival (OS) although accompanied by the risk of increased toxicity[7,8]. The median survival for patients with metastatic disease still remains less than 1 year[7,8].

**MOLECULAR THERAPEUTICS IN PDAC**

The dawn of the era of precision-medicine in oncology has led to tremendous gains in understanding various molecular mechanisms of PDAC oncogenesis, but translating this knowledge to the bedside with targeted therapy has been a daunting task. The complex biology of PDAC has posed a formidable challenge against successful targeted interventions (summarized in Table 1). However, in recent years several innovative approaches have achieved early success to pave the way for impactful molecular therapeutic strategies.

**GENETIC HETEROGENEITY OF PDAC**

Similar to the adenoma-carcinoma sequence in colon cancer, the development of PDAC represents the culmination of progressive increments in dysplasia in precursor lesions collectively termed pancreatic intra-epithelial neoplasia (PanIN)[9]. Molecular profiling studies in genetically engineered mouse models (GEMM) have demonstrated that histological progression of PanINs from low to high-grade occurs in tandem with successive accumulation of gene mutations such as activation of the *KRAS* oncogene, inactivation of the tumor suppressor cyclin dependent kinase-N2A (CDKN2A) gene and the eventual inactivation of TP53 and deleted in pancreatic cancer 4 (DPC4/SMAD4) genes[10]. All of the same genetic alterations also occur in established PDAC but at a higher frequency (Table 2). Patients with familial forms of PDAC also harbor germ-line mutations in BRCA2 and partner and localizer of BRCA2 (*PALB2*) genes[11,12]. In a sentinel genomic analysis of 24 pancreatic tumors, Jones *et al*[13] classified the genetic alterations in PDAC into a core set of 12 cellular signaling pathways that encompass an incredibly high 63 gene mutations within an individual tumor. A recent study of 109 micro-dissected pancreatic tumors by whole-exome sequencing corroborated the high mutational burden and also identified other novel genetic mutations that confer adverse prognosis such as MYC amplification[14]. Abnormalities in Wnt and Hedgehog signaling, chromatin remodeling and DNA repair mechanisms occur at a high frequency in PDAC[14,15]. In addition to remarkable variations in genetic abnormalities in individual tumors, the realization that PDAC genes function through a relatively small number of pathways confers a level of genetic heterogeneity that makes molecular targeting exceptionally difficult.

***Targeting KRAS and downstream signal transduction***

The four human *RAS* genes encode for small guanosine triphosphatases (GTPases) and under normal circumstances cycle between an active GTP-bound and an inactive guanosine diphosphate (GDP) bound state[16]. Upwards of 95% of PDACs possess activating mutations of the *KRAS* gene, most commonly at the G12 residue[17]. Mutant *KRAS* remains persistently active in the GTP-bound state and results in uninterrupted downstream signal transduction of growth signals such as rapidly activated fibrosarcoma homolog B (BRAF), mitogen activated protein kinase (MAPK) and phosphatidyl inositol-3 kinase (PI3K)/mammalian target of rapamycin (mTOR)[16].

Despite intensive efforts, direct pharmacologic inhibition of *KRAS* has been unsuccessful because of the high binding affinity of the oncoprotein to GTP and inability to identify an easily accessible active site within *KRAS* that is susceptible to competitive allosteric inhibition[18]. To overcome these difficulties, alternate approaches have been attempted but with limited success. Van Cutsem *et al*[19] attempted inhibition of farnesylation, a crucial step in post-translational modification of *KRAS* proteins that is essential for membrane anchorage of RAS, using the farnesyl-transferase inhibitor tipifarnib in combination with gemcitabine. But no improvement in OS was observed when compared to gemcitabine plus placebo in patients with advanced PDAC. Likewise, other strategies such as dislodging *KRAS* from the plasma membrane and preventing interactions with *KRAS* activating proteins have been effective in pre-clinical models but are yet to be translated to the clinical setting[20,21].

Substantial efforts have also been devoted to inhibition of downstream signal transduction, especially the PI3K and MAPK (RAF/Mek/ERK) pathways, as they are more amenable to pharmacological inhibition[22–24]. Disconcertingly, this strategy has proven unsuccessful because inhibition of the MEK pathway resulted in feedback activation of the PI3K pathway mediated by the epidermal growth factor receptor (EGFR)[25]. To counter this Ko *et al*[26] investigated the effect of dual inhibition of EGFR and MEK with erlotinib and selumetinib respectively. In this phase II non-randomized trial of 41 patients with chemotherapy refractory PDAC, 26% had stable disease for 12 wk or more and 38% of patients had a greater than 50% decline in CA 19-9 levels[26]. Though this combination approach showed promise, it needs to be validated in larger studies.

***Targeting BRCA2 and PALB2***

The main role of the tumor suppressor genes *BRCA2* and *PALB2* is to repair double stranded DNA breaks by homologous recombination. In patients with germ-line mutations of these genes, DNA repair occurs by alternate means, predominantly by base-excision repair mediated by the enzyme poly(ADP-ribose) polymerase (PARP). Inhibition of PARP in patients with BRCA mutations renders tumor cells incapable of repairing DNA damage and cell death ensues. This concept is known as “synthetic lethality” and represents a great example of targeted therapy in PDAC[27].

In a preclinical study, the PARP inhibitor 3-aminobenzamide in combination with gemcitabine showed strong anti-tumor activity by inducing apoptosis in PDAC cell lines[28]. Remarkably, neoadjuvant iniparib plus gemcitabine induced a complete pathological response in a patient with recurrent PDAC harboring the BRCA2 mutation[29]. In a phase I study of olaparib plus gemcitabine in patients with advanced solid tumors that also included 15 patients with PDAC, no differences in efficacy endpoints were noted with the combination[30]. However, these patients were genetically unselected and were unable to receive full-dose gemcitabine due to myelotoxicity attributed to olaparib. The role of *PARP* inhibitors in PDAC might thus be limited to patients with *BRCA2* mutations. The combination of PARP inhibitors with cytotoxic chemotherapy is also being investigated (ClinicalTrials.gov identifiers: NCT01585805 and NCT01296763, Table 3).

**OVEREXPRESSION OF GROWTH-FACTOR RECEPTORS ON TUMOR CELLS**

PDAC cells overexpress the EGFR and its ligand transforming growth factor-α (TGF-α)[31]. In transgenic mouse models, EGFR signaling mediated by TGF-α has been shown to be essential for the onset of ductal metaplasia, a precursor lesion that progresses to PanIN and eventually to PDAC[32]. EGFR signaling is perceived to be a vital cog in mediating the oncogenic effects of *KRAS* as evidenced in mouse models wherein genetic or pharmacological inhibition of EGFR signaling eliminates tumorigenesis[33,34]. The exact mechanism by which EGFR overexpression contributes to the development of PDAC is unclear but could be related to induction of the Notch pathway[35]. EGFR overexpression is also associated with increased propensity for liver metastasis and poor prognosis[36]. Paradoxically, despite compelling pre-clinical evidence, the EGFR inhibitor erlotinib showed only a marginal clinical benefit for patients with advanced PDAC by prolonging OS by a mere 2 wk[37]. It is hypothesized that EGFR signaling might be essential earlier in tumorigenesis while advanced PDAC cells escape EGFR dependence[38].

***Targeting insulin-like growth factor-1 receptor***

PDAC cells also overexpress insulin-like growth factor-1 receptor (IGF-1R)[39]. IGF-1R signaling promotes tumorigenesis by activating PI3K, MAPK, AKT and Rac pathways[40]. This results in uncontrolled cellular proliferation, survival and metastasis. Based on exciting and positive results from preclinical[41] and early-phase trials[42,43]. ganitumab, a fully humanized monoclonal antibody against IGF-1R combined with gemcitabine was investigated in a phase III, double-blind, placebo-controlled randomized controlled trial (RCT) as first-line therapy for patients with metastatic PDAC[44]. Though the ganitumab-gemcitabine combination was safe, the study was terminated early based on results of a pre-planned futility analysis which revealed no improvement in the primary objective of OS, and the reason for lack of efficacy is yet unclear[44]. Inhibition of IGF-1R using small interfering RNA (siRNA) had an anti-proliferative effect on HPAC and Panc-1 pancreatic cancer cell lines invoking the possibility of a novel target for future clinical studies[45].

**DESMOPLASTIC STROMA**

The stroma of PDAC is characterized by an intense fibrotic reaction termed “desmoplasia”[46]. This is attributed to collagen, laminin, fibronectin, hyaluronan and various other components of the extracellular matrix (ECM) secreted by activated pancreatic myofibroblasts (stellate cells) in response to stimuli from TGF-β, platelet derived growth factor (PDGF) and fibroblast growth factors (FGF) produced by the tumor microenvironment (TME)[46]. The accumulation of ECM components renders the tumor milieu rigid, and the ensuing increase in extracellular fluid pressure results in collapse of blood vessels in the tumor stroma. The resultant hypoxic peri-tumoral milieu is thus a significant impediment to the effective delivery of chemotherapy to the tumor[47]. Furthermore, matrix metalloproteinases (MMP) produced in the ECM damages the structural integrity of the ECM to self-perpetuate tumor invasion and metastasis[48]. The desmoplastic stroma in PDAC represents an ever-changing compartment that not only functions as a mechanical barrier to drug delivery, but also favors tumorigenesis and invasion.

**STROMAL TARGETING STRATEGIES**

***Pegylated recombinant hyaluronidase***

Hyaluronan is a visco-elastic glycosaminoglycan found in abundance in normal tissues, notably in joint cartilage[49]. It is also found in the stroma of PDAC, where it contributes to significantly elevated interstitial fluid pressure (IFP) and vascular collapse[47]. In the KPC mouse model, enzymatic depletion of hyaluronan with pegylated recombinant hyaluronidase (PEGPH20, Halozyme, San Diego, CA) rapidly normalized the IFP and hence restored normal vascular caliber[50]. Importantly, co-administration of PEGPH20 and gemcitabine resulted in an 83% increase in survival and a dramatic decrease in metastatic burden in mice, owing to the enhanced delivery of gemcitabine to the tumor[50]. Based on the encouraging results from a phase Ib trial that combined PEGPH20 with gemcitabine[51], this strategy is now being investigated in phase II trials in combination with conventional chemotherapy regimens for metastatic PDAC (ClinicalTrials.gov identifiers: NCT01959139 and NCT01839487, Table 3). Initial reports demonstrate that patients with high hyaluronan expressing tumors have greater clinical benefit[52].

***Nanoparticle albumin-bound (nab)-paclitaxel with gemcitabine***

Though cytotoxic agents are not considered to be within the realm of targeted therapy, nab-paclitaxel might be an exception. In a small yet novel study, the combination of nab-paclitaxel and gemcitabine was administered to 16 patients in a neo-adjuvant fashion[53]. The effects on tumor stroma were determined by endoscopic ultrasound (EUS) elastography and examination of surgically resected tumor specimens. Not only was there a significant decrease in tumor stiffness on EUS elastography, but also a decrease in cancer associated fibroblasts (CAF) and significant disruption of the intense collagen architecture[53]. Similarly, stromal disruption was also noted in a patient-derived xenograft mouse model treated with the same combination[54]. In this study, genetically engineered mice bearing tumors received nab-paclitaxel, gemcitabine or the combination of the two. The intra-tumoral concentration of gemcitabine was nearly 3-fold higher in mice treated with nab-paclitaxel plus gemcitabine than in those receiving gemcitabine alone. The exact mechanism of action of nab-paclitaxel in depleting tumor stroma has not been elucidated, but could be mediated by secreted protein acidic and rich in cysteine (SPARC) - a matrix glycoprotein and marker of activated fibroblasts[55] proposed to be a crucial driver of PDAC invasiveness[54,56,57].

***Targeting myofibroblasts/stellate cells***

Though the anti-inflammatory properties of 1,25(OH)2D3 have been well established[58], the finding that activated myofibroblasts (also known as stellate cells) overexpress the vitamin D receptor (VDR) was an unexpected finding[59]. The VDR plays an important role in the transcriptional regulation of activated myofibroblasts by converting them back to their quiescent state[59]. This is substantiated by a preclinical study in mouse models in which calcipotriol, a VDR agonist resulted in stromal depletion, facilitated intra-tumoral delivery of gemcitabine and caused reduction in tumor volume[59]. Unlike other therapies that focus on stromal ablation, reprogramming the stroma using vitamin D analogs might be a useful adjunct to PDAC therapy.

***Hedgehog pathway inhibition***

The hedgehog (Hh) signaling cascade activates the Gli family of receptors when the sonic Hh ligands bind to its receptor Patched1 that in-turn relieves the repression on Smoothened1 (Smo)[60]. This paracrine signaling is vital for the proliferation of the desmoplastic stroma in PDAC[60]. IPI-926 is a powerful inhibitor of Smo, which when administered in combination with gemcitabine to KPC mice resulted in increased mean vessel density in the stroma and increased intra-tumoral concentration of gemcitabine[61]. However, when the combination of gemcitabine with IPI-926 resulted in worse progression free survival (PFS) and OS compared to gemcitabine plus placebo in a phase II trial that had to be terminated early[62]. More recent studies have shown a possible protective effect of the stroma, which when depleted resulted in a more aggressive and hypervascular phenotype[63,64]. The incongruity in outcomes between pre-clinical and clinical trials is a fine example to exemplify the complexity of targeting the TME in PDAC.

**ANGIOGENESIS**

Tumor cells can activate quiescent endothelial cells through an “angiogenic switch” which causes overexpression of pro-angiogenic factors, chiefly vascular endothelial growth factor (VEGF)[65]. VEGF and its two high-affinity tyrosine kinase receptors namely flk-1/KDR and flt-1 are overexpressed in PDAC and associated with disease progression[66]. VEGF enhances MAPK phosphorylation in pancreatic cancer cell lines, and PD9805 an inhibitor of MAPK inhibits the proliferative effects of VEGF[67]. In contrast to pancreatic cancer cell lines, actual pancreatic tumors have a much lower microvessel density compared to normal pancreatic tissue[68]. Unsurprisingly, anti-angiogenic therapy directed against circulating VEGF using bevacizumab in combination either in combination with gemcitabine alone or with gemcitabine and erlotinib has been unsuccessful[69,70]. Likewise, VEGF receptor targeted agents such as axitinib and aflibercept have not improved outcomes either[71,72]. As explained previously, the desmoplastic stroma contributes significantly to altered vasculature. Additionally, the abundance of angiostatic factors such as angiostatin and endostatin that are secreted in the TME also explains the discordance between VEGF overexpression and lack of clinical benefit with VEGF inhibition[46,73].

**TUMOR STEM CELLS**

Cancer stem cells (CSC) constitute a very small proportion of pancreatic tumors (< 1%), but have the potential for unlimited proliferation[74]. They were identified in PDAC using a xenograft model of immunocompromised mice and proven to have a 100-fold higher tumorigenic potential compared to non-tumorigenic cancer cells[74]. A distinct population of CD133+ PDAC stem cells also predicts propensity to metastasis[75]. Moreover, cancer stem cells are extremely resistant to chemotherapy and radiation[76,77], attributed to the overexpression of the early developmental sonic hedgehog (SHH) pathway[78]. The self-renewing nature of CSCs poses a significant challenge in molecular therapeutics of PDAC.

***Targeting CSCs in PDAC***

Data emerging from preclinical studies have demonstrated that it is indeed possible to target and eliminate CSCs. Salinomycin, an antibiotic with a greater than 100-fold efficacy against CSCs compared to paclitaxel, inhibited the growth of CD133+ pancreatic CSCs and the effects were synergistic with gemcitabine, which curbed the growth of non-CSC cells[79]. Pancreatic CSCs also overexpress epithelial cell adhesion molecule (EpCAM) and this feature has been the focus of immunotherapy directed against CSCs[80]. MT110 is a bi-specific T cell engaging antibody (BiTE) that simultaneously targets EpCAM on CSCs and T cell-CD3 complexes on T cells to effectively eliminate the highly tumorigenic CSCs both in vivo and in vitro in a mouse model of PDAC[80]. Natural agents such as isoflavones, 3,3’-diindolylmethane (DIM) and curcumin analogues have also garnered attention because of their inhibitory effects on CSCs through cell-signaling molecules and microRNAs (miRNA)[81]. Pancreatic CSCs also overexpress Nodal and Activin belonging to the TGF-β superfamily and pharmacological inhibition or knockdown of their receptor activin-like 4 and 7 (Alk 4/7) reversed gemcitabine resistance in an orthotopic mouse model and dramatically reduced their tumorigenicity[82]. In addition to newer agents, the anti-neoplastic effects of the timeworn drug metformin are also attributed to its activity against pancreatic CSCs[83]. Results from these preclinical studies await clinical translation.

**IMMUNE BIOLOGY OF PDAC**

The immune system serves as an innate defense against tumorigenesis and metastasis. To counteract immune-surveillance, tumors develop adaptive mechanisms and PDAC is adept at immune evasion because of its inherently low immunogenicity[84,85]. The lack of anti-tumor effector T lymphocytes in preclinical mouse models of PDAC compared to a very high proportion of immunosuppressive cells such as regulatory T cells (Tregs), tumor-associated macrophages and myeloid derived suppressor cells tips the balance in favor of tumorigenesis[85]. Tumor and stromal cells also secrete several inflammatory mediators, notably TGF-β and interleukin 10 (IL-10) which down-regulate T cell and antigen presenting cell (APC) proliferation in the PDAC microenvironment[86,87]. Despite the purported low immunogenicity of PDAC, the presence of CD4+ helper T cells and CD8+ cytotoxic T cells (CTL) in resected pancreatic tumors was associated with longer OS, suggestive of a definite immune response against PDAC[88]. Though it has been a challenging endeavor to devise effective strategies to harness the host’s immune system against PDAC, results of recent vaccine trials and immune checkpoint inhibitors in PDAC have been quite encouraging.

**VACCINE THERAPY**

Immune mediated anti-tumor response occurs in two steps; first, tumor associated antigens (TAA) are presented by APC, notably dendritic cells to effector/CTL, which in turn recognize antigenic epitopes bound to major histocompatibility (MHC) molecules. Next, concomitant binding of co-stimulatory molecules such as B7-1 on APCs and CD28 on T cells results in T cell activation. However, tumor cells lack the additional co-stimulatory molecules and immune evasion ensues[89]. Vaccine-based therapies are designed to circumvent immune evasion by delivering TAAs to APCs and stimulate a robust cell-mediated immune response to attack and eliminate tumor cells.

Initial vaccine designs for PDAC utilized peptide antigens such as mucin-1 (MUC1), carcinoembryonic antigen (CEA) and protein products of *KRAS* oncogene that are capable of binding exact MHC molecules[89]. Because peptide vaccines contain only single antigenic epitopes, it leads to immune tolerance with minimal and transient efficacy[90]. The expansion of proteonomics and gene expression based assays has led to the identification of several TAAs that are selectively expressed by pancreatic cancer cells and has widened the scope for development of whole-cell vaccines that utilize these antigens to trigger tumor-specific immunity. Mesothelin is one such example of a TAA that is overexpressed in nearly all PDACs (but not in normal cells) and is implicated in cell adhesion and metastases[91,92]. Mesothelin-specific CD8+ T cell responses have been associated with improved OS following vaccine therapy[93].

***Granulocyte-macrophage colony-stimulating factor vaccines***

**GVAX:** GVAX is a whole-cell irradiated allogeneic vaccine that is composed of tumor cells from two pancreatic cell lines (Panc 10.05 and Panc 6.03) that have been genetically modified using a plasmid vector encoding for the granulocyte-macrophage colony-stimulating factor (*GM-CSF*) gene[94]. When injected transdermally, high GM-CSF secretion at the vaccine site causes mobilization and differentiation of APCs, a feature that patients with PDAC typically lack. APCs subsequently migrate to regional lymph nodes and activate CD4+ and CD8+ T cells to mount an effective anti-tumor response[95].

Initial trials demonstrated the safety and tolerability of GVAX when administered in the adjuvant setting followed by conventional chemoradiation. Delayed-type hypersensitivity (DTH) reactions and induction of mesothelin-specific CD8+ cells correlated with prolonged disease free survival in the phase I and phase II trials respectively[94,96]. Based on the favorable results in the adjuvant setting, GVAX was studied in the metastatic setting in patients who had progressive disease after gemcitabine[97]. In this open-label phase II study, the combination of GVAX with immune-modulating dose of cyclophosphamide (Cy) was compared to GVAX alone. The rationale for adding Cy was to enhance treatment related immune response by inhibiting immunosuppressive Tregs. Although OS was better in the combination arm compared to GVAX alone, the results were not statistically significant (median OS - 4.7 mo *vs* 2.3 mo). CD8+ T cell responses to mesothelin were enhanced in the combination arm and associated with a trend towards prolonged PFS[97]. Whether metronomic Cy plus GVAX will counter immune tolerance mediated by Tregs is being evaluated in a randomized clinical trial (NCT00727441, Table 3).

***GVAX-prime and CRS-207-boost***

CRS-207 is a live-attenuated strain of Listeria monocytogenes, genetically engineered to secrete mesothelin into the cytosol of APCs. In addition to activating effector T cells by delivering TAAs directly to the APCs, the cytokine mediated inflammatory response that is triggered by CRS-207 also serves to recruit more APCs[95]. The synergy between GVAX and CRS-207 was demonstrated in a phase I trial[98] which led to a multi-center, randomized phase II trial among 90 patients exclusively with PDAC[99]. Patients with previously treated metastatic PDAC were randomized 2:1 to either 2 doses of GVAX immune priming followed by 4 doses of CRS-207 as a boost (arm A) or 6 doses of GVAX alone (arm B). All patients received Cy to inhibit Tregs. After a median duration of follow-up of 6.6 mo, the OS was 6.1 mo in arm A compared to 3.9 mo in arm B (HR for death, 0.59; 95%CI: 0.36 to 0.91, *P* = 0.02). Toxicity with the combination was minimal and included transient fevers, fatigue, lymphopenia and elevated liver enzymes. As with previous studies, detection of enhanced mesothelin specific CD8+ T cell responses was associated with longer OS regardless of treatment arm[99]. A larger phase IIb trial is currently underway to compare the combination of GVAX plus CRS-207 to CRS-207 alone or chemotherapy alone in the metastatic setting (NCT02004262, Table 3).

***Algenpantucel-L***

Algenpantucel-L (NewLink Genetics Corporation, Ames, IA) is an allogeneic vaccine that contains two PDAC cell lines (HAPa-1 and HAPa-2) that have been genetically engineered to express α(1,3)-galactosyl epitopes (α-Gal)[100]. Though human cells lack the α-Gal epitopes, the gut flora stimulates antibodies against it. These antibodies are the primary mediators of hyperacute rejection characterized by rapid organ destruction through complement activation within minutes of organ transplantation[100]. When these antibodies are coupled with tumor cells such as in algenpantucel-L, it promotes opsonization and phagocytosis of tumor cells by APCs and results in T cell activation. In a phase II study of 70 patients with resected PDAC, algenpantucel-L was added to either gemcitabine or 5-fluorouracil based chemoradiotherapy[100]. After a median follow-up of 21 mo, the DFS and OS at 1 year were 62% and 86% respectively. Notably, the OS in this trial was better than the reported 81% in the sentinel RTOG-9704 trial using the same chemoradiotherapy regimen. Patients who received a higher dose of 300 million cells/dose fared better than those who received 100 million cells/dose with regard to both DFS (81% *vs* 51%) and OS (81% *vs* 68%) at 12 mo respectively, suggesting a strong dose-response effect. Apart from mild adverse events such as injection site pain and induration the vaccine was well tolerated. Phase III trials evaluating algenpantucel-L in the adjuvant (NCT01072981) and neoadjuvant setting (NCT01836432) are ongoing (Table 3).

***Immune checkpoint inhibitors***

Cytotoxic T lymphocyte antigen-4 **(**CTLA-4) is expressed on the surface of activated T cells and down-regulates immune activation by competitively inhibiting the binding of CD28 to B7-1 and turning off the intracellular signaling cascade of B7-1[101]. Monotherapy with ipilimumab (Yervoy, Bristol-Myers Squibb Company), an anti-CTLA-4 mAb, was ineffective in the treatment of locally advanced or metastatic PDAC[102]. However, the combination of GVAX with ipilimumab showed striking clinical and immunological synergy in previously treated patients with advanced PDAC[103]. Compared to single-agent ipilimumab, patients in the combination therapy arm had better OS at 1 year (27% *vs* 7%) although the study was not powered to detect differences in OS. Significantly however, combination therapy was not associated with increased adverse events, despite the higher dose of ipilimumab (10 mg/kg) used in this study. Increase in peak mesothelin-specific T cells and enhancement of the T cell repertoire was associated with longer OS[103]. Most responders in this study required at least 12 wk of therapy, thus underscoring the need for selecting patients with early stage disease in future trials to evaluate delayed responses often seen with immunotherapy.

Programmed cell death ligand-1 (PD-L1) and its receptor PD-1 are expressed on the cell surface of tumor cells as well as activated T cells. This receptor-ligand interaction down-regulates CD4+ and CD8+ T cells and is a natural immune checkpoint to prevent excessive immune mediated tissue damage[104]. PD-L1 expression is up regulated in PDAC cells and results in a blunted T cell response against the tumor[104]. Blocking the interaction between PD-1 and PD-L1 successfully augmented anti-tumor immune responses in vitro and formed the basis for investigating the efficacy of BMS-936559, an anti-PD-L1 mAb in various solid tumors[105]. Durable responses were noted in patients with melanoma, non-small cell lung cancer and renal-cell carcinoma but disappointingly no responses were seen in 14 patients with PDAC[105]. The resistance to PD-L1 inhibition in PDAC is due to the high expression of fibroblast activation protein (FAP) by carcinoma-associated fibroblasts (CAF) in the tumor stroma[106]. These FAP+ CAFs produce chemokine (C-X-C motif) ligand 12 (CXCL12) that binds with chemokine receptor 4 (CXCR4) to prevent T cells from infiltrating the tumor and causing local immunosuppression. Inhibition of CXCR4 by AMD3100 in a GEMM of PDAC caused cancer regression synergistically with PD-L1 inhibition[106]. Therefore it might be feasible to overcome tumoral immunosuppression through a combined approach. A clinical trial to test this hypothesis has recently opened to enrollment as a phase I study of ulocuplumab (anti-CXCR4) and nivolumab (anti-PD1) (NCT02472977, Table 3). Since these immune-modulating agents are not cancer T cell specific and can cause activation of other quiescent T cell populations, autoimmune toxicities occur frequently[107]. Hence future studies will also need to focus on effective management of these toxicities.

***CD40 agonist therapy***

CD40 belongs to the tumor necrosis factor (TNF) receptor superfamily and is expressed by multiple APCs including dendritic cells, B cells and macrophages[108]. Activated CD40 plays a crucial role in the priming and activation of tumor-specific T cells, but also mediates T cell independent antitumor immunity by activating macrophages[108]. CP-870, 893 is a CD40 agonistic mAb that potentiates anti-tumor immunity by these aforementioned mechanisms. In the initial pre-clinical study CP-870, 893 when administered in combination with gemcitabine in the KPC mouse model caused rapid regression of tumors mediated by T cell-independent macrophage infiltration[109]. Notably, depletion of tumor stroma was also noted and attributed to the effect of stromal infiltrating macrophages. In a phase I trial conducted subsequently, 22 patients with previously untreated advanced PDAC were administered CP-870, 893 with gemcitabine[110]. The radiological response rate (19% *vs* 9.4%) and median OS (8.6 mo *vs* 6.8 mo) were better than expected with single agent gemcitabine. The addition of gemcitabine is postulated to cause antigenic release akin to that of a vaccine with co-stimulation of APCs by CD40 agonist therapy[111]. Apart from transient cytokine release syndrome and depletion of B cells, none of the auto-immune toxicities seen with the immune check-point inhibitors were noted[110].

**FUTURE STRATEGIES**

***Adoptive T cell transfer***

Stemming from the successes in hematological malignancies notably acute lymphoblastic leukemia[112], adoptive T cell transfer is an exciting new paradigm that holds tremendous promise in PDAC. This therapeutic strategy involves ex vivo genetic engineering of T cells collected from patients to produce chimeric antigen receptors (CAR) capable of recognizing mesothelin expressed on PDAC cells[113,114]. Infusion of CAR-T cells back to the patient results in immediate recognition of tumor cells and obviates antigen processing and HLA expression. In preclinical studies, CAR-T cells exhibited potent anti-tumor activity[115]. Beatty *et al*[116] have also reported a marked decline in ascitic fluid malignant cell burden in a patient with metastatic PDAC, in addition to transient decline in [18F] fluorodeoxyglucose (FDG) uptake on positron emission tomography (PET) scan after infusion of CAR-T cells. CAR-T cell therapy is a subject of active research in PDAC and studies are ongoing (ClinicalTrials.gov identifiers: NCT01897415 and NCT01583686, Table 3).

***Targeting epigenetic regulators***

Epigenetics is the study of changes in gene expression by mechanisms other than changes in the DNA code. Histone modification by acetylation or methylation, DNA methylation and miRNA expression are the main mechanisms of epigenetic regulation[117]. Histone acetylation by histone acetyltransferase (HAT) promotes transcriptional activity but histone deacetylases (HDAC) repress transcription of tumor suppressor genes and are overexpressed in PDAC[118]. HDAC inhibitors serve to abrogate the transcriptional repression and impair tumorigenesis by playing a crucial role in differentiation, cell-cycle inhibition and apoptosis in tumor cells[117]. Multiple HDAC inhibitors such as hydroxamic acid derivatives (vorinostat), cyclic peptides (romidepsin), short-chain fatty acids (valproic acid) and benzamides have been studied, but results in PDAC have been disappointing[119,120]. However, the recognition that miRNAs play an important role in PDAC has resulted in increased attention towards exploiting them as potential therapeutic targets[121]. Acting at the post-transcriptional level, these non-coding RNAs play a crucial role in apoptosis, differentiation and proliferation. Aberrant overexpression of multiple miRNAs, particularly miRNA-21 has been demonstrated in PDAC and its inhibition by Lentiviral vectors has shown promising antitumor effects in preclinical studies[121,122]. miRNA targeted therapy especially in combination with chemotherapy is in its early stages and expected to gain momentum in the future (ClinicalTrials.gov identifier: NCT01274455, Table 3).

***Targeting signal transduction***

As described previously, targeting signaling pathways downstream from *KRAS* has been unsuccessful so far. However, there is renewed interest in targeting the effects of Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway after its importance in PDAC and associated cachexia became apparent[123,124]. The addition of the JAK inhibitor ruxolitinib to capecitabine in patients with refractory metastatic PDAC in a phase II trial showed OS benefit for a subgroup of patients with elevated levels of C-reactive protein (CRP)[125] and has formed the rationale for phase III trials evaluating ruxolitinib in metastatic PDAC (ClinicalTrials.gov identifiers: NCT02119663 and NCT02117479, Table 3). Global genomic analysis data also revealed alterations in genes in the Wnt/Notch and TGF-β signaling pathways in all PDACs[13]. Ongoing clinical trials to evaluate the efficacy of specific inhibitors of these pathways are currently underway (ClinicalTrials.gov identifiers: NCT02050178, NCT01764477: Wnt inhibitors, NCT01647828: mAb against Notch, NCT01373164: oral anti TGF-β receptor type 1, Table 3).

**CONCLUSION**

As evinced in this review, with improved understanding of the biology, genetic basis and molecular mechanisms that initiate and propagate PDAC carcinogenesis, the focus has shifted from identifying effective cytotoxic chemotherapy regimens to molecularly targeted therapies. These efforts have been further burnished by significant strides in the field of onco-immunology that now allows for cautious optimism that effective therapeutic options for PDAC are finally within reach.

**REFERENCES**

1 **Siegel R**, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 9-29 [PMID: 24399786 DOI: 10.3322/caac.21208]

2 **Rahib L**, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74**: 2913-2921 [PMID: 24840647 DOI: 10.1158/0008-5472.CAN-14-0155]

3 **Yeo CJ**, Cameron JL, Lillemoe KD, Sitzmann JV, Hruban RH, Goodman SN, Dooley WC, Coleman J, Pitt HA. Pancreaticoduodenectomy for cancer of the head of the pancreas. 201 patients. *Ann Surg* 1995; **221**: 721-731; discussion 731-733 [PMID: 7794076 DOI: 10.1097/00000658-199506000-00011]

4 **Shaib Y**, Davila J, Naumann C, El-Serag H. The impact of curative intent surgery on the survival of pancreatic cancer patients: a U.S. Population-based study. *Am J Gastroenterol* 2007; **102**: 1377-1382 [PMID: 17403071 DOI: 10.1111/j.1572-0241.2007.01202.x]

5 **Li D**, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet* 2004; **363**: 1049-1057 [PMID: 15051286 DOI: 10.1016/S0140-6736(04)15841-8]

6 **Burris HA**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413 [PMID: 9196156]

7 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]

8 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

9 **Hruban RH**, Maitra A, Goggins M. Update on pancreatic intraepithelial neoplasia. *Int J Clin Exp Pathol* 2008; **1**: 306-316 [PMID: 18787611]

10 **Feldmann G**, Beaty R, Hruban RH, Maitra A. Molecular genetics of pancreatic intraepithelial neoplasia. *J Hepatobiliary Pancreat Surg* 2007; **14**: 224-232 [PMID: 17520196 DOI: 10.1007/s00534-006-1166-5]

11 **Couch FJ**, Johnson MR, Rabe KG, Brune K, de Andrade M, Goggins M, Rothenmund H, Gallinger S, Klein A, Petersen GM, Hruban RH. The prevalence of BRCA2 mutations in familial pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 342-346 [PMID: 17301269 DOI: 10.1158/1055-9965.EPI-06-0783]

12 **Jones S**, Hruban RH, Kamiyama M, Borges M, Zhang X, Parsons DW, Lin JC, Palmisano E, Brune K, Jaffee EM, Iacobuzio-Donahue CA, Maitra A, Parmigiani G, Kern SE, Velculescu VE, Kinzler KW, Vogelstein B, Eshleman JR, Goggins M, Klein AP. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science* 2009; **324**: 217 [PMID: 19264984 DOI: 10.1126/science.1171202]

13 **Jones S**, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]

14 **Witkiewicz AK**, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, Mollaee M, Wagner KU, Koduru P, Yopp A, Choti MA, Yeo CJ, McCue P, White MA, Knudsen ES. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nat Commun* 2015; **6**: 6744 [PMID: 25855536 DOI: 10.1038/ncomms7744]

15 **Waddell N**, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek K, Quinn MC, Robertson AJ, Fadlullah MZ, Bruxner TJ, Christ AN, Harliwong I, Idrisoglu S, Manning S, Nourse C, Nourbakhsh E, Wani S, Wilson PJ, Markham E, Cloonan N, Anderson MJ, Fink JL, Holmes O, Kazakoff SH, Leonard C, Newell F, Poudel B, Song S, Taylor D, Waddell N, Wood S, Xu Q, Wu J, Pinese M, Cowley MJ, Lee HC, Jones MD, Nagrial AM, Humphris J, Chantrill LA, Chin V, Steinmann AM, Mawson A, Humphrey ES, Colvin EK, Chou A, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Pettitt JA, Merrett ND, Toon C, Epari K, Nguyen NQ, Barbour A, Zeps N, Jamieson NB, Graham JS, Niclou SP, Bjerkvig R, Grützmann R, Aust D, Hruban RH, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Falconi M, Zamboni G, Tortora G, Tempero MA, Gill AJ, Eshleman JR, Pilarsky C, Scarpa A, Musgrove EA, Pearson JV, Biankin AV, Grimmond SM. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* 2015; **518**: 495-501 [PMID: 25719666 DOI: 10.1038/nature14169]

16 **Bryant KL**, Mancias JD, Kimmelman AC, Der CJ. KRAS: feeding pancreatic cancer proliferation. *Trends Biochem Sci* 2014; **39**: 91-100 [PMID: 24388967 DOI: 10.1016/j.tibs.2013.12.004]

17 **Sousa CM**, Kimmelman AC. The complex landscape of pancreatic cancer metabolism. *Carcinogenesis* 2014; **35**: 1441-1450 [PMID: 24743516 DOI: 10.1093/carcin/bgu097]

18 **Stephen AG**, Esposito D, Bagni RK, McCormick F. Dragging ras back in the ring. *Cancer Cell* 2014; **25**: 272-281 [PMID: 24651010 DOI: 10.1016/j.ccr.2014.02.017]

19 **Van Cutsem E**, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, Schoffski P, Post S, Verslype C, Neumann H, Safran H, Humblet Y, Perez Ruixo J, Ma Y, Von Hoff D. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J Clin Oncol* 2004; **22**: 1430-1438 [PMID: 15084616 DOI: 10.1200/JCO.2004.10.112]

20 **Laheru D**, Shah P, Rajeshkumar NV, McAllister F, Taylor G, Goldsweig H, Le DT, Donehower R, Jimeno A, Linden S, Zhao M, Song D, Rudek MA, Hidalgo M. Integrated preclinical and clinical development of S-trans, trans-Farnesylthiosalicylic Acid (FTS, Salirasib) in pancreatic cancer. *Invest New Drugs* 2012; **30**: 2391-2399 [PMID: 22547163 DOI: 10.1007/s10637-012-9818-6]

21 **Patgiri A**, Yadav KK, Arora PS, Bar-Sagi D. An orthosteric inhibitor of the Ras-Sos interaction. *Nat Chem Biol* 2011; **7**: 585-587 [PMID: 21765406 DOI: 10.1038/nchembio.612]

22 **Gysin S**, Salt M, Young A, McCormick F. Therapeutic strategies for targeting ras proteins. *Genes Cancer* 2011; **2**: 359-372 [PMID: 21779505 DOI: 10.1177/1947601911412376]

23 **Infante JR**, Somer BG, Park JO, Li CP, Scheulen ME, Kasubhai SM, Oh DY, Liu Y, Redhu S, Steplewski K, Le N. A randomised, double-blind, placebo-controlled trial of trametinib, an oral MEK inhibitor, in combination with gemcitabine for patients with untreated metastatic adenocarcinoma of the pancreas. *Eur J Cancer* 2014; **50**: 2072-2081 [PMID: 24915778 DOI: 10.1016/j.ejca.2014.04.024]

24 **Bodoky G**, Timcheva C, Spigel DR, La Stella PJ, Ciuleanu TE, Pover G, Tebbutt NC. A phase II open-label randomized study to assess the efficacy and safety of selumetinib (AZD6244 [ARRY-142886]) versus capecitabine in patients with advanced or metastatic pancreatic cancer who have failed first-line gemcitabine therapy. *Invest New Drugs* 2012; **30**: 1216-1223 [PMID: 21594619 DOI: 10.1007/s10637-011-9687-4]

25 **Mirzoeva OK**, Collisson EA, Schaefer PM, Hann B, Hom YK, Ko AH, Korn WM. Subtype-specific MEK-PI3 kinase feedback as a therapeutic target in pancreatic adenocarcinoma. *Mol Cancer Ther* 2013; **12**: 2213-2225 [PMID: 23918833 DOI: 10.1158/1535-7163.MCT-13-0104]

26 **Ko AH**, Tempero MA, Bekaii-Saab TB, Kuhn P, Courtin R, Ziyeh S, Tahiri S, Kelley RK, Dito E, Ong A, Linetskaya R, Mirzoeva OK, Wu CSY, Venook AP, Korn WM. Dual MEK/EGFR inhibition for advanced, chemotherapy-refractory pancreatic cancer: A multicenter phase II trial of selumetinib (AZD6244; ARRY-142886) plus erlotinib. Proceedings of the ASCO Annual Meeting 2013. *J Clin Oncol* 2013; **31**: (suppl; abstr 4014)

27 **Lowery MA**, Kelsen DP, Stadler ZK, Yu KH, Janjigian YY, Ludwig E, D'Adamo DR, Salo-Mullen E, Robson ME, Allen PJ, Kurtz RC, O'Reilly EM. An emerging entity: pancreatic adenocarcinoma associated with a known BRCA mutation: clinical descriptors, treatment implications, and future directions. *Oncologist* 2011; **16**: 1397-1402 [PMID: 21934105 DOI: 10.1634/theoncologist.2011-0185]

28 **Jacob DA**, Bahra M, Langrehr JM, Boas-Knoop S, Stefaniak R, Davis J, Schumacher G, Lippert S, Neumann UP. Combination therapy of poly (ADP-ribose) polymerase inhibitor 3-aminobenzamide and gemcitabine shows strong antitumor activity in pancreatic cancer cells. *J Gastroenterol Hepatol* 2007; **22**: 738-748 [PMID: 17444865 DOI: 10.1111/j.1440-1746.2006.04496.x]

29 **Fogelman DR**, Wolff RA, Kopetz S, Javle M, Bradley C, Mok I, Cabanillas F, Abbruzzese JL. Evidence for the efficacy of Iniparib, a PARP-1 inhibitor, in BRCA2-associated pancreatic cancer. *Anticancer Res* 2011; **31**: 1417-1420 [PMID: 21508395]

30 **Bendell J**, O'Reilly EM, Middleton MR, Chau I, Hochster H, Fielding A, Burke W, Burris H. Phase I study of olaparib plus gemcitabine in patients with advanced solid tumours and comparison with gemcitabine alone in patients with locally advanced/metastatic pancreatic cancer. *Ann Oncol* 2015; **26**: 804-811 [PMID: 25573533 DOI: 10.1093/annonc/mdu581]

31 **Lemoine NR**, Hughes CM, Barton CM, Poulsom R, Jeffery RE, Klöppel G, Hall PA, Gullick WJ. The epidermal growth factor receptor in human pancreatic cancer. *J Pathol* 1992; **166**: 7-12 [PMID: 1538276]

32 **Reichert M**, Rustgi AK. Pancreatic ductal cells in development, regeneration, and neoplasia. *J Clin Invest* 2011; **121**: 4572-4578 [PMID: 22133881 DOI: 10.1172/JCI57131.4572]

33 **Ardito CM**, Grüner BM, Takeuchi KK, Lubeseder-Martellato C, Teichmann N, Mazur PK, Delgiorno KE, Carpenter ES, Halbrook CJ, Hall JC, Pal D, Briel T, Herner A, Trajkovic-Arsic M, Sipos B, Liou GY, Storz P, Murray NR, Threadgill DW, Sibilia M, Washington MK, Wilson CL, Schmid RM, Raines EW, Crawford HC, Siveke JT. EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* 2012; **22**: 304-317 [PMID: 22975374 DOI: 10.1016/j.ccr.2012.07.024]

34 **Navas C**, Hernández-Porras I, Schuhmacher AJ, Sibilia M, Guerra C, Barbacid M. EGF receptor signaling is essential for k-ras oncogene-driven pancreatic ductal adenocarcinoma. *Cancer Cell* 2012; **22**: 318-330 [PMID: 22975375 DOI: 10.1016/j.ccr.2012.08.001]

35 **Miyamoto Y**, Maitra A, Ghosh B, Zechner U, Argani P, Iacobuzio-Donahue CA, Sriuranpong V, Iso T, Meszoely IM, Wolfe MS, Hruban RH, Ball DW, Schmid RM, Leach SD. Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. *Cancer Cell* 2003; **3**: 565-576 [PMID: 12842085 DOI: 10.1016/S1535-6108(03)00140-5]

36 **Tobita K**, Kijima H, Dowaki S, Kashiwagi H, Ohtani Y, Oida Y, Yamazaki H, Nakamura M, Ueyama Y, Tanaka M, Inokuchi S, Makuuchi H. Epidermal growth factor receptor expression in human pancreatic cancer: Significance for liver metastasis. *Int J Mol Med* 2003; **11**: 305-309 [PMID: 12579331]

37 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677 DOI: 10.1200/JCO.2006.07.9525]

38 **Perera RM**, Bardeesy N. Ready, set, go: the EGF receptor at the pancreatic cancer starting line. *Cancer Cell* 2012; **22**: 281-282 [PMID: 22975369 DOI: 10.1016/j.ccr.2012.08.019]

39 **Nair PN**, De Armond DT, Adamo ML, Strodel WE, Freeman JW. Aberrant expression and activation of insulin-like growth factor-1 receptor (IGF-1R) are mediated by an induction of IGF-1R promoter activity and stabilization of IGF-1R mRNA and contributes to growth factor independence and increased survival of the pancreatic cancer cell line MIA PaCa-2. *Oncogene* 2001; **20**: 8203-8214 [PMID: 11781836 DOI: 10.1038/sj.onc.1205044]

40 **Kamrava M**, Gius D, Casagrande G, Kohn E. Will targeting insulin growth factor help us or hurt us?: An oncologist's perspective. *Ageing Res Rev* 2011; **10**: 62-70 [PMID: 19896561 DOI: 10.1016/j.arr.2009.10.007]

41 **Beltran PJ**, Mitchell P, Chung YA, Cajulis E, Lu J, Belmontes B, Ho J, Tsai MM, Zhu M, Vonderfecht S, Baserga R, Kendall R, Radinsky R, Calzone FJ. AMG 479, a fully human anti-insulin-like growth factor receptor type I monoclonal antibody, inhibits the growth and survival of pancreatic carcinoma cells. *Mol Cancer Ther* 2009; **8**: 1095-1105 [PMID: 19366899 DOI: 10.1158/1535-7163.MCT-08-1171]

42 **Rosen LS**, Puzanov I, Friberg G, Chan E, Hwang YC, Deng H, Gilbert J, Mahalingam D, McCaffery I, Michael SA, Mita AC, Mita MM, Mulay M, Shubhakar P, Zhu M, Sarantopoulos J. Safety and pharmacokinetics of ganitumab (AMG 479) combined with sorafenib, panitumumab, erlotinib, or gemcitabine in patients with advanced solid tumors. *Clin Cancer Res* 2012; **18**: 3414-3427 [PMID: 22510349 DOI: 10.1158/1078-0432.CCR-11-3369]

43 **Kindler HL**, Richards DA, Garbo LE, Garon EB, Stephenson JJ, Rocha-Lima CM, Safran H, Chan D, Kocs DM, Galimi F, McGreivy J, Bray SL, Hei Y, Feigal EG, Loh E, Fuchs CS. A randomized, placebo-controlled phase 2 study of ganitumab (AMG 479) or conatumumab (AMG 655) in combination with gemcitabine in patients with metastatic pancreatic cancer. *Ann Oncol* 2012; **23**: 2834-2842 [PMID: 22700995 DOI: 10.1093/annonc/mds142]

44 **Fuchs CS**, Azevedo S, Okusaka T, Van Laethem JL, Lipton LR, Riess H, Szczylik C, Moore MJ, Peeters M, Bodoky G, Ikeda M, Melichar B, Nemecek R, Ohkawa S, Świeboda-Sadlej A, Tjulandin SA, Van Cutsem E, Loberg R, Haddad V, Gansert JL, Bach BA, Carrato A. A phase 3 randomized, double-blind, placebo-controlled trial of ganitumab or placebo in combination with gemcitabine as first-line therapy for metastatic adenocarcinoma of the pancreas: the GAMMA trial. *Ann Oncol* 2015; **26**: 921-927 [PMID: 25609246 DOI: 10.1093/annonc/mdv027]

45 **Subramani R**, Lopez-Valdez R, Arumugam A, Nandy S, Boopalan T, Lakshmanaswamy R. Targeting insulin-like growth factor 1 receptor inhibits pancreatic cancer growth and metastasis. *PLoS One* 2014; **9**: e97016 [PMID: 24809702 DOI: 10.1371/journal.pone.0097016]

46 **Feig C**, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. *Clin Cancer Res* 2012; **18**: 4266-4276 [PMID: 22896693 DOI: 10.1158/1078-0432.CCR-11-3114]

47 **Mahadevan D**, Von Hoff DD. Tumor-stroma interactions in pancreatic ductal adenocarcinoma. *Mol Cancer Ther* 2007; **6**: 1186-1197 [PMID: 17406031]

48 **Comoglio PM**, Trusolino L. Cancer: the matrix is now in control. *Nat Med* 2005; **11**: 1156-1159 [PMID: 16270068]

49 **Toole BP**. Hyaluronan: from extracellular glue to pericellular cue. *Nat Rev Cancer* 2004; **4**: 528-539 [PMID: 15229478 DOI: 10.1038/nrc1391]

50 **Provenzano PP**, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* 2012; **21**: 418-429 [PMID: 22439937 DOI: 10.1016/j.ccr.2012.01.007]

51 **Hingorani SR**, Harris WP, Beck JT, Berdov BA, Wagner SA, Pshevlotsky SM, Tjulandin S, Gladkov O, Holcombe RF, Jiang P, Maneval DC, Zhu J, Devoe CE. A phase 1b multi-center international study of gemcitabine combined with PEGPH20 (pegylated recombinant human hyaluronidase) in patients with stage IV previously untreated pancreatic cancer. Proceedings of the ASCO Annual Meeting 2013. *J Clin Oncol* 2013; **31**: (suppl; abstr 4010)

52 **Hingorani SR**, Harris WP, Hendifar AE, Bullock AJ, Xionghua WW, Huang Y JP. High response rate and PFS with PEGPH20 added to nab-paclitaxel/gemcitabine in stage IV previously untreated pancreatic cancer patients with high-HA tumors: Interim results of a randomized phase II study. Proceedings of the ASCO Annual Meeting 2015. *J Clin Oncol* 2015; **33**: (suppl; abstr 4006)

53 **Alvarez R**, Musteanu M, Garcia-Garcia E, Lopez-Casas PP, Megias D, Guerra C, Muñoz M, Quijano Y, Cubillo A, Rodriguez-Pascual J, Plaza C, de Vicente E, Prados S, Tabernero S, Barbacid M, Lopez-Rios F, Hidalgo M. Stromal disrupting effects of nab-paclitaxel in pancreatic cancer. *Br J Cancer* 2013; **109**: 926-933 [PMID: 23907428 DOI: 10.1038/bjc.2013.415]

54 **Von Hoff DD**, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE, Korn RL, Desai N, Trieu V, Iglesias JL, Zhang H, Soon-Shiong P, Shi T, Rajeshkumar NV, Maitra A, Hidalgo M. Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J Clin Oncol* 2011; **29**: 4548-4554 [PMID: 21969517 DOI: 10.1200/JCO.2011.36.5742]

55 **Kalluri R**, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer* 2006; **6**: 392-401 [PMID: 16572188 DOI: 10.1038/nrc1877]

56 **Infante JR**, Matsubayashi H, Sato N, Tonascia J, Klein AP, Riall TA, Yeo C, Iacobuzio-Donahue C, Goggins M. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. *J Clin Oncol* 2007; **25**: 319-325 [PMID: 17235047 DOI: 10.1200/JCO.2006.07.8824]

57 **Sato N**, Fukushima N, Maehara N, Matsubayashi H, Koopmann J, Su GH, Hruban RH, Goggins M. SPARC/osteonectin is a frequent target for aberrant methylation in pancreatic adenocarcinoma and a mediator of tumor-stromal interactions. *Oncogene* 2003; **22**: 5021-5030 [PMID: 12902985 DOI: 10.1038/sj.onc.1206807]

58 **Nagpal S**, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev* 2005; **26**: 662-687 [PMID: 15798098 DOI: 10.1210/er.2004-0002]

59 **Sherman MH**, Yu RT, Engle DD, Ding N, Atkins AR, Tiriac H, Collisson EA, Connor F, Van Dyke T, Kozlov S, Martin P, Tseng TW, Dawson DW, Donahue TR, Masamune A, Shimosegawa T, Apte MV, Wilson JS, Ng B, Lau SL, Gunton JE, Wahl GM, Hunter T, Drebin JA, O'Dwyer PJ, Liddle C, Tuveson DA, Downes M, Evans RM. Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. *Cell* 2014; **159**: 80-93 [PMID: 25259922 DOI: 10.1016/j.cell.2014.08.007]

60 **Yauch RL**, Gould SE, Scales SJ, Tang T, Tian H, Ahn CP, Marshall D, Fu L, Januario T, Kallop D, Nannini-Pepe M, Kotkow K, Marsters JC, Rubin LL, de Sauvage FJ. A paracrine requirement for hedgehog signalling in cancer. *Nature* 2008; **455**: 406-410 [PMID: 18754008 DOI: 10.1038/nature07275]

61 **Olive KP**, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Rückert F, Grützmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009; **324**: 1457-1461 [PMID: 19460966 DOI: 10.1126/science.1171362.Inhibition]

62 Infinity Pharmaceuticals. Update from phase 2 study of saridegib plus gemcitabine in patients with metastatic pancreatic cancer. ID: 1653550. [accessed 2015 Jul 28]. Available from: URL: http: //www.infi.com

63 **Rhim AD**, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA, Dekleva EN, Saunders T, Becerra CP, Tattersall IW, Westphalen CB, Kitajewski J, Fernandez-Barrena MG, Fernandez-Zapico ME, Iacobuzio-Donahue C, Olive KP, Stanger BZ. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell* 2014; **25**: 735-747 [PMID: 24856585 DOI: 10.1016/j.ccr.2014.04.021.Stromal]

64 **Özdemir BC**, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR, Laklai H, Sugimoto H, Kahlert C, Novitskiy SV, De Jesus-Acosta A, Sharma P, Heidari P, Mahmood U, Chin L, Moses HL, Weaver VM, Maitra A, Allison JP, LeBleu VS, Kalluri R. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* 2014; **25**: 719-734 [PMID: 24856586 DOI: 10.1016/j.ccr.2014.04.005]

65 **Hanahan D**, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; **86**: 353-364 [PMID: 8756718]

66 **Itakura J**, Ishiwata T, Shen B, Kornmann M, Korc M. Concomitant over-expression of vascular endothelial growth factor and its receptors in pancreatic cancer. *Int J Cancer* 2000; **85**: 27-34 [PMID: 10585578]

67 **von Marschall Z**, Cramer T, Höcker M, Burde R, Plath T, Schirner M, Heidenreich R, Breier G, Riecken EO, Wiedenmann B, Rosewicz S. De novo expression of vascular endothelial growth factor in human pancreatic cancer: evidence for an autocrine mitogenic loop. *Gastroenterology* 2000; **119**: 1358-1372 [PMID: 11054395]

68 **Olson P**, Chu GC, Perry SR, Nolan-Stevaux O, Hanahan D. Imaging guided trials of the angiogenesis inhibitor sunitinib in mouse models predict efficacy in pancreatic neuroendocrine but not ductal carcinoma. *Proc Natl Acad Sci U S A* 2011; **108**: E1275-E1284 [PMID: 22084065 DOI: 10.1073/pnas.1111079108]

69 **Kindler HL**, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, Wozniak TF, Picus J, Bhargava P, Mayer RJ, Schilsky RL, Goldberg RM. Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J Clin Oncol* 2010; **28**: 3617-3622 [PMID: 20606091 DOI: 10.1200/JCO.2010.28.1386]

70 **Van Cutsem E**, Vervenne WL, Bennouna J, Humblet Y, Gill S, Van Laethem JL, Verslype C, Scheithauer W, Shang A, Cosaert J, Moore MJ. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**: 2231-2237 [PMID: 19307500 DOI: 10.1200/JCO.2008.20.0238]

71 **Kindler HL**, Ioka T, Richel DJ, Bennouna J, Létourneau R, Okusaka T, Funakoshi A, Furuse J, Park YS, Ohkawa S, Springett GM, Wasan HS, Trask PC, Bycott P, Ricart AD, Kim S, Van Cutsem E. Axitinib plus gemcitabine versus placebo plus gemcitabine in patients with advanced pancreatic adenocarcinoma: a double-blind randomised phase 3 study. *Lancet Oncol* 2011; **12**: 256-262 [PMID: 21306953 DOI: 10.1016/S1470-2045(11)70004-3]

72 **Rougier P**, Riess H, Manges R, Karasek P, Humblet Y, Barone C, Santoro A, Assadourian S, Hatteville L, Philip PA. Randomised, placebo-controlled, double-blind, parallel-group phase III study evaluating aflibercept in patients receiving first-line treatment with gemcitabine for metastatic pancreatic cancer. *Eur J Cancer* 2013; **49**: 2633-2642 [PMID: 23642329 DOI: 10.1016/j.ejca.2013.04.002]

73 **Kisker O**, Onizuka S, Banyard J, Komiyama T, Becker CM, Achilles EG, Barnes CM, O'Reilly MS, Folkman J, Pirie-Shepherd SR. Generation of multiple angiogenesis inhibitors by human pancreatic cancer. *Cancer Res* 2001; **61**: 7298-7304 [PMID: 11585769]

74 **Li C**, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. *Cancer Res* 2007; **67**: 1030-1037 [PMID: 17283135 DOI: 10.1158/0008-5472.CAN-06-2030]

75 **Hermann PC**, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007; **1**: 313-323 [PMID: 18371365 DOI: 10.1016/j.stem.2007.06.002]

76 **Izumiya M**, Kabashima A, Higuchi H, Igarashi T, Sakai G, Iizuka H, Nakamura S, Adachi M, Hamamoto Y, Funakoshi S, Takaishi H, Hibi T. Chemoresistance is associated with cancer stem cell-like properties and epithelial-to-mesenchymal transition in pancreatic cancer cells. *Anticancer Res* 2012; **32**: 3847-3853 [PMID: 22993328]

77 **Du Z**, Qin R, Wei C, Wang M, Shi C, Tian R, Peng C. Pancreatic cancer cells resistant to chemoradiotherapy rich in "stem-cell-like" tumor cells. *Dig Dis Sci* 2011; **56**: 741-750 [PMID: 20683663 DOI: 10.1007/s10620-010-1340-0]

78 **Huang FT**, Zhuan-Sun YX, Zhuang YY, Wei SL, Tang J, Chen WB, Zhang SN. Inhibition of hedgehog signaling depresses self-renewal of pancreatic cancer stem cells and reverses chemoresistance. *Int J Oncol* 2012; **41**: 1707-1714 [PMID: 22923052 DOI: 10.3892/ijo.2012.1597]

79 **Zhang GN**, Liang Y, Zhou LJ, Chen SP, Chen G, Zhang TP, Kang T, Zhao YP. Combination of salinomycin and gemcitabine eliminates pancreatic cancer cells. *Cancer Lett* 2011; **313**: 137-144 [PMID: 22030254 DOI: 10.1016/j.canlet.2011.05.030]

80 **Cioffi M**, Dorado J, Baeuerle PA, Heeschen C. EpCAM/CD3-Bispecific T-cell engaging antibody MT110 eliminates primary human pancreatic cancer stem cells. *Clin Cancer Res* 2012; **18**: 465-474 [PMID: 22096026 DOI: 10.1158/1078-0432.CCR-11-1270]

81 **Li Y**, Kong D, Ahmad A, Bao B, Sarkar FH. Pancreatic cancer stem cells: emerging target for designing novel therapy. *Cancer Lett* 2013; **338**: 94-100 [PMID: 22445908 DOI: 10.1016/j.canlet.2012.03.018]

82 **Lonardo E**, Hermann PC, Mueller MT, Huber S, Balic A, Miranda-Lorenzo I, Zagorac S, Alcala S, Rodriguez-Arabaolaza I, Ramirez JC, Torres-Ruíz R, Garcia E, Hidalgo M, Cebrián DÁ, Heuchel R, Löhr M, Berger F, Bartenstein P, Aicher A, Heeschen C. Nodal/Activin signaling drives self-renewal and tumorigenicity of pancreatic cancer stem cells and provides a target for combined drug therapy. *Cell Stem Cell* 2011; **9**: 433-446 [PMID: 22056140 DOI: 10.1016/j.stem.2011.10.001]

83 **Bao B**, Wang Z, Ali S, Ahmad A, Azmi AS, Sarkar SH, Banerjee S, Kong D, Li Y, Thakur S, Sarkar FH. Metformin inhibits cell proliferation, migration and invasion by attenuating CSC function mediated by deregulating miRNAs in pancreatic cancer cells. *Cancer Prev Res* (Phila) 2012; **5**: 355-364 [PMID: 22086681 DOI: 10.1158/1940-6207.CAPR-11-0299]

84 **von Bernstorff W**, Voss M, Freichel S, Schmid A, Vogel I, Jöhnk C, Henne-Bruns D, Kremer B, Kalthoff H. Systemic and local immunosuppression in pancreatic cancer patients. *Clin Cancer Res* 2001; **7**: 925s-932s [PMID: 11300493]

85 **Clark CE**, Beatty GL, Vonderheide RH. Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer. *Cancer Lett* 2009; **279**: 1-7 [PMID: 19013709 DOI: 10.1016/j.canlet.2008.09.037]

86 **Bierie B**, Moses HL. Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer* 2006; **6**: 506-520 [PMID: 16794634 DOI: 10.1038/nrc1926]

87 **Harizi H**, Gualde N. Pivotal role of PGE2 and IL-10 in the cross-regulation of dendritic cell-derived inflammatory mediators. *Cell Mol Immunol* 2006; **3**: 271-277 [PMID: 16978535]

88 **Fukunaga A**, Miyamoto M, Cho Y, Murakami S, Kawarada Y, Oshikiri T, Kato K, Kurokawa T, Suzuoki M, Nakakubo Y, Hiraoka K, Itoh T, Morikawa T, Okushiba S, Kondo S, Katoh H. CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. *Pancreas* 2004; **28**: e26-e31 [PMID: 14707745]

89 **Soares KC**, Zheng L, Edil B, Jaffee EM. Vaccines for pancreatic cancer. *Cancer J* 2012; **18**: 642-652 [PMID: 23187853 DOI: 10.1097/PPO.0b013e3182756903]

90 **Melief CJ**, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. *Nat Rev Cancer* 2008; **8**: 351-360 [PMID: 18418403 DOI: 10.1038/nrc2373]

91 **Argani P**, Iacobuzio-Donahue C, Ryu B, Rosty C, Goggins M, Wilentz RE, Murugesan SR, Leach SD, Jaffee E, Yeo CJ, Cameron JL, Kern SE, Hruban RH. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). *Clin Cancer Res* 2001; **7**: 3862-3868 [PMID: 11751476]

92 **Pastan I**, Hassan R. Discovery of mesothelin and exploiting it as a target for immunotherapy. *Cancer Res* 2014; **74**: 2907-2912 [PMID: 24824231 DOI: 10.1158/0008-5472.CAN-14-0337]

93 **Thomas AM**, Santarsiero LM, Lutz ER, Armstrong TD, Chen YC, Huang LQ, Laheru DA, Goggins M, Hruban RH, Jaffee EM. Mesothelin-specific CD8(+) T cell responses provide evidence of in vivo cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients. *J Exp Med* 2004; **200**: 297-306 [PMID: 15289501 DOI: 10.1084/jem.20031435]

94 **Jaffee EM**, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, Goemann M, Coleman J, Grochow L, Donehower RC, Lillemoe KD, O'Reilly S, Abrams RA, Pardoll DM, Cameron JL, Yeo CJ. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. *J Clin Oncol* 2001; **19**: 145-156 [PMID: 11134207]

95 **Springett GM**. Novel pancreatic cancer vaccines could unleash the army within. *Cancer Control* 2014; **21**: 242-246 [PMID: 24955709]

96 **Lutz E**, Yeo CJ, Lillemoe KD, Biedrzycki B, Kobrin B, Herman J, Sugar E, Piantadosi S, Cameron JL, Solt S, Onners B, Tartakovsky I, Choi M, Sharma R, Illei PB, Hruban RH, Abrams RA, Le D, Jaffee E, Laheru D. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A Phase II trial of safety, efficacy, and immune activation. *Ann Surg* 2011; **253**: 328-335 [PMID: 21217520 DOI: 10.1097/SLA.0b013e3181fd271c.A]

97 **Laheru D**, Lutz E, Burke J, Biedrzycki B, Solt S, Onners B, Tartakovsky I, Nemunaitis J, Le D, Sugar E, Hege K, Jaffee E. Allogeneic granulocyte macrophage colony-stimulating factor-secreting tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: a pilot study of safety, feasibility, and immune activation. *Clin Cancer Res* 2008; **14**: 1455-1463 [PMID: 18316569 DOI: 10.1158/1078-0432.CCR-07-0371]

98 **Le DT**, Brockstedt DG, Nir-Paz R, Hampl J, Mathur S, Nemunaitis J, Sterman DH, Hassan R, Lutz E, Moyer B, Giedlin M, Louis JL, Sugar EA, Pons A, Cox AL, Levine J, Murphy AL, Illei P, Dubensky TW, Eiden JE, Jaffee EM, Laheru DA. A live-attenuated Listeria vaccine (ANZ-100) and a live-attenuated Listeria vaccine expressing mesothelin (CRS-207) for advanced cancers: phase I studies of safety and immune induction. *Clin Cancer Res* 2012; **18**: 858-868 [PMID: 22147941]

99 **Le DT**, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G, Morse M, Zeh H, Cohen D, Fine RL, Onners B, Uram JN, Laheru DA, Lutz ER, Solt S, Murphy AL, Skoble J, Lemmens E, Grous J, Dubensky T, Brockstedt DG, Jaffee EM. Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. *J Clin Oncol* 2015; **33**: 1325-1333 [PMID: 25584002 DOI: 10.1200/JCO.2014.57.4244]

100 **Hardacre JM**, Mulcahy M, Small W, Talamonti M, Obel J, Krishnamurthi S, Rocha-Lima CS, Safran H, Lenz HJ, Chiorean EG. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: a phase 2 study. *J Gastrointest Surg* 2013; **17**: 94-100; discussion p. 100-101 [PMID: 23229886 DOI: 10.1007/s11605-012-2064-6]

101 **Korman AJ**, Peggs KS, Allison JP. Checkpoint blockade in cancer immunotherapy. *Adv Immunol* 2006; **90**: 297-339 [PMID: 16730267]

102 **Royal RE**, Levy C, Turner K, Mathur A, Hughes M, Kammula US, Sherry RM, Topalian SL, Yang JC, Lowy I, Rosenberg SA. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J Immunother* 2010; **33**: 828-833 [PMID: 20842054 DOI: 10.1097/CJI.0b013e3181eec14c]

103 **Le DT**, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, Zheng L, Diaz LA, Donehower RC, Jaffee EM, Laheru DA. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. *J Immunother* 2013; **36**: 382-389 [PMID: 23924790 DOI: 10.1097/CJI.0b013e31829fb7a2]

104 **Topalian SL**, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr Opin Immunol* 2012; **24**: 207-212 [PMID: 22236695 DOI: 10.1016/j.coi.2011.12.009.Targeting]

105 **Brahmer JR**, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; **366**: 2455-2465 [PMID: 22658128 DOI: 10.1056/NEJMoa1200694]

106 **Feig C**, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, Connell CM, Roberts EW, Zhao Q, Caballero OL, Teichmann SA, Janowitz T, Jodrell DI, Tuveson DA, Fearon DT. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci USA* 2013; **110**: 20212-20217 [PMID: 24277834 DOI: 10.1073/pnas.1320318110]

107 **Wang J**, Reiss KA, Khatri R, Jaffee E, Laheru D. Immune Therapy in GI Malignancies: A Review. *J Clin Oncol* 2015; **33**: 1745-1753 [PMID: 25918295 DOI: 10.1200/JCO.2015.60.7879]

108 **Schoenberger SP**, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 1998; **393**: 480-483 [PMID: 9624005 DOI: 10.1038/31002]

109 **Beatty GL**, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, Huhn RD, Song W, Li D, Sharp LL, Torigian DA, O'Dwyer PJ, Vonderheide RH. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 2011; **331**: 1612-1616 [PMID: 21436454 DOI: 10.1126/science.1198443]

110 **Beatty GL**, Torigian DA, Chiorean EG, Saboury B, Brothers A, Alavi A, Troxel AB, Sun W, Teitelbaum UR, Vonderheide RH, O'Dwyer PJ. A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2013; **19**: 6286-6295 [PMID: 23983255 DOI: 10.1158/1078-0432.CCR-13-1320]

111 **Le DT**, Jaffee EM. Harnessing immune responses in the tumor microenvironment: all signals needed. *Clin Cancer Res* 2013; **19**: 6061-6063 [PMID: 24097857 DOI: 10.1158/1078-0432.CCR-13-2424]

112 **Grupp SA**, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, Milone MC, Levine BL, June CH. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013; **368**: 1509-1518 [PMID: 23527958 DOI: 10.1056/NEJMoa1215134]

113 **Kalos M**, June CH. Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. *Immunity* 2013; **39**: 49-60 [PMID: 23890063 DOI: 10.1016/j.immuni.2013.07.002]

114 **Beatty GL**. Engineered chimeric antigen receptor-expressing T cells for the treatment of pancreatic ductal adenocarcinoma. *Oncoimmunology* 2014; **3**: e28327 [PMID: 25050204 DOI: 10.4161/onci.28327]

115 **Zhao Y**, Moon E, Carpenito C, Paulos CM, Liu X, Brennan AL, Chew A, Carroll RG, Scholler J, Levine BL, Albelda SM, June CH. Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor. *Cancer Res* 2010; **70**: 9053-9061 [PMID: 20926399 DOI: 10.1158/0008-5472.CAN-10-2880]

116 **Beatty GL**, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G, Chew A, Zhao Y, Levine BL, Albelda SM, Kalos M, June CH. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. *Cancer Immunol Res* 2014; **2**: 112-120 [PMID: 24579088 DOI: 10.1158/2326-6066.CIR-13-0170]

117 **van Kampen JG**, Marijnissen-van Zanten MA, Simmer F, van der Graaf WT, Ligtenberg MJ, Nagtegaal ID. Epigenetic targeting in pancreatic cancer. *Cancer Treat Rev* 2014; **40**: 656-664 [PMID: 24433955 DOI: 10.1016/j.ctrv.2013.12.002]

118 **Schneider G**, Krämer OH, Schmid RM, Saur D. Acetylation as a transcriptional control mechanism-HDACs and HATs in pancreatic ductal adenocarcinoma. *J Gastrointest Cancer* 2011; **42**: 85-92 [PMID: 21271301 DOI: 10.1007/s12029-011-9257-1]

119 **Koutsounas I**, Giaginis C, Theocharis S. Histone deacetylase inhibitors and pancreatic cancer: are there any promising clinical trials? *World J Gastroenterol* 2013; **19**: 1173-1181 [PMID: 23482354 DOI: 10.3748/wjg.v19.i8.1173]

120 **Kozak G**, Blanco FF, Brody JR. Novel Targets in Pancreatic Cancer Research. *Semin Oncol* 2015; **42**: 177–187 [DOI: 10.1053/j.seminoncol.2014.12.015]

121 **Sicard F**, Gayral M, Lulka H, Buscail L, Cordelier P. Targeting miR-21 for the therapy of pancreatic cancer. *Mol Ther* 2013; **21**: 986-994 [PMID: 23481326 DOI: 10.1038/mt.2013.35]

122 **du Rieu MC**, Torrisani J, Selves J, Al Saati T, Souque A, Dufresne M, Tsongalis GJ, Suriawinata AA, Carrère N, Buscail L, Cordelier P. MicroRNA-21 is induced early in pancreatic ductal adenocarcinoma precursor lesions. *Clin Chem* 2010; **56**: 603-612 [PMID: 20093556 DOI: 10.1373/clinchem.2009.137364]

123 **Lesina M**, Kurkowski MU, Ludes K, Rose-John S, Treiber M, Klöppel G, Yoshimura A, Reindl W, Sipos B, Akira S, Schmid RM, Algül H. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *Cancer Cell* 2011; **19**: 456-469 [PMID: 21481788 DOI: 10.1016/j.ccr.2011.03.009]

124 **Gilabert M**, Calvo E, Airoldi A, Hamidi T, Moutardier V, Turrini O, Iovanna J. Pancreatic cancer-induced cachexia is Jak2-dependent in mice. *J Cell Physiol* 2014; **229**: 1437-1443 [PMID: 24648112 DOI: 10.1002/jcp.24580]

125 **Hurwitz H**, Uppal N WS. A randomized double-blind phase 2 study of ruxolitinib or placebo with capecitabine as second-line therapy in patients with metastatic pancreatic cancer. *J Clin Oncol* 2014; **32**: (suppl; abstr 4000)

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**Table 1 Barriers to effective molecularly targeted therapy in pancreatic ductal adenocarcinoma**

|  |  |
| --- | --- |
| **PDAC biology**  | **Barrier**  |
| **Genetic heterogeneity** | Inability to directly inhibit *KRAS*  |
|  | Convergence of signal transduction pathways downstream from *KRAS* with feedback inhibitory loops |
| **Overexpression of EGFR, IGF-1R** | Escape from growth factor dependence in later stages of tumorigenesis  |
| **Desmoplastic stroma**  | Hypoxic tumor milieu impairs effective drug delivery  |
| **Overexpression of angiogenic factors**  | Secretion of angiostatic factors in tumor microenvironment  |
| **PDAC stem cells** | Difficult to eradicate subpopulation of cells capable of self-renewal  |
|  | Resistance to chemotherapy, radiation  |
| **Low immunogenicity** | Evasion of host immunity Abundance of immunosuppressive cells in tumor milieu |

PDAC: Pancreatic ductal adenocarcinoma; *KRAS*: Kirsten rat sarcoma oncogene; EGFR: Epidermal growth factor receptor; IGF-1R: Insulin like growth factor-1 receptor.

**Table 2 Frequency and consequences of common genetic mutations in pancreatic ductal adenocarcinoma**

|  |  |  |  |
| --- | --- | --- | --- |
| **Mutation category**  | **Frequency in PDAC** | **Effects of mutation** | **Consequence**  |
| **Gain of function**  |  |  |  |
| *KRAS* | > 95%  | Continuous transduction of downstream growth signals (BRAF/MAPK, PI3K/mTOR)  | Enhanced cell growth and survival  |
| **Loss of function** |  |  |  |
| **CDKN2A** | 95%  | Disruption of RB1 by CDK4  | Uncontrolled cellular proliferation |
| **TP53** | 75%-85%  | Impaired DNA damage repair, loss of cell cycle checkpoint activation | Chromosomal instability, aneuploidy  |
| **DPC4/SMAD4** | 50%  | Loss of inhibition of TGF-β | Loss of cell growth inhibition  |
| **BRCA2**  | 6%-17%  | Impaired DNA damage repair by homologous recombination, loss of cell-cycle checkpoint activation  | Genomic instability  |
| **PALB2** | 1%-3%  | Impaired BRCA2 function  | Genomic instability  |

*KRAS*: Kirsten rat sarcoma oncogene; BRAF: Rapidly activated fibrosarcoma homolog B; MAPK: Mitogen activated protein kinase; PI3K: Phosphatidyl inositol-3 kinase; mTOR: Mammalian target of rapamycin; CDK: Cyclin dependent kinase; DPC4: Deleted in pancreatic cancer 4; TGF-β: Transforming growth factor-β; BRCA2: Breast cancer 2; PALB2: Partner and localizer of BRCA.

**Table 3 Summary of selected ongoing clinical trials evaluating molecular therapies in pancreatic ductal adenocarcinoma (according to** [**www.clinicalTrials.gov**](http://www.clinicalTrials.gov)**, accessed July 2015)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Category**  | **Clinical Trial number**  | **PDA setting**  | **Medications studied**  | **Phase**  | **Status**  | **Estimated completion**  |
| **Tumor suppressor genes** |  |  |  |  |  |  |
|  | NCT01585805 | Locally advanced/metastatic | Gem and Cisplatin ± Veliparib *vs* Veliparib alone | II  | Recruiting  | 07/2017 |
|  | NCT01296763 | Advanced  | Irinotecan + Cisplatin + Mitomycin C ± Olaparib | I/II | Ongoing, not recruiting  | 01/2014 |
| **Recombinant hyaluronidase** |  |  |  |  |  |  |
|  | NCT01959139 | Metastatic  | FOLFIRINOX ± PEGPH20  | I/II | Recruiting  | 12/2017 |
|  | NCT01839487 | Metastatic  | Gem + Nab-paclitaxel *vs* Gem + Nab-paclitaxel + PEGPH20  | II | Recruiting  | 04/2016  |
| **Vaccine therapy** |  |  |  |  |  |  |
|  | NCT02004262 | Metastatic  | Cy + GVAX + CRS-207 *vs* Chemotherapy *vs* CRS-207  | II | Recruiting  | 12/2016 |
|  | NCT01072981 | Adjuvant  | Chemotherapy *vs* Chemo-radiotherapy ± Algenpantucel-L | III | Ongoing, not recruiting | 06/2016 |
|  | NCT01836432 | Neoadjuvant  | FOLFIRINOX ± Algenpantucel-L | III | Recruiting | 09/2015 |
| **Immune checkpoint** | NCT02472977 | Metastatic | ulocuplumab (CXCR4) and nivolumab (PD1) | IB | Recruiting | 7/2017 |
| **CAR-T cell therapy** |  |  |  |  |  |  |
|  | NCT01897415 | Metastatic  | Autologous Redirected RNA Mesothelin specific CAR-T cells  | I | Not recruiting  | 01/2015 |
|  | NCT01583686 | Metastatic  | CAR-T cell Receptor  | I/II | Recruiting  | 12/2018  |
| **Micro-RNA-21 targeted therapy** |  |  |  |  |  |  |
|  | NCT01274455 | Locally advanced  | Gem + Plasmid DNA CYL-02 | I | Not recruiting  | 12/2013  |
| **Signal transduction inhibitors** |  |  |  |  |  |  |
| **Janus Kinase targeted**  | NCT02119663 | Locally advanced/metastatic  | Capecitabine + Ruxolitinib *vs* Capecitabine + Placebo | III | Recruiting | 06/2017 |
|  | NCT02117479 | Locally advanced/metastatic | Capecitabine + Ruxolitinib *vs* Capecitabine + Placebo | III | Recruiting  | 12/2015 |
| **Wnt targeted**  | NCT02050178 | Metastatic  | OMP-54F28 + Gem-Nab-paclitaxel | I | Recruiting  | 12/2016  |
|  | NCT01764477 | Metastatic  | PRI-724 + Gem  | I  | Recruiting  | 03/2016  |
| **Notch inhibitor**  | NCT01647828 | Locally advanced/metastatic | OMP-59R5 + Gem-Nab-paclitaxel | I/II | Recruiting  | 01/2016  |
| **TGF-β inhibitor** | NCT01373164 | Locally advanced/metastatic | LY2157299 + Gem  | I/II | Not recruiting  | 11/2015 |

PDA: Pancreas ductal adenocarcinoma; Gem: Gemcitabine; FOLFIRINOX: 5-fluorouracil, leucovorin, irinotecan, oxaliplatin; CAR-T: Chimeric antigen receptor T cell; TGF-β: Transforming growth factor-β; Cy: Cyclophosphamide.