Tumor microenvironment in pancreatic ductal adenocarcinoma: Implications in immunotherapy

Caitlyn Smith, Wei Zheng, Jixin Dong, Yaohong Wang, Jinping Lai, Xiuli Liu, Feng Yin

Abstract
Pancreatic ductal adenocarcinoma is one of the most aggressive and lethal cancers. Surgical resection is the only curable treatment option, but it is available for only a small fraction of patients at the time of diagnosis. With current therapeutic regimens, the average 5-year survival rate is less than 10% in pancreatic cancer patients. Immunotherapy has emerged as one of the most promising treatment options for multiple solid tumors of advanced stage. However, its clinical efficacy is suboptimal in most clinical trials on pancreatic cancer. Current studies have suggested that the tumor microenvironment is likely the underlying barrier affecting immunotherapy drug efficacy in pancreatic cancer. In this review, we discuss the role of the tumor microenvironment in pancreatic cancer and the latest advances in immunotherapy on pancreatic cancer.
Key Words: Pancreatic ductal adenocarcinoma; Tumor microenvironment; Immunotherapy; Clinical trial; Chemotherapy; Treatment

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Core Tip: Despite advances in basic and translational research, pancreatic cancer remains one of the most lethal cancers. Recent breakthroughs in immunotherapy have revolutionized cancer therapy and have shown great potential to transform pancreatic cancer treatment. However, due to the barrier related to the tumor microenvironment, pancreatic cancer has shown inferior treatment outcomes toward various immunotherapy regimens. Further efforts, such as combinatorial immunotherapy or molecular tumor subtyping, are warranted to overcome immunotherapy resistance in pancreatic cancer.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) develops in the exocrine compartment of the pancreas and accounts for approximately 90% of pancreatic malignancies, making it the most common pancreatic neoplasm. Due to the lack of early diagnosis and limited treatment response, PDAC remains a highly aggressive and lethal malignancy and is the fourth leading cause of cancer-related death worldwide[1]. Although there has been notable progress in understanding tumor biology and the development of novel therapeutic regimens, the average 5-year survival rate is still less than 5%-10% in PDAC patients [1,2]. The clinical manifestations of pancreatic cancers are generally nonspecific, including weight loss, abdominal pain, thromboembolic disease, and type 2 diabetes[3,4]. In approximately 60%-70% of PDAC cases, the tumor arises from the head of the pancreas and could present as pancreatitis and obstructive jaundice[5]. Tumors of the pancreatic body and tail frequently have a poor prognosis due to their late presentation and associated advanced tumor stage[6].

The standard of care for resectable PDAC is surgical resection followed by adjuvant chemotherapy. Surgical resection remains the only curative therapy, but it is available for merely 10%-20% of patients at the time of diagnosis. Moreover, even with curative surgical resection, local recurrence and distal metastasis of PDAC are still quite common[7]. Advanced-stage PDAC is routinely treated with neoadjuvant chemotherapy, and the current first-line therapy regimens include gemcitabine, gemcitabine plus nab-paclitaxel, and FOLFIRINOX (the combination of oxaliplatin, leucovorin, fluorouracil, and irinotecan) [8]. Recently, the poly(adenosine diphosphate-ribose) polymerase inhibitor (PARPi) olaparib (Lynparza) has been approved for patients with germline BRCA-mutated metastatic pancreatic cancer[9]. The development of these neoadjuvant chemotherapy regimens has greatly improved patient survival and quality of life. However, a significant portion of PDAC eventually relapses despite surgical resection and/or neoadjuvant chemotherapy and leads to patient death[10,11].

The difficulties in treating pancreatic cancer lie at the cellular and genetic levels[12]. Mutational changes in pancreatic tumors lead to gene instability, tumor growth, and resistance to treatments[13]. In addition to the characteristic molecular landmarks, including oncogenic KRAS mutation and inactivation of the tumor suppressor genes CDKN2A/P16, TP53, and SMAD4, PDAC also frequently harbors mutations involving diverse cell signaling pathways[14]. The molecular heterogeneity likely accounts for its drug resistance in chemotherapy[15]. In addition, pancreatic cancer stem cells, accounting for approximately 1% of all pancreatic cancer cells, have the capability for self-renewal and exhibit chemoresistance properties[16].

Immunotherapy has emerged as one of the most promising treatment options for advanced solid tumors, including lung, kidney, bladder, liver, and colorectal cancers[17]. Unfortunately, PDAC is notoriously resistant to immunotherapy, and thus far, most phase I/II clinical trials on PDAC have failed to demonstrate the desirable clinical efficacy of immunotherapy[18]. Of note, microsatellite instability (MSI), one of the predictive biomarkers for immune checkpoint blockade therapy, is only detected in a rare small portion of PDAC patients (less than 1%) [19,20]. On the other hand, emerging evidence has pinpointed the tumor microenvironment (TME) in PDAC as a critical component of treatment resistance toward immunotherapy[21,22].

In this review, we discuss the role of the tumor microenvironment and the latest advances in immunotherapy on pancreatic cancer through the search of peer-reviewed clinical and basic research articles related to this topic on PubMed, as well as the publicly accessible information on relevant
clinical trials through ClinicalTrials.gov.

**TUMOR MICROENVIRONMENT IN PANCREATIC CANCER**

PDAC is a type of stromal-rich cancer that frequently presents with a prominent desmoplastic reaction and is characterized by fibrogenic connective stromal tissue surrounding invasive carcinoma[23] (Figure 1). Desmoplastic reaction, or desmoplasia, is considered as the morphological basis of the TME. In general, the TME in PDAC demonstrates extensive desmoplasia, decreased stromal vascularization, and altered immune cell infiltration that lead to reduced drug activity and advancement of tumor progression. This process is characterized by an increase in the deposition of noncellular components, such as extracellular matrix (ECM), as well as an increase in the proliferation of cellular components, such as cancer-associated fibroblasts (CAFs) and immune cells[24,25]. Various cytokines, including interferons, interleukins, tumor necrosis factor (TNF), and transforming growth factor β (TGF-β), also play essential roles linking the TME cellular and noncellular components to regulate tumor growth, metastases, and drug resistance. Of note, the overall stroma is responsible for most of the tumor mass, but the stromal cellular components make up a relatively small fraction, approximately 10%-30%, of the tumor mass[26].

**Noncellular components of the tumor microenvironment**

The ECM is a significant factor in the initiation and progression of PDAC, and its deposition is associated with tumor migration, invasion, and poor prognosis[27]. The ECM is predominantly produced by cancer-associated pancreatic stellate cells (PSCs), a subtype of CAFs[28]. In PDAC, the ECM comprises most of the tumor mass and various matrix proteins, including collagen, fibronectin, proteoglycans, hyaluronan, proteolytic matrix metalloproteinases (MMPs), and tissue inhibitors of MMPs [29]. Among ECM components of particular interest are hyaluronan and MMPs in tumor progression and prognosis in PDAC.

In general, ECM provides a rigid barrier leading to increased tumor pressure, decreased vascularization, and reduced drug delivery. A significant cause of drug resistance is the inability of conventional chemotherapeutic drugs such as gemcitabine to penetrate the thick stromal layer[30]. Therefore, it is rational to propose a combinatory therapeutic strategy for PDAC by targeting the tumor ECM. Hyaluronan, or hyaluronic acid (HA), is a glycosaminoglycan polymer and a major component of the ECM. Increased deposition of HA is associated with tumor metastases, drug resistance, and poor prognosis in PDAC[27,31]. Since stromal HA levels are dynamically regulated by synthases (to produce HA) and hyaluronidases (to degrade HA), hyaluronidase-based drug development has been a promising field in targeted therapy against the TME. The enzymatic depletion of hyaluronan through recombinant hyaluronidase (PEGPH20) has led to significantly increased overall survival when combined with neoadjuvant chemotherapy[32]. This is attributed mainly to improved delivery of systemic therapy through degradation of HA and remodeling of the TME. However, a recent phase IB/II randomized study (NCT01959139) of FOLFIRINOX plus pegylated recombinant PEGPH20 showed increased toxicity with this combination therapy and decreased overall survival (OS) (7.7 mo vs 14.4 mo) compared with FOLFIRINOX monotherapy[33]. Moreover, despite promising results of PEGPH20 in phase I-II studies, in a recent phase III randomized study (HALO 109-301), the addition of PEGPH20 to nab-paclitaxel/gemcitabine did not improve OS and progression-free survival (PFS) in patients with hyaluronan-high metastatic PDAC, and additional development of PEHPH20 in metastatic PDAC was halted[34].

MMPs are calcium-dependent metalloproteinases responsible for ECM protein degradation and are implicated in cancer initiation, growth, and metastasis. Clinical trial results with broad-spectrum MMP inhibitors were discouraging due to lack of specificity, associated toxicity, and insufficient clinical benefit[35], warranting further basic and translational studies to classify the role of individual MMPs in PDAC. Among MMP family members, the expression levels of MMP-2, MMP-7, MMP-9, and MMP-11 were significantly elevated in PDAC tumor tissues compared with normal pancreas samples[36,37]. Increased MMP-2 expression in PDAC leads to tumor invasion and progression[38-40]. MMP-7 expression is also associated with PDAC initiation and progression[41] and has been shown to be an independent prognostic factor for PDAC in a multivariate analysis. MMP-9 is significantly associated with pancreatic cancer progression and poor prognosis[37] and has emerged as a prognostic biomarker and potential therapeutic target. Highly selective and potent MMP-9 inhibitory antibodies have been developed for ulcerative colitis and colorectal cancer[42]. However, in a preclinical study, systemic ablation of MMP-9 facilitated pancreatic cancer growth and metastasis by creating a tumor-promoting TME[43]. This study has suggested a controversial role for MMP-9 in pancreatic cancer progression.

Additional studies have also demonstrated conflicting results in drugs targeting the tumor stroma. Olive et al[44] demonstrated that depletion of ECM in PDAC, through inhibition of the Sonic Hedgehog signaling pathway, promoted gemcitabine efficacy and improved survival. However, the involvement of the Sonic Hedgehog-dependent tumor stroma in PDAC has been controversial, as evidence shows that some components of the tumor stroma could actually act to restrain, rather than support, tumor...
growth[45]. All these failures indicate that targeting desmoplasia alone is insufficient for treating advanced PDAC. The tumor stroma has both tumor-promoting and tumor-suppressing functions, which are probably context dependent. The stromal heterogeneity should be considered for the development of targeted therapy.

Cellular components of the tumor microenvironment

PDAC displays unique immunologic hallmarks. The TME in PDAC consists of diverse cellular components, including CAFs, regulatory and cytotoxic lymphocytes, macrophages, and endothelial cells [46]. CAFs are the major TME cellular component responsible for the production and deposition of ECM proteins. The involvement of CAFs in the progression of PDAC has been a hot and controversial topic. Similar to the observations made with tumor stroma, CAFs also play dual functions in regulating PDAC progression. On the one hand, CAFs promote cancer progression and drug resistance through the deposition of dense ECM, the release of exosomes (extracellular vesicles), and metabolic support [47-49]. On the other hand, depletion of CAFs leads to accelerated PDAC progression and reduced survival in multiple preclinical studies[50,51]. These discrepancies are likely associated with the heterogeneity of CAFs[52,53], a concept supported by recent studies demonstrating the existence of multiple distinct and mutually exclusive CAF subtypes in pancreatic cancer[54,55]. CAF subtypes with diverse biomarkers, including α-smooth muscle actin (αSMA), fibroblast activation protein (FAP), S100A4, and platelet-derived growth factor receptor-β (PDGFRβ), have been identified [56]. Specifically, FAP-positive active CAFs have been linked to tumor-promoting functions by maintaining an immunosuppressive TME[57]. FAP is a type-II transmembrane serine protease, and its expression has been detected in both the tumor stroma and cancer cells in PDAC, with the highest expression in the tumor stroma at the tumor front [58]. FAP-positive CAFs potently shape the immune landscape in the TME by secreting TGF-β, VEGF, and multiple matrix processing enzymes[59,60], recruiting circulating myeloid-derived suppressor cells (MDSCs) into the tumor stroma[57], and inhibiting natural killer cell (NK) cytotoxicity and cytokine production[61]. FAP has been suggested as an ideal target for the TME, and its specific therapeutic reagents are in development[62].

In addition to CAFs, the TME also consists of multiple types of immunosuppressive cells, including regulatory T cells (Tregs), MDSCs, and tumor-associated macrophages (TAMs)[63]. These cells correlate to provide an immunosuppressive TME and have been under extensive preclinical and clinical investigation.

Tregs, defined as CD4+/CD25+/FOXP3+ T cells, are a subtype of repressive T cells that play an essential role in maintaining immune tolerance and preventing autoimmune disorders. Tregs can be found in PDAC and premalignant lesion intraductal papillary mucinous neoplasms (IPMNs). The

Figure 1 Pancreatic ductal adenocarcinoma with an associated tumor microenvironment. Please note the desmoplastic stromal reaction surrounding the tumor glands, decreased stromal vascularization, and scattered infiltrating inflammatory cells (HE stain, 200 x).
prevalence of Tregs in CD4+ T lymphocytes correlates significantly with the progression and invasion of IPMNs and is associated with poor prognosis in PDAC. The immunosuppressive function of Tregs has been attributed to the secretion of suppressive cytokines, including IL-10 and TGF-β1, and the induction of CD4+ T-cell death [64, 65]. Preoperative chemoradiation therapy has been shown to decrease Tregs in PDAC [66]. However, in a recent study, depletion of Tregs in a mouse model caused accelerated tumor progression due to unexpected crosstalk between Tregs and CAFs in PDAC [67]. This study has challenged the current view and posed uncertainties in developing Treg-based targeted therapy.

MDSCs and TAMs have also been suggested as potential therapeutic targets against the TME. Even though these two cell types are considered as separate entities, they have no demarcated boundaries and share many common characteristics [68]. MDSCs are a group of heterogeneous immature myeloid cells and can potently suppress T-cell function in tumors [69]. The levels of MDSCs correlate with the progression of PDAC and have been proposed as a predictive biomarker of chemotherapy failure [70, 71]. TAMs are circulating monocyte-derived macrophages in the tumor stroma and represent a significant population of immune cells within the TME. TAMs can be further subclassified into the M1 and M2 subtypes, with M1 being proinflammatory (antitumorigenic) and M2 being anti-inflammatory (protumorigenic) [72]. M2-polarized TAMs are associated with an unfavorable prognosis in PDAC [73]. Liu et al [74] revealed progressive accumulations of MDSCs and M2-polarized TAMs accompanied by dynamic reductions in cytotoxic T cells (CTLs) and helper T cells (Ths) in PDAC progression. Gemcitabine affects the TME by inhibiting the expansion of MDSCs and the induction of Th2 cells while promoting M2-polarized TAMs [74]. M2-polarized TAMs can also be induced by other chemotherapeutic agents, such as carboplatin and cisplatin, leading to increased secretion of interleukin-6 (IL-6), IL-10, and prostaglandin E2 [75]. In addition, interferon-γ upregulates the expression of programmed death-ligand 1 (PD-L1) in MDSCs, resulting in an immunosuppressive environment [76]. Further investigations and clinical trials are needed to test the efficacy of targeting MDSCs and TAMs in pancreatic cancer.

**IMMUNOTHERAPY IN PANCREATIC CANCER**

Current treatment options for PDAC have limited effects on patient survival. The recent development of immunotherapy has improved clinical outcomes for various types of solid tumors [17] and can revolutionize cancer treatment in PDAC. Activating the patient’s T cells is the principal basis for cancer immunotherapy. This is accomplished through multiple mechanisms, such as decreased tumor-specific antigen presentation, T-cell activation, T-cell infiltration into the pancreatic tumor, and elimination of cancer cells by T cells [77]. Multiple cancer immunotherapies have been introduced, including immune checkpoint inhibitors, cancer vaccines, and adoptive cell transfer.

**Immune checkpoint inhibitors**

Immune checkpoint molecules are a group of surface receptors expressed on various immune cells that transduce inhibitory signals to T cells upon ligand binding. These molecules play an important role in preventing an autoimmune attack against self-antigens. Due to strong immune selective pressure, cancer cells frequently adopt the power of immune checkpoint molecules to avoid immune destruction. Initially approved for the treatment of metastatic melanoma, immune checkpoint inhibitors (ICIs) have been cleared to treat various solid tumors, including advanced or metastatic urothelial carcinoma, non-small-cell lung cancer, colorectal cancer, triple-negative breast cancer, and head and neck squamous cell carcinoma [78, 79]. Currently, FDA-approved immune checkpoint inhibitors (ICIs) include anti-CTLA-4 agents (ipilimumab), anti-PD-1 agents (nivolumab, pembrolizumab, cemiplimab) and anti-PD-L1 agents (atezolizumab, avelumab, durvalumab) [79].

ICIs have emerged as a new therapeutic option for pancreatic cancer. Unfortunately, most phase I and II clinical trials on ICI treatment have failed to show the desired beneficial effect in PDAC. Two independent phase II clinical trials have demonstrated unsatisfactory clinical outcomes on monotherapy with anti-CTLA-4 mAb (Table 1). Single-agent ipilimumab, an anti-CTLA-4 mAb, was ineffective for the treatment of advanced PDAC (NCT00112580) (https://clinicaltrials.gov/ct2/show/NCT00112580) [80, 81]. Monotherapy with tremelimumab, another anti-CTLA-4 mAb, also yielded poor clinical outcomes in PDAC, with 18 out of 20 patients demonstrating progressive disease and a poor median OS of 4 mo (95% CI: 2.83-5.42 mo) (NCT02527434) (https://clinicaltrials.gov/ct2/show/NCT02527434).

Combination therapy with ipilimumab and gemcitabine, on the other hand, has demonstrated promising results due to the increased immune response by enhancing naïve T-cell activation [82]. In a phase 1b clinical trial (NCT01473940) (https://clinicaltrials.gov/ct2/show/NCT01473940), initial results on combination therapy with ipilimumab and gemcitabine showed that the treatment was tolerable, with a median PFS of 2.5 mo (95% CI: 0.8-4.8 mo) and a median OS of 8.5 mo (95% CI: 2.2-10.3 mo). In this study, five out of the 11 patients had stable disease, while two had a partial response. An ongoing clinical trial (NCT01928394) (https://clinicaltrials.gov/ct2/show/NCT01928394) is comparing nivolumab (anti-PD-1 mAb) monotherapy and combination therapy with nivolumab plus ipilimumab in patients with advanced or metastatic PDAC, and the results will be released in 2023.
A high tumor mutational burden (TMB) in cancer cells tends to produce more immunogenic neoantigens and may predict immunotherapy response[87]. A Phase II clinical trial (NCT05093231) [https://clinicaltrials.gov/ct2/show/NCT05093231] investigating the efficacy of pembrolizumab plus olaparib in metastatic pancreatic adenocarcinoma patients exhibiting high tumor mutation burden is ongoing, and the results will be released in 2026. Based on the results from current clinical trials, further studies need to focus on the combined approaches using ICIs with different therapeutic approaches, including chemotherapy, radiotherapy, or additional innovative platforms of immunotherapy, such as cancer vaccine and adoptive cell transfer.

**Therapeutic cancer vaccines**

Therapeutic cancer vaccines include whole-cell vaccines, dendritic cells, DNA, and peptide vaccines that activate cancer antigen-specific cytotoxic T lymphocytes (CTLs), eliciting immunogenic antigen presentation and leading to an anticancer response[88]. One such pancreatic cancer vaccine is GVAX,
which is generated from irradiated pancreatic cancer cells expressing granulocyte-macrophage colony-stimulating factor (GM-CSF)[89] (Table 2). Upon vaccination, GVAX secretes GM-CSF, induces subsequent activation of antigen-presenting cell and T-cell priming, and stimulates the patient’s immune system against pancreatic cancer cells[90]. GVAX was tolerable even at high doses, and the vaccine-induced increased delayed-type hypersensitivity response to autologous tumor cells[91]. In a phase II clinical trial on GVAX (NCT0084383) (https://clinicaltrials.gov/ct2/show/NCT0084383), sixty patients received GVAX 8-10 wk after surgical intervention, followed by adjuvant 5-FU-based chemoradiotherapy. The median PFS was 17.3 mo (95% CI: 14.6-22.8 mo), with a median OS of 24.8 mo (95% CI: 21.2-31.6 mo), which compares favorably with published data for resected PDAC[92]. Combinatory immunotherapy has aimed to induce a much more sustained antitumor T-cell response[93]. In a phase Ib trial for locally advanced, unresectable or metastatic PDAC (NCT00836407) (https://clinicaltrials.gov/ct2/show/NCT00836407), thirty patients received either ipilimumab monotherapy or ipilimumab plus GVAX cancer vaccine, and the median OS was 3.6 mo for the ipilimumab monotherapy group, compared to 5.7 mo in the group with combination therapy[94]. Although combinatory immunotherapy has shown its potential for advanced PDAC, more studies are needed to fully explore this novel therapeutic strategy’s capability.

Few human leukocyte antigen (HLA)-A(*2)42042-restricted tumor-associated antigens, including the KIF20A-10-66 peptide, have been identified in PDAC[95]. A phase I/II clinical trial in Japan showed a better prognosis in patients with metastatic PDAC and HLA-A*2402-positive status who received KIF20A-10-66 peptide vaccination as second-line treatment after failure of gemcitabine chemotherapy[96]. In two separate phase II clinical trials, KIF20A-derived peptide was evaluated in combination with two antiangiogenic cancer vaccines targeting vascular endothelial growth factor receptor 1 (VEGFR1) and VEGFR2. In the HLA-A*2402-matched group, patients with peptide-specific CTL induction had improved prognosis and increased OS[97,98]. Another HLA-A24-restricted antigenic peptide, SVN-2B, also functions as an immunogenic molecule. A vaccination protocol of SVN-2B in combination with interferon-α has demonstrated effective clinical and immunological responses for advanced PDAC[99].

Algenpantucel-L is a whole-cell pancreatic cancer vaccine with two irradiated allogeneic human pancreatic cell lines (HAPa-1 and HAPa-2) expressing the murine enzyme (1,3)-galactosyltransferase (αGT)[100]. Of note, the αGT enzyme is the critical barrier to xenotransplantation due to hyperacute rejection[101]. As a result, Algenpantucel-L will induce a hyperacute rejection of the allograft cells through rapid activation of antibody-dependent cell-mediated cytotoxicity (ADCC), leading to a response against the patient’s pancreatic cancer cells through epitope spreading[102]. A phase II, open-label trial (NCT00569387) (https://clinicaltrials.gov/ct2/show/NCT00569387) evaluated the use of the Algenpantucel-L tumor vaccine in combination with gemcitabine plus 5-FU chemoradiotherapy in patients with resected PDAC. Seventy patients were recruited in the study, and the 12-mo disease-free survival (DFS) and OS were 63% and 86%, respectively, suggesting that the Algenpantucel-L tumor vaccine could be administered with standard chemotherapy following surgical resection of pancreatic cancer[101]. Unfortunately, in a recent phase 3, open-label, randomized clinical trial (NCT01836432) (https://clinicaltrials.gov/ct2/show/NCT01836432), Algenpantucel-L failed to improve survival on borderline resectable or locally advanced PDAC receiving neoadjuvant chemoradiation therapy[103].

Overexpression of Mucin 1 (MUC-1), a type I transmembrane protein with O-linked glycosylation, plays a crucial role in oncogenic signaling to promote metastasis, angiogenesis, and invasion[104]. MUC-1 has served as a target for cancer vaccine immunotherapy[105]. Following surgical resection, a phase I/II study of a MUC1 peptide-loaded dendritic cell vaccine was conducted in 12 pancreaticobiliary cancer patients. Four out of twelve (33.3%) patients who received this MUC-1-based tumor vaccine were alive after four years without evidence of recurrence[106]. An optimized construct with MUC-1-variable number tandem repeats has been designed with much more potent immunogenicity[107].

Dendritic cell vaccines have been introduced to enhance the antitumor immune response through the stimulation of naïve T cells[108]. In a study evaluating the effectiveness of a dendritic cell vaccine in patients with advanced PDAC (NCT01410968) (https://clinicaltrials.gov/ct2/show/NCT01410968), autologous dendritic cells were isolated in HLA-A2-positive patients, loaded with three A-2 restricted peptides, and readministered as a cellular vaccine. The results were promising with the generation of antigen-specific T cells in three patients, as well as tolerable adverse effects[109]. In a phase I study in Japan, a Wilms' tumor 1 (WT1)-pulsed dendritic cell vaccine combined with chemotherapy showed safety and potential acquisition of immunity in resected PDAC[110]. Multiple associated studies have further supported the clinical benefits of dendritic cell-based vaccines in PDAC[111-113].

Approximately 95% of PDAC patients have mutations in the KRAS oncogene. Despite an early study suggesting an unproven efficacy by targeting mutated KRAS in PDAC[114], multiple subsequent clinical studies have demonstrated the clinical potential for such a therapeutic approach. A phase I/II clinical trial (NCT02261714) (https://clinicaltrials.gov/ct2/show/NCT02261714) evaluated the efficacy of a synthetic mutant RAS peptide vaccine with GM-CSF in PDAC. TG01, a mixture of 7 synthetic RAS peptides representing the most common KRAS mutations, combined with GM-CSF and gemcitabine was well tolerated with a robust immune response and improved clinical outcome[115]. One study demonstrated a long-term immune response and improved survival in patients with resected PDAC after KRAS vaccination[116]. An alternative KRAS-based tumor vaccine is GI-4000, a recombinant heat-
Table 2 Complete vaccine immunotherapy-based clinical trials in pancreatic ductal adenocarcinoma

| Treatment                          | Phase | Number       | Cancer stage               | Outcomes                                                                                     |
|------------------------------------|-------|--------------|----------------------------|---------------------------------------------------------------------------------------------|
| GVAX, 5-FU, chemotherapy           | II    | NCT010084363 | Resected stage             | Median OS 24.8 mo (95% CI: 21.2-31.6 mo)                                                    |
|                                    |       |              | 1/II PDAC                  |                                                                                             |
| GVAX, cyclophosphamide, CRS-207    | II    | NCT01417000  | Metastatic PDAC            | Cy/GVAX and CRS-207 extended OS for PDAC patients, with minimal toxicity                   |
| GVAX, cyclophosphamide, CRS-207    | II    | NCT02004262  | Metastatic PDAC            | Cy/GVAX and CRS-207 did not show survival benefit over chemotherapy in patients with previously treated metastatic PDAC |
| GVAX, Ipilimumab, FOLFIRINOX       | II    | NCT01896669  | Metastatic PDAC            | Ipilimumab + GVAX group did not show survival benefit over chemotherapy                     |
|                                    |       |              |                            | [median OS 9.38 mo (95% CI: 5.0-12.2 mo) vs 14.7 mo (95% CI: 11.6-20.0 mo)]                  |
| Algenpantucel-L                    | II    | NCT00569387  | Surgically resected PDAC   | The addition of algenpantucel-L to standard adjuvant therapy for resected pancreatic cancer may improve survival (12-mo DFS 62%, 12-mo OS 86%) |
| Gemcitabine, SFU                   | III   | NCT01072981  | Surgically resected PDAC   | No results reported yet                                                                    |
| Chemoradiation, Algenpantucel-L    |       |              |                            |                                                                                             |
| Dendritic cells pulsed with MUC-1/WT-1 | I/II  | NCT03114631  | PDAC                       | Dendritic cells immunotherapy provided a favorable outcome in PDAC patients (12-mo OS 78.2% vs 33.8%) |
| GI-4000 (KRAS), Gemcitabine        | II    | NCT00300950  | Non-metastatic, Post-resection PDAC | Overall, GI-4000 group showed a similar pattern of recurrence-free survival and OS compared with the placebo group. For stratified R1 resection subgroup, there was a trend in 1 year OS (72% vs 56%), an improvement in OS (523.5 vs 443.5 d) (hazard ratio: 1.06; 95%CI: 0.53–2.13, P = 0.872), and increased frequency of immune responders (40% vs 8%; P = 0.062) for GI-4000 vs placebo. |
| Ras-peptide vaccine, IL-2, GM-CSF  | II    | NCT00019331  | Metastatic PDAC            | No results reported yet                                                                    |
| GV1001 (telomerase peptide vaccine), Gemcitabine, Capectabine | III | NCT00425360  | Locally Advanced or Metastatic PDAC | Adding GV1001 vaccination to chemotherapy did not improve OS. |

https://clinicaltrials.gov/. PDAC: Pancreatic ductal adenocarcinoma; Cy: cyclophosphamide; DFS: Disease-free survival; OS: Overall survival.

inactivated Saccharomyces cerevisiae yeast-derived vaccine expressing mutated KRAS proteins. A phase I trial revealed a favorable safety profile and immunogenicity of the GI-4000 cancer vaccine[17]. A subsequent phase II trial (NCT00300950) (https://clinicaltrials.gov/ct2/show/NCT00300950) compared GI-4000 plus gemcitabine with placebo plus gemcitabine alone in patients with resected PDAC carrying KRAS mutation. GI-4000 was well tolerated. It led to a similar median OS compared with placebo. However, compared with the placebo group, the GI-4000 group had a trend of improved OS (523.5 vs 443.5 d) and an increased frequency of immune responders (40% vs 8%) in the stratified R1 resection subgroup[18].

The GV1001 tumor vaccine consists of a fragment (16 amino acids) of human telomerase reverse transcriptase (hTERT) found in a high proportion in PDAC cancer cells and has been introduced as a novel therapeutic regimen[19]. In a phase I/II clinical trial evaluating the clinical outcomes in patients with unresectable PDAC, GV1001 plus GM-CSF elicited an immune response in 63% of patients, resulting in a median OS of 7.2 mo for immune responders compared to 2.9 mo for nonimmune responders[20]. However, in a randomized phase III study of patients with locally advanced and metastatic PDAC, combination therapy consisting of GV1001, gemcitabine, and capecitabine chemotherapy showed no improvement in OS compared to chemotherapy alone [6.9 mo (95% CI: 6.4–7.6 mo) vs 7.9 mo (95% CI: 7.1–8.8 mo)] (NCT00425360) (https://clinicaltrials.gov/ct2/show/NCT00425360) [121]. Another GV1001-based phase III clinical trial (NCT00358566) (https://clinicaltrials.gov/ct2/show/NCT00358566) was terminated early because of a lack of survival advantage.

Adoptive cell transfer

Adoptive cell transfer, also known as cellular immunotherapy, includes chimeric antigen receptor T-cell (CAR T cell) therapy and tumor-infiltrating lymphocyte (TIL) therapy[122,123]. CAR T-cell therapy is the most common type of adoptive cell transfer. Generally, it involves harvesting the patient’s T cells, genetic modification to express surface chimeric antigen receptor, ex vivo expansion, and then transferring the cells back to enhance tumor immunity. In forty-three patients with PDAC who underwent radical pancreatectomy, gemcitabine plus adoptive cell transfer with T cells stimulated by the MUC1-expressing human pancreatic cancer cell line demonstrated a median OS of 14.7 mo[124]. Mesothelin is a tumor antigen highly expressed in PDAC[125]. In a preclinical study, CAR T-cell therapy targeting mesothelin demonstrated promising tumor-suppressive effects[126]. Amatuximab
**Table 3 Complete adoptive cell transfer-based clinical trials in pancreatic ductal adenocarcinoma**

| Treatment                                      | Phase | Number          | Cancer stage                  | Outcomes                                                                 |
|------------------------------------------------|-------|-----------------|-------------------------------|--------------------------------------------------------------------------|
| MORAb-009, Gemcitabine                         | II    | NCT00570713     | Advanced PDAC                 | MORAb-009 did not show survival benefit over placebo group [median OS 6.5 mo, 95% CI: 4.5–8.10 mo vs 6.9 mo 95% CI: 5.4–8.8 mo] |
| MORAb-009                                      | I     | NCT00325494     | PDAC                          | No results reported yet                                                  |
| Radiolabeled Amatuximab (MORAb-009)            | I     | NCT01521325     | PDAC                          | No results reported yet                                                  |
| Autologous Redirected RNA Mesothelin CAR T cells| I     | NCT01897415     | PDAC                          | No results reported yet                                                  |
| CART-133 T cells                               | I/II  | NCT02541370     | Relapsed and/or Chemotherapy Refractory Advanced PDAC | No results reported yet                                                  |

https://clinicaltrials.gov/. PDAC: Pancreatic ductal adenocarcinoma; OS: Overall survival.

(MORAb-009), a chimeric mAb targeting mesothelin, also led to reduced growth of mesothelin-expressing tumors, including PDAC[127]. In a phase I trial, the efficacy of MORAb-009 was tested in seven PDAC patients, and one patient had disease control for greater than six months[128]. However, in a phase II randomized placebo-controlled clinical trial (NCT00570713) (https://clinicaltrials.gov/show/NCT00570713) evaluating the efficacy of MORAb-009 plus gemcitabine, no significantly improved clinical outcome was observed [median OS: 6.5 mo (95% CI: 4.5–8.10 mo) vs 6.9 mo (95% CI: 5.4–8.8 mo)]. Compared with the development of ICIs and cancer vaccines, adoptive cell transfer therapy is still in the early development phase against pancreatic cancer (Table 3); more preclinical and clinical studies are needed to further explore its full clinical potential.

**IMMUNOTHERAPY AND THE TUMOR MICROENVIRONMENT**

Immunotherapy has thus far failed to fulfill its promise in PDAC. The underlying mechanisms appear to be complex and multifactorial primarily due to their unique genetic signatures, metabolic features, and immunosuppressive TME. Pancreatic cancers carry unique molecular genetic backgrounds. MSI in pancreatic cancer is extremely rare (approximately 1%). Oncogenic \*KRAS\* mutations, the most common mutation in PDAC, have also contributed to PDAC initiation and maintenance by producing an immunosuppressive TME[129].

Furthermore, altered metabolism of glucose, amino acids, and lipids and their crosstalk with the TME play essential roles in PDAC tumor progression[130]. Multiple lines of evidence have pinpointed the TME as one of the significant barriers to developing effective immunotherapy for PDAC. It is of great clinical interest to sensitize PDAC to immunotherapy through modification of the TME.

One such effort has been focused on CAFs in the TME. As an immunosuppressive component of the TME, FAP-positive CAFs potentially account for the ineffectiveness of immunotherapy in PDAC[131]. Another subtype of CAFs, characterized by the expression of the leucine-rich repeat-containing 15 (LRRC15) protein, could only be detected in pancreatic cancer tissue and is associated with poor response to anti-PD-L1 therapy[132]. Notably, FAP-positive CAFs are the only CAF subtype that expresses CX motif chemokine ligand 12 (CXCL12). Ablation of FAP-positive CAFs or inhibition of CXCL12 uncovers the antitumor activity of CTLA-4 and PD-L1-based immunotherapy[133]. A phase I/II clinical trial (NCT03168139) (https://clinicaltrials.gov/ct2/show/NCT03168139) was conducted to evaluate the treatment effect of pembrolizumab in patients receiving docetaxel (NOX-A12), an agent targeting CXCL12 and TME in metastatic PDAC. No results have been reported yet.

Cellular components in the TME, including MDSCs and TAMs, are also promising targets in the combinatorial strategy for immunotherapy. MDSCs and TAMs induce an immunosuppressive TME, partially through colony-stimulating factor 1 receptor (CSF1R) and focal adhesion kinase (FAK)[134]. Small molecular inhibitors of CSF1R or FAK can reprogram the TME and improve T lymphocyte-mediated pancreatic cancer destruction[135,136]. Multiple clinical trials with CSF1R or FAK inhibitors combined with immunotherapy are currently ongoing (Table 4).

**CONCLUSION**

Despite advances in translational research, PDAC remains a highly lethal malignancy. Recent breakthroughs in immunotherapy have revolutionized cancer therapy and have shown great potential to transform future PDAC treatment. However, PDAC has shown inferior treatment outcomes toward
Table 4 Ongoing clinical trials with immunotherapy plus agents targeting the tumor microenvironment in pancreatic ductal adenocarcinoma

| Strategy | Treatment | Phase | Number | Cancer stage |
|----------|-----------|-------|--------|--------------|
| Immune checkpoint inhibitor (target) + CAFs/CXCL12 targeted agents | Pembrolizumab (PD-1) + Olaptesed pegol | I/II | NCT03168139 | Metastatic PDAC |
| Immune checkpoint inhibitor (target) + CSF1R targeted agent | Durvalumab (PD-L1) + Pexidartinib | I | NCT02777710 | Metastatic/Advanced PDAC |
| | Nivolumab (PD-1) + Cabiralizumab | I | NCT02526017 | Advanced PDAC |
| | Nivolumab (PD-1) + Cabiralizumab + Gemcitabine | II | NCT03697564 | Advanced PDAC (Stage IV) |
| Immune checkpoint inhibitor (target) + FAK targeted agent | Pembrolizumab (PD-1) + Defactinib + Gemcitabine | I/IIa | NCT02758587 | Advanced PDAC |
| | Pembrolizumab (PD-1) + Defactinib + Gemcitabine | I | NCT02546531 | Advanced PDAC |
| | Pembrolizumab (PD-1) + Defactinib | II | NCT03727880 | Resectable PDAC |

https://clinicaltrials.gov/. PDAC: Pancreatic ductal adenocarcinoma; CTLA-4: Cytotoxic T lymphocyte-associated antigen-4; PD-1: Programmed cell death protein 1; PD-L1: Programmed cell death ligand 1; CAFs: Cancer-associated fibroblasts; CXCL12: CXC motif chemokine ligand 12; CSF1R: Colony-stimulating factor 1 receptor; FAK: Focal adhesion kinase.

various immunotherapy regimens compared to other cancer types. The TME has been considered as the fundamental underlying barrier to therapy resistance. To overcome this therapeutic resistance, further investigations with innovative treatment strategies will be needed.

FOOTNOTES

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Country/Territory of origin: United States

ORCID number: Caitlyn Smith 0000-0001-8944-0480; Wei Zheng 0000-0003-3193-2655; Jixin Dong 0000-0002-2757-4464; Yaohong Wang 0000-0001-5964-0465; Jinping Lai 0000-0001-5791-2017; Feng Yin 0000-0002-8444-1123.

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REFERENCES

1 Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin 2021; 71: 7-33 [PMID: 33433946 DOI: 10.3322/caac.21654]
2 Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, Stein KD, Alteri R, Jemal A. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin 2016; 66: 271-289 [PMID: 27253694 DOI: 10.3322/caac.21349]
3 Porta M, Fabregat X, Malats N, Guarner L, Carrato A, de Miguel A, Ruiz L, Jariod M, Costafreda S, Coll S, Alguacil J, Corominas JM, Solá R, Salas A, Real FX. Exocrine pancreatic cancer: symptoms at presentation and their relation to tumour site and stage. Clin Transl Oncol 2005; 7: 189-197 [PMID: 15960930 DOI: 10.1007/BF02712816]
4 De Souza A, Khawaja KI, Masud F, Saif MW. Metformin and pancreatic cancer: Is there a role? Cancer Chemother Pharmacol 2016; 77: 235-242 [PMID: 26740120 DOI: 10.1007/s00280-015-2948-8]
5 Corbu V, Tortora G, Scarpa A. Molecular pathology of pancreatic cancer: from bench-to-bedside translation. Curr Drug
MA, Tuveson DA, Frost GI, Shepard HM, Huang Z. Accumulation of extracellular hyaluronan by hyaluronan synthase 3

Kultti A

Binenbaum Y

Description of a Complex Network and Promising Therapeutic Options.

Ferrara B

Karagiannis GS

[PMID: 10.1038/nrgastro.2012.115]

Desmoplasia in Primary Tumors and Metastatic Lesions of Pancreatic Cancer.

Hessmann E

Whatcott CJ

DOI: 10.1016/S0140-6736(20)30974-6

Ettrich TJ, Seefflerlein T. Systemic Therapy for Metastatic Pancreatic Cancer. Curr Treat Options Oncol 2021; 22: 106

[PMID: 34665339 DOI: 10.1007/s11864-021-00895-4]

Oberstein PE, Olive KP. Pancreatic cancer: is it so hard to treat? Therap Adv Gastroenterol 2013; 6: 321-337

[PMID: 23184611 DOI: 10.1177/1756283X13478860]

Conroy T, Desesseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouquiérière C, Bennouna J, Bachelot JF, Khemissa-Akouz F, Pérez-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M; Groupe Tumeurs Digestives de Unicancer; PRODIGE Intergroup. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011; 364: 1817-1825

[PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

Sarantis P, Koustantis E, Papadimitropoulos A, Papavassiliou AG, Karamouzis MV. Pancreatic ductal adenocarcinoma: Treatment hurdles, tumor microenvironment and immunotherapy. J World J Gastrointest Oncol 2020; 12: 173-181

[PMID: 32104548 DOI: 10.4251/wjgo.v12.i2.173]

Storz P, Crawford HC. Carcinogenesis of Pancreatic Ductal Adenocarcinoma. Gastroenterology 2020; 158: 2072-2081

[PMID: 32199881 DOI: 10.1053/j.gastro.2020.02.059]

Grant TJ, Hua K, Singh A. Molecular Pathogenesis of Pancreatic Cancer. Prog Mol Biol Transl Sci 2016; 144: 241-275

[PMID: 27865459 DOI: 10.1016/bs.pmbts.2016.09.008]

Samuel N, Hudson TJ. The molecular and cellular heterogeneity of pancreatic ductal adenocarcinoma. Nat Rev Gastroenterol Hepatol 2011; 9: 77-87

[PMID: 22183185 DOI: 10.1038/nrgastro.2011.215]

Lee CJ, Li C, Simeone DM. Human pancreatic cancer stem cells: implications for how we treat pancreatic cancer. Transl Oncol 2008; 1: 14-18

[PMID: 18607507 DOI: 10.1593/doi.08013]

Pham T, Roth S, Kong J, Guerra G, Narasimhan V, Pereira L, Desai J, Heriot A, Ramsay R. An Update on Immunotherapy for Solid Tumors: A Review. Ann Surg Oncol 2018; 25: 3404-3412

[PMID: 30093324 DOI: 10.1245/s10434-018-6658-4]

Katayama ES, Hue JJ, Bajor DL, Ocun LM, Ammori JB, Hardacre JM, Winter JM. A comprehensive analysis of clinical trials in pancreatic cancer: what is coming down the pike? Oncotarget 2020; 11: 3489-3501

[PMID: 33014285 DOI: 10.18632/oncotarget.27727]

Goggin M, Offerhaus GJ, Hilgers W, Griffin CA, Shekher M, Tang D, Sohn TA, Yeo CJ, Kern SE, Hruban RH. Pancreatic adenocarcinomas with DNA replication errors (RER+) are associated with wild-type K-ras and characteristic histopathology. Poor differentiation, a syncytial growth pattern, and pushing borders suggest RER+. Am J Pathol 1998; 152: 1501-1507

[PMID: 9862054]

Wilentz RE, Goggin M, Redston M, Marcus VA, Adsay NV, Sohn TA, Kadkol SS, Yeo CJ, Choti M, Zaharak M, Johnson K, Tasci T, Offerhaus GJ, Hruban RH, Kern SE. Genetic, immunohistochemical, and clinical features of medullary carcinoma of the pancreas: A newly described and characterized entity. Am J Pathol 2000; 156: 1641-1651

[PMID: 10793575 DOI: 10.1016/S0002-9440(10)6035-3]

Foucher ED, Gibgo C, Chouaib S, Galon J, Iovanna L, Olive D. Pancreatic Ductal Adenocarcinoma: A Strong Imbalance of Good and Bad Immunological Cops in the Tumor Microenvironment. Front Immunol 2018; 9: 1044

[PMID: 29868007 DOI: 10.3389/fimmu.2018.01044]

Ren B, Cui M, Yang G, Wang H, Feng M, You L, Zhao Y. Tumor microenvironment participates in metastasis of pancreatic cancer. Mol Cancer 2018; 17: 108

[PMID: 30060755 DOI: 10.1186/s12943-018-0858-1]

Waghray M, Yalamanchili M, di Magliano MP, Simeone DM. Deciphering the role of stroma in pancreatic cancer. Curr Opin Gastroenterol 2013; 29: 537-543

[PMID: 23892539 DOI: 10.1097/MOG.0b013e328363af6e]

Ho WJ, Jaffeem EM, Zheng L. The tumour microenvironment in pancreatic cancer - clinical challenges and opportunities. Nat Rev Clin Oncol 2020; 17: 527-540

[PMID: 32398706 DOI: 10.1038/s41571-020-0363-5]

Hessmann E, Buchholz SM, Demir IE, Singh SK, Gress TM, Ellenrieder V, Neesse A. Microenvironmental Determinants of Pancreatic Cancer. Physiol Rev 2020; 100: 1707-1751

[PMID: 32297835 DOI: 10.1152/physrev.00042.2019]

Erkan M, Hausmann S, Michalski CW, Fingerle AA, Dobritz M, Kleeff J, Fries H. The role of stroma in pancreatic cancer: diagnostic and therapeutic implications. Nat Rev Gastroenterol Hepatol 2012; 9: 454-467

[PMID: 22710659 DOI: 10.1038/nrgastro.2012.115]

Whatcott CJ, Diep CH, Jiang P, Watanabe A, LoBello J, Sima C, Hostetter G, Shepard HM, Von Hoff DD, Han H. Desmoplasia in Primary Tumors and Metastatic Lesions of Pancreatic Cancer. Clin Cancer Res 2015; 21: 3561-3568

[PMID: 25469592 DOI: 10.1158/1078-0432.CCR-14-1051]

Karakannissis GS, Postahidis T, Erdogan SE, Kirsch R, Riddell RH, Diamandis EP. Cancer-associated fibroblasts drive the progression of metastasis through both paracrine and mechanical pressure on cancer tissue. Mol Cancer Res 2012; 10: 1403-1418

[PMID: 23024188 DOI: 10.1158/1541-7786.MCR-12-0307]

Ferrara B, Pigatelli C, Cossutta M, Citro A, Courty J, Piemonti L. The Extracellular Matrix in Pancreatic Cancer: Description of a Complex Network and Promising Therapeutic Options. Cancers (Basel) 2021; 13 [PMID: 34503252 DOI: 10.3390/cancers13144442]

Binnenbaum Y, N'ara'a S, Gil Z. Gemcitabine resistance in pancreatic ductal adenocarcinoma. Drug Resist Updat 2015; 23: 55-68

[PMID: 26690340 DOI: 10.1016/j.drup.2015.10.002]

Kultti A, Zhao C, Singh NC, Zimmerman S, Osgood RJ, Symons R, Jiang P, Li X, Thompson CB, Infante JR, Jacobetz MA, Tuveson DA, Frost GI, Shepard HM, Huang Z. Accumulation of extracellular hyaluronan by hyaluronan synthase 3
promotes tumor growth and modulates the pancreatic cancer microenvironment. *Biomed Res Int* 2014; **2014**: 817613 [PMID: 25147816 DOI: 10.1155/2014/817613]

32 **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]

33 **Guille J**, Borre M, Vogelzang NJ, Ng S, Agarwal N, Parker CC, Pook DW, Ratneshburg P, Flajg TW, Carles J, Saad F, Shore ND, Chen L, Heery CR, Gerritsen WR, Prisu F, Langkilde NC, Novikov A, Kantoff PW. Phase III Trial of PROSTVAC in Asymptomatic or Minimally Symptomatic Metastatic Castration-Resistant Prostate Cancer. *J Clin Oncol* 2019; **37**: 1051-1061 [PMID: 30912751 DOI: 10.1200/JCO.18.02031]

34 **Von Cutsen E**, Tempero MA, Sigal D, Oh DY, Fazio N, Macarulla T, Hitre E, Hammell P, Hendifae AF, Bates SE, Li CP, Hingorani SR, de la Fouchardiere C, Kasi A, Heinemann V, Maraveyas A, Bahgary N, Layos L, Sahavi V, Zheng L, Lacy J, Pack JO, Portales F, Oberstein P, Wu W, Chondros D, Bullock AJ; HALO 109-301 Investigators. Randomized Phase III Trial of Pegvorhyaluronidase Alfa With Nab-Paclitaxel Plus Gemcitabine for Patients With Hyaluronan-High Metastatic Adenocarcinoma. *J Clin Oncol* 2020; **38**: 3185-3194 [PMID: 32706635 DOI: 10.1200/JCO.20.00590]

35 **Coussens LM**, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* 2002; **295**: 2387-2392 [PMID: 11923519 DOI: 10.1126/science.1067100]

36 **Bramhall SR**, Neoptolemos JP, Stamp GW, Lemoine NR. Imbalance of expression of matrix metalloproteinases (MMPs) and tissue inhibitors of the matrix metalloproteinases (TIMPs) in human pancreatic carcinoma. *J Pathol* 1997; **182**: 347-355 [PMID: 9349239 DOI: 10.1002/(SICI)1096-9896(199707)182:3<347::AID-PATH486>3.0.CO;2-J]

37 **Xu Y**, Li Z, Jiang P, Wu G, Chen K, Zhang X, Li X. The co-expression of MMP-9 and Tenascin-C is significantly associated with the progression and prognosis of pancreatic cancer. *Diagn Pathol* 2015; **10**: 211 [PMID: 26652622 DOI: 10.1186/s13005-015-0445-3]

38 **Okada Y**, Eibl G, Guha S, Duffy JP, Reber HA, Hines OJ. Nerve growth factor stimulates MMP-2 expression and activity and increases invasion by human pancreatic cancer cells. *Clin Exp Metastasis* 2004; **21**: 285-292 [PMID: 15554384 DOI: 10.1023/b:clin.0000046311.24625.54]

39 **Schneiderhan W**, Diaz F, Fundel M, Zhou S, Siech M, Hasel C, Möller P, Gschwend JE, Seufferlein T, Tress G, Adler G, Bachem MG. Pancreatic stem cells are an important source of MMP-2 in human pancreatic cancer and accelerate tumor progression in a murine xenograft model and CAM assay. *J Cell Sci* 2007; **120**: 512-519 [PMID: 17227797 DOI: 10.1242/jcs.03347]

40 **Ellenrieder V**, Alber B, Lacher U, Hendler SF, Menke A, Boeck W, Wagner M, Wilda M, Friess H, Bührer M, Adler G, Gress TM. Role of MT-MMPs and MMP-2 in pancreatic cancer progression. *Int J Cancer* 2000; **85**: 14-20 [PMID: 10585576 DOI: 10.1002/(sici)1097-0215(20000115)85:1<14::aid-ijc3.0.co;2-o]

41 **Crawford HC**, Scoggins CR, Washington MK, Matrisian LM, Leach SD. Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas. *J Clin Invest* 2002; **109**: 1437-1444 [PMID: 12045257 DOI: 10.1172/JCI15051]

42 **Marshall DC**, Lyman SK, McCauley S, Kovalenko M, Spangler R, Liu C, Lee M, O'Sullivan C, Barry-Hamilton V, Ghermaiazeh N, Mikelsen-Vigdal A, Garcia CA, Jorgensen B, Velayo AC, Wang R, Adamek-Wilson JL, Smith V. Selective AllostERIC Inhibition of MMP9 Is Efficacious in Preclinical Models of Ulcerative Colitis and Colorectal Cancer. *PLoS One* 2015; **10**: e0127063 [PMID: 25961843 DOI: 10.1371/journal.pone.0127063]

43 **Grünewald B**, Vandooren J, Gerg M, Ahomaa K, Hunger A, Berchtold S, Akbarieh S, Schafner S, Knolle P, Edwards DR, Shaw PJ, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR, Laklai H, Sugimoto H, Kahlert C, Okada Y, Eibl G, Guha S, Duffy JP, Reber HA, Hines OJ. Nerve growth factor stimulates MMP-2 expression and activity and increases invasion by human pancreatic cancer cells. *Clin Exp Metastasis* 2004; **21**: 285-292 [PMID: 15554384 DOI: 10.1023/b:clin.0000046311.24625.54]

44 **Oliver KP**, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Alard A, Fresco KK, Denicola G, Feig C, Combs C, Bührer M, Adler G, Gress TM. Role of MT-MMPs and MMP-2 in pancreatic cancer progression. *Int J Cancer* 2000; **85**: 14-20 [PMID: 10585576 DOI: 10.1002/(sici)1097-0215(20000115)85:1<14::aid-ijc3.0.co;2-o]

45 **Cheng X**, Kim JY, Ghafoory S, Duvaci T, Rafiee R, Theobald J, Alborzinha H, Holena P, Fredebohm J, Merz KH, Mehrabi A, Hafezi M, Saffari A, Eisenbrand G, Hoheisel JD, Wölfl S. Methylisoindigo preferentially kills cancer stem cells by interfering cell metabolism *J Natl Cancer Inst* 2014; **106**: 824-834 [PMID: 26887594 DOI: 10.1093/jnci/dju060]

46 **Tuong LH**, Pauklin S. Pancreatic Cancer Microenvironment and Cellular Composition: Current Understandings and Therapeutic Approaches. *Cancers* (Basel) 2021; **13**: 3185-3194 [PMID: 34638513 DOI: 10.3390/cancers13195028]

47 **Richards KE**, Zeleniak AE, Fishel ML, Wu J, Littlepage LE, Hill R. Cancer-associated fibroblast exosomes suppress survival and proliferation of pancreatic cancer cells. *Oncogene* 2009; **28**: 324-334 [PMID: 19469066 DOI: 10.1126/science.1171362]

48 **Olivers O**, Mayers JR, Gourian V, Torrence ME, Gicquel T, Borge L, Lac S, Roques J, Lavaut MN, Berthezène P, Rubin M, Seqc V, Garcia S, Moutardier V, Lombardo D, Iovanna JL, Tommasi R, Guillaumond F, Vander Heiden MG, Vasseur S. Collagen-derived proline promotes pancreatic ductal adenocarcinoma cell survival under nutrient limited conditions. *Nat Commun* 2017; **8**: 16031 [PMID: 28685754 DOI: 10.1038/ncomms16031]

49 **Sousa CM**, Biancur DE, Wang X, Halbrook CJ, Sherman MH, Zhang L, Kremer D, Hwang RF, Witkiewicz AK, Ying H, Arasa JM, Evans RM, Cantley LC, Lyssiotis CA, Kimmelman AC. Pancreatic stellate cells support tumour metabolism through autophagic alanine secretion. *Nature* 2016; **536**: 479-483 [PMID: 27509858 DOI: 10.1038/nature19084]

50 **Özdemir BC**, Pentcheva-Hoang T, Carstens JL, Zhou X, Wu CC, Simpson TR, Laklai H, Sugimoto H, Kahlert C, Novitskyy SV, de Jesus-Acosta A, Sharma P, Heidari P, Mahmood U, Chin L, Moses HL, Weaver VM, Maitra A, Allison JP, LeBlouc 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.crc.2014.04.005]

51 Lee JJ, Perera RM, Wang H, Wu DC, Liu XS, Han S, Fitamant J, Jones PD, Ghanta KS, Kawano S, Nagle JM, Deshpande V, Boucher Y, Kato T, Chen JK, Willmann JK, Bardeesy N, Beachy P.A. Stromal response to Hedgehog signaling restrain pancreatic cancer progression. *Proc Natl Acad Sci U S A* 2014; 111: E3091-E3100 [PMID: 25024225 DOI: 10.1073/pnas.1411679111]

52 Biffi G, Tuveson DA. Diversity and Biology of Cancer-Associated Fibroblasts. *Physiol Rev* 2021; 101: 147-176 [PMID: 32466724 DOI: 10.1152/physrev.00048.2019]

53 Helms E, Onate MK, Sherman MH. Fibroblast Heterogeneity in the Pancreatic Tumor Microenvironment. *Cancer Discov* 2020; 10: 648-656 [PMID: 32014869 DOI: 10.1158/2326-7278.CD-19-1353]

54 Öhliund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, Corbo V, Oni TE, Heam SA, Lee EJ, Chio II, Hwang CI, Tiriach H, Boker LA, Engle DD, Feig C, Kulti A, Egeblad M, Fearon DT, Crawford JM, Clevers H, Park Y, Tuveson DA. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med* 2017; 214: 579-596 [PMID: 28232471 DOI: 10.1084/jem.20162024]

55 Sahai E, Asatsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, Fearon D, Goren T, Hingorani SR, Hunter T, Hynes RO, Jain RK, Janowitz T, Jorgensen C, Kimmelman AC, Kolonin MG, Maki RG, Powers RS, Puré E, Ramirez DC, Scherz-Shouval R, Sherman MH, Stewart S, Tlsty TD, Tuveson DA, Watt FM, Weaver V, Weeraratna AT, Werb Z. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat Rev Cancer* 2020; 20: 174-186 [PMID: 31980749 DOI: 10.1038/s41568-019-0238-1]

56 Kalluri R. The biology and function of fibroblasts in cancer. *Nat Rev Cancer* 2016; 16: 582-598 [PMID: 27550820 DOI: 10.1038/nrc.2016.73]

57 Yang X, Lin Y, Shi Y, Li B, Liu W, Yin W, Dang Y, Chu Y, Fan J, He R. FAP Promotes Immunosuppression by Cancer-Related Fibroblasts in the Tumor Microenvironment via STAT3-CCL2 Signaling. *Cancer Res* 2016; 76: 4124-4135 [PMID: 27271761 DOI: 10.1158/0008-5472.CAN-15-2973]

58 Shi M, Yu DH, Chen Y, Zhao CY, Zhang J, Liu QH, Ni CR, Zhu MH. Expression of fibroblast activation protein in human pancreatic adenocarcinoma and its clinicopathological significance. *World J Gastroenterol* 2012; 18: 840-846 [PMID: 22371645 DOI: 10.3748/wjg.v18.i8.840]

59 Ziani L, Chouaib S, Thiery J. Alteration of the Antitumor Immune Response by Cancer-Associated Fibroblasts. *Front Immunol* 2018; 9: 414 [PMID: 29545811 DOI: 10.3389/fimmu.2018.00414]

60 Kobayashi H, Enomoto A, Woods SL, Burt AD, Takahashi M, Worthing D. Cancer-associated fibroblasts in gastrointestinal cancer. *Nat Rev Gastroenterol Hepatol* 2019; 16: 282-295 [PMID: 30778141 DOI: 10.1038/s41575-019-0115-0]

61 Li T, Yi S, Liu W, Jia C, Wang G, Hua X, Tai Y, Zhang Q, Chen G. Colorectal carcinoma-derived fibroblasts modulate natural killer cell phenotype and antitumor cytotoxicity. *Med Oncol* 2013; 30: 663 [PMID: 23873014 DOI: 10.1007/s12032-013-0663-z]

62 Fitzgerald AA, Weiner LM. The role of fibroblast activation protein in health and malignancy. *Cancer Metastasis Rev* 2020; 39: 783-803 [PMID: 32601975 DOI: 10.1007/s10555-020-09909-3]

63 Aliru ML, Schoenhals JE, Venkatesulu BP, Anderson CC, Barosmian HB, Younes AI, K Mahadevan LS, Soeung M, Aziz KE, Welsh JW, Krishnan S. Radiation therapy and immunotherapy: what is the optimal timing or sequencing? *Immunotherapy* 2018; 10: 299-316 [PMID: 29421979 DOI: 10.2217/imt-2017-0082]

64 Ren X, Ye F, Jiang Z, Chu Y, Xiong S, Wang Y. Involvement of cellular death in TRAIL-DR5-dependent suppression induced by CD4(+)/CD25(-) regulatory T cells. *Cell Death Dis* 2007; 14: 2067-2068 [PMID: 17762882 DOI: 10.1038/sj.cdd.4402220]

65 Gondek DC, Lu LF, Quezada SA, Sakkaschi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J Immunol* 2005; 174: 1783-1786 [PMID: 15699103 DOI: 10.4049/jimmunol.174.4.1783]

66 Tsuchikawa T, Hirano S, Tanaka E, Matsumoto J, Kato K, Nakamura T, Ebihara Y, Shichinohe T. Novel aspects of preoperative chemoradiation therapy improving anti-tumor immunity in pancreatic cancer. *Cancer Sci* 2013; 104: 531-535 [PMID: 23363422 DOI: 10.1111/cas.12119]

67 Zhang Y, Lazarus J, Steele NG, Yan W, Lee HJ, Nwosu ZC, Hallbrook CJ, Menjivar RE, Kemp SB, Siriharachai VR, Velez-Delgado A, Donahue EA, Carpenter ES, Brown KL, Iriarrey-Negron V, Nevison AC, Vinta A, Anderson MA, Crawford HC, Lyssiotis CA, Frankel TL, Bednar F, Pasca di Magliano M. Regulatory T-cell Depletion Alters the Tumor Microenvironment and Accelerates Pancreatic Carcinogenesis. *Cancer Discov* 2020; 10: 422-439 [PMID: 31911451 DOI: 10.1158/2326-7278.CD-19-0958]

68 Uglet S, De Sanctis F, Mandruzziato S, Bronte V. Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages. *J Clin Invest* 2015; 125: 3365-3376 [PMID: 26325033 DOI: 10.1172/JCI80006]

69 De Cico P, Ercolano G, Iannaro A. The New Era of Cancer Immunotherapy: Targeting Myeloid-Derived Suppressor Cells to Overcome Immune Evasion. *Front Immunol* 2020; 11: 1600 [PMID: 32849585 DOI: 10.3389/fimmu.2020.01680]

70 Markowitz J, Brooks TR, Duggan MC, Paul BK, Pan X, Wei L, Abrams Z, Luedde E, Lesinski GB, Mundy-Bosse B, Bekaii-Saab T, Carson WE. 3rd. Patients with pancreatic adenocarcinoma exhibit elevated levels of myeloid-derived suppressor cells upon progression of disease. *Cancer Immunol Immunother* 2015; 64: 149-159 [PMID: 25365035 DOI: 10.1007/s00262-014-1618-8]

71 Di Caro G, Cortese N, Castino GF, Grizzi F, Gavazzi F, Ridolfi C, Capretti G, Mineri R, Todoric J, Zerbi A, Allavena P, Mantovani A, Marchesi F. Dual prognostic significance of tumour-associated macrophages in human pancreatic adenocarcinoma treated or untreated with chemotherapy. *Gut* 2016; 65: 1710-1720 [PMID: 26139690 DOI: 10.1136/gutjnl-2015-309193]

72 Lankadasari MB, Mukhopadhyay P, Mohammad S, Harikumar KB. TAMing pancreatic cancer: combat with a double edged sword. *Mol Cancer* 2019; 18: 48 [PMID: 30925924 DOI: 10.1186/s12943-019-0966-6]
73 Hu H, Hang JJ, Han T, Zhuo M, Jiao F, Wang LW. The M2 phenotype of tumor-associated macrophages in the stroma confers a poor prognosis in pancreatic cancer. *Tumour Biol* 2016; 37: 8657-8664 [PMID: 26738860 DOI: 10.1007/s13277-015-4741-z]

74 Liu Q, Li Y, Niu Z, Zong Y, Wang M, Yao L, Lu Z, Liao Q, Zhao Y. Atorvastatin (Lipitor) attenuates the effects of aspirin on pancreatic cancerogenesis and the chemotherapeutic efficacy of gemcitabine on pancreatic cancer by promoting M2 polarized tumor associated macrophages. *J Exp Clin Cancer Res* 2016; 35: 33 [PMID: 26579926 DOI: 10.1186/s13046-016-0304-4]

75 Dijkstra EM, Heusinkveld M, Tummers B, Vogelpoel LT, Goedemans R, Jha V, Nortier JW, Walters MJ, Kroep JR, van der Burg SH. Chemotherapy alters monocyte differentiation to favor generation of cancer-supporting M2 macrophages in the tumor microenvironment. *Cancer Res* 2013; 73: 2480-2492 [PMID: 23436796 DOI: 10.1158/0008-5472.CAN-12-3542]

76 Young K, Hughes DJ, Cunningham D, Starling N. Immunotherapy and pancreatic cancer: unique challenges and potential opportunities. *Ther Adv Med Oncol* 2018; 10: 3574212 DOI: 10.1177/1758891818821861

77 Zhang J, Wolfgang CL, Zheng L. Precision Immuno-Oncology: Prospects of Individualized Immunotherapy for Pancreatic Cancer. *Cancers (Basel)* 2018; 10: 29385739 DOI: 10.3390/cancers1020039

78 Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A* 2002; 99: 12293-12297 [PMID: 12218188 DOI: 10.1073/pnas.192461099]

79 Twomey JD, Zhang B. Cancer Immunotherapy Update: FDA-Approved Checkpoint Inhibitors and Companion Diagnostics. *AAPS J* 2021; 23: 39 [PMID: 33677681 DOI: 10.1208/s12248-022-02437-6]

80 Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kamnula US, Sherry RM, Topalian SL, Yang JC, Lowy I, Rosenberg SA. Phase 1 trial of single agent Ipilimumab (anti-CTLA-4) in heavily-advanced or metastatic pancreatic adenocarcinoma. *J Immunother* 2010; 33: 828-833 [PMID: 20842054 DOI: 10.1007/JCI401338131ec14]

81 Torphy RJ, Zhu Y, Schullick RD. Immunotherapy for pancreatic cancer: Barriers and breakthroughs. *Ann Gastroenterol Surg* 2018; 2: 274-281 [PMID: 30003190 DOI: 10.1002/asg.12176]

82 Plate JM, Plate AE, Shott S, Bograd S, Harris JE. Effect of gemcitabine on immune cells in subjects with adenocarcinoma of the pancreas. *Cancer Immunol Immunother* 2005; 54: 915-925 [PMID: 15782312 DOI: 10.1007/s00262-004-0638-1]

83 Aglietta M, Barone C, Sawyer MB, Moore MJ, Miller WH Jr, Bagalà C, Colombi F, Cagnazzo C, Gioeni L, Wang E, Huang B, Fly KD, Leone F. A phase I dose escalation trial of tremelimumab (CP-675,206) in combination with gemcitabine, nab-paclitaxel, and pembrolizumab in metastatic pancreatic adenocarcinoma. *Cancer Immunol Immunother* 2018; 68: 99-102 [PMID: 29119276 DOI: 10.1007/s00262-017-1909-5]

84 Weiss GJ, Blaydorn L, Beck J, Bornemann-Kolatzki K, Uronvitz H, Schütz E, Khemka V. Phase I/II study of immunotherapy, nab-paclitaxel, and pembrolizumab in metastatic pancreatic adenocarcinoma. *Invest New Drugs* 2018; 36: 96-102 [PMID: 29119276 DOI: 10.1007/s10637-017-0525-1]

85 Mizugaki H, Yamanoto N, Murakami H, Kennotsu H, Fujiyara W, Ishida Y, Kawakami T, Takahashi T. Phase I dose-finding study of monotherapy with atezolizumab, an engineered immunoglobulin monoclonal antibody targeting PD-L1, in Japanese patients with advanced solid tumors. *Invest New Drugs* 2016; 34: 596-603 [PMID: 27363843 DOI: 10.1007/s10637-016-0371-6]

86 O'Reilly EM, Oh DY, Dhani N, Renouf DJ, Lee MA, Sun W, Fisher G, Hezel A, Chang SC, Vladovic G, Takahashi O, Yang Y, Fitts D, Philip PA. Darvulamab With or Without Tremelimumab for Patients With Metastatic Pancreatic Ductal Adenocarcinoma: A Phase 2 Randomized Clinical Trial. *JAMA Oncol* 2019; 5: 1431-1438 [PMID: 31318392 DOI: 10.1001/jamaoncol.2019.1588]

87 Jardim DL, Goodman A, de Melo Gagliato D, Kurzrock R. The Challenges of Tumor Mutational Burden as an Immunotherapy Biomarker. *Cancer Cell* 2021; 39: 154-173 [PMID: 33125859 DOI: 10.1016/j.ccell.2020.10.001]

88 Salman B, Zhou D, Jaffee EM, Edil BH, Zheng L. Vaccine therapy for pancreatic cancer. *Oncoimmunology* 2013; 2: e26662 [PMID: 24498551 DOI: 10.4161/onci.26662]

89 Rosenberg A, Mahalingam D. Immunotherapy in pancreatic adenocarcinoma-overcoming barriers to response. *J Gastrointest Oncol* 2018; 9: 143-153 [PMID: 29564181 DOI: 10.21037/jgo.2018.01.13]

90 Laheru D, Biedrzycki B, Jaffee EM. Development of a cytotoxic-modified allogeneic whole cell pancreatic cancer vaccine. *Methods Mol Biol* 2013; 980: 175-203 [PMID: 23359154 DOI: 10.1007/978-1-62703-287-2_9]

91 Jaffee EM, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, Goemann M, Coleman J, Grochow L, Donehower RC, Lillemoen 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 DOI: 10.1200/JCO.2001.19.1.145]

92 Lutz E, Yeo CJ, Lillemoen KD, Biedrzycki B, Kobrin B, Herman J, Sugar E, Piantadosi S, Cameron JL, Solt S, Omners B, Tartakovsky I, Choi M, Sharma R, Ihee 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. *Clin Cancer Res* 2011; 17: 328-335 [PMID: 21217520 DOI: 10.1158/1078-0432.CAN-10-0744]

93 Chung V, Kos FJ, Hardwick N, Yuan Y, Chao J, Li D, Waisman J, Li M, Zurcher K, Frankel P, Diamond DJ. Evaluation of safety and efficacy of p53MAA vaccine combined with pembrolizumab in patients with advanced solid cancers. *Clin Transl Oncol* 2019; 21: 363-372 [PMID: 30094792 DOI: 10.1007/s12094-018-1932-2]

94 Le DT, Lutz E, Uram JN, Sugar EA, Omners B, Solt S, Zheng L, Díaz LA Jr, 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.0b013e31829f7a2j]

95 Osawa R, Tsumoda T, Yoshimura S, Watanabe T, Miyazawa M, Tani M, Takada K, Nakagawa H, Nakamura Y, Yamaue H. Identification of HLA-A24-restricted novel T Cell epitope peptides derived from P-cadherin and kinesin family member 20A. *J Biomed Biotechnol* 2012; 2012: 848042 [PMID: 22778556 DOI: 10.1155/2012/848042]
Aksnes AK, Miller R, Dueland S. TG01/GM-CSF and adjuvant gemcitabine in patients with resected RAS-mutant pancreatic cancer. *Am J Clin Oncol* 2011; 34: 771-778 [PMID: 21589970 DOI: 10.1097/SLA.0b013e31822b39f7]

Kobayashi M, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. *Trends Mol Med* 2014; 20: 332-342 [PMID: 24667139 DOI: 10.1016/j.molmed.2014.02.007]

Behrens ME, Mucigur E, Yamasaki T, Uemura C, Koido S, Kan S, Yoshida K, Mori M, Hirano Y, Ito Z, Kobayashi H, Takami S, Matsumoto Y, Kajihara M, Misawa T, Okamoto M, Sugiyama H, Shimodaira S. Prognostic markers for patient outcome following pancreatic cancer receiving chemotherapy: a multicenter analysis. *Hum Vaccin Immunother* 2015; 11: 2945-2954 [PMID: 26619245 DOI: 10.1089/hum.2015.0193263]

Hewitt DB, Nissen N, Hatoun H, Mushber B, Seng J, Covelier AL, Al-Raijahi R, Yeo CJ, Leiby B, Banks J, Balducci L, Vaccaro G, LoConte N, George TJ, Brenner W, Elquza E, Vahanian N, Rossi G, Kennedy E, Link C, Lavu H. A Phase 3 Randomized Clinical Trial of Chemotherapy With or Without Algenpantucel-L (HyperAcute-Pancreas) Immunotherapy in Subjects With Borderline Resectable or Locally Advanced Unresectable Pancreatic Cancer. *Ann Surg Oncol* 2022; 29: 475-483: [PMID: 36304573 DOI: 10.1007/s10434-020-09257-x]

Nath S, Mukherjee P, MUC1: a multifaceted oncoprotein with a key role in cancer progression. *Trends Mol Med* 2014; 20: 332-342 [PMID: 24667139 DOI: 10.1016/j.molmed.2014.02.007]

Behrens ME, Grandgenett PM, Bailey JM, Singh PK, Yi CH, Yu F, Hollingsworth MA. The reactive tumor microenvironment: MUC1 signaling directly regulates expression of CTGF. *Oncogene* 2010; 29: 5667-5677 [PMID: 20697347 DOI: 10.1038/onc.2010.327]

Lepisto AJ, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, Geller BA, Schmotzer A, Potter DP, Whiteside T, Finn OJ, Ramanathan RK. A phase I/I study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. *Hum Vaccin Immunother* 2019; 15: 2217-2225 [PMID: 29599342 DOI: 10.2217/imt.18.113]

Kimura Y, Zhou QB, Liao YD, Mai C, Chen TJ, Tang YQ, Chen RF. Optimized construction of MUC1-VNTR, DNA vaccine and its anti-pancreatic cancer efficacy. *Oncol Lett* 2017; 13: 2198-2206 [PMID: 28454381 DOI: 10.3892/ol.2017.5717]

Palucka K, Ueno H, Fay J, Banchereau J. Dendritic cells and immunity against cancer. *J Intern Med* 2011; 269: 64-73 [PMID: 21558979 DOI: 10.1111/j.1365-2796.2010.02317.x]

Mehrotra S, Britten CD, Chin S, Garrett-Mayer E, Cloud CA, Li M, Scurti G, Salem ML, Nelson MH, Thomas MB, Paulos CM, Salazar AM, Nishimura MI, Rubinstein MP, Li Z, Cole DJ. Vaccination with poly(IC:LC) and peptide-pulsed dendritic cells for patients with resected pancreatic and biliary tumors. *J Gastrointest Surg* 2012; 16: 64-73 [PMID: 2217-2225 [PMID: 23078230 DOI: 10.1111/j.1365-2796.2010.02317.x]

Kobayashi M, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. *Trends Mol Med* 2014; 20: 332-342 [PMID: 24667139 DOI: 10.1016/j.molmed.2014.02.007]

Behrens ME, Grandgenett PM, Bailey JM, Singh PK, Yi CH, Yu F, Hollingsworth MA. The reactive tumor microenvironment: MUC1 signaling directly regulates expression of CTGF. *Oncogene* 2010; 29: 5667-5677 [PMID: 20697347 DOI: 10.1038/onc.2010.327]

Lepisto AJ, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, Geller BA, Schmotzer A, Potter DP, Whiteside T, Finn OJ, Ramanathan RK. A phase I/I study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. *Hum Vaccin Immunother* 2019; 15: 2217-2225 [PMID: 29599342 DOI: 10.2217/imt.18.113]

Kimura Y, Tsukada J, Tomoda T, Takahashi H, Imai K, Shimamura K, Sunamura M, Yonemitsu Y, Shimodaira S, Koido S, Homma S, Okamoto M. Clinical and immunologic evaluation of dendritic cell-based immunotherapy in combination with gemcitabine and/or S-1 in patients with advanced pancreatic cancer. *Pancreas* 2012; 41: 195-205 [PMID: 21792083 DOI: 10.1097/MPA.0b013e31822b39f7]

Kobayashi M, Shimodaira S, Nagai K, Ogawasara M, Takahashi H, Abe H, Tanii M, Okamoto M, Tsujitani S, Yusa S, Ishida T, Kishimoto J, Shibamoto Y, Nagaya M, Yonemitsu Y, DC Vaccine Study Group at the Japan Society of Innovative Cell Therapy (J-SICT). Prognostic factors related to add-on dendritic cell vaccines on patients with inoperable pancreatic cancer receiving chemotherapy: a multicenter analysis. *Cancer Immunol Immunother* 2014; 63: 797-806 [PMID: 24777613 DOI: 10.1007/s00262-014-1554-7]

Takakura K, Koido S, Kan S, Yoshida K, Mori M, Hirano Y, Ito Z, Kobayashi H, Takami S, Matsumoto Y, Kajihara M, Misawa T, Okamoto M, Sugiyama H, Homma S, Ohkusa T, Taji H. Prognostic markers for patient outcome following vaccination with multiple MHC Class I/II-restricted WT1 peptide-pulsed dendritic cell genes plus chemotherapy for pancreatic cancer. *Anticancer Res* 2015; 35: 555-562 [PMID: 25550020]

Abou-Alfa GK, Chapman PB, Feilchenfeldt J, Brennan MF, Capano M, Gansukh B, Jacobs G, Levin A, Neville D, Kelsen DP, O'Reilly EM. Targeting mutated K-ras in pancreatic adenocarcinoma using an adjuvant vaccine. *Am J Clin Oncol* 2011; 34: 321-325 [PMID: 20686403 DOI: 10.1097/COC.0b013e3181e84b1f]

Palmer DH, Valle JW, Ma YT, Faluyi O, Neoptolemos JP, Jensen Gjertsen T, Iversen B, Amund Eriksen J, Møller AS, Valle JW, Ma YT, Faluyi O, Neoptolemos JP, Jensen Gjertsen T, Iversen B, Amund Eriksen J, Møller AS, Nakano A, Sasaki F, Shibamoto Y, Yamasaki T, Uemura C, Koido S, Kan S, Yoshida K, Mori M, Hirano Y, Ito Z, Kobayashi H, Takami S, Matsumoto Y, Kajihara M, Misawa T, Okamoto M, Sugiyama H, Homma S, Ohkusa T, Taji H. Prognostic markers for patient outcome following vaccination with multiple MHC Class I/II-restricted WT1 peptide-pulsed dendritic cell genes plus chemotherapy for pancreatic cancer. *Anticancer Res* 2015; 35: 555-562 [PMID: 25550020]
Smith C et al. Tumor microenvironment in pancreatic cancer

116 Wedén S, Klemp M, Gladhaug IP, Møller S, Erikson JA, Gaudernack G, Buunes T. Long-term follow-up of patients with resected pancreatic cancer following vaccination against mutant K-ras. \textit{Int J Cancer} 2011; \textbf{128}: 1120-1128 [PMID: 20473937 DOI: 10.1002/ijc.25449]

117 Cohn A, Morse MA, O'Neil B, Whiting S, Coeshott C, Ferraro J, Bellgrau D, Apelian D, Rodell TC. Whole Recombinant Saccharomyces cerevisiae Yeast Expressing Ras Mutations as Treatment for Patients With Solid Tumors Bearing Ras Mutations: Results From a Phase 1 Trial. \textit{J Immunother} 2018; \textbf{41}: 141-150 [PMID: 29252891 DOI: 10.1007/s10375-018-00219]

118 Muscarella P, Bekaii-Saab T, McIntyre K, Rosemurgy A, Ross SB, Richards DA, Fisher WE, Flynn PJ, Mattson A, Coeshott C, Roder H, Roder J, Harrell FE, Cohn A, Rodell TC, Apelian D. A Phase 2 Randomized Placebo-Controlled Adjuvant Trial of GI-4000, a Recombinant Yeast Expressing Mutated Ras Proteins in Patients with Resected Pancreas Cancer. \textit{J Pancreat Cancer} 2021; 7: 8-19 [PMID: 33768412 DOI: 10.1089/panc.2020.0021]

119 Suedara N, Mizumoto K, Muta T, Tominaga Y, Shimura H, Kitajima S, Hamasaki N, Tsuchiyoshi M, Tanaka M. Telomerase elevation in pancreatic ductal carcinoma compared to nonmalignant pathological states. \textit{Clin Cancer Res} 1997; 3: 993-998 [PMID: 9815776]

120 Bernhardt SL, Gjertsen MK, Trachsel S, Møller S, Erikson JA, Meo M, Buunes T, Gaudernack G. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/I study. \textit{Br J Cancer} 2006; \textbf{95}: 1474-1482 [PMID: 17060934 DOI: 10.1038/sj.bjc.6603437]

121 Middleton G, SilcockS, Cox T, Valle J, Wadsley J, Propper D, Coxon F, Ross P, Madhusudan S, Roques T, Cunningham D, Falk S, Wadd N, Harrison M, Conrie P, Iverson T, Robinson A, McAdam K, Eatoek M, Evans J, Archer C, Hickish T, García-Alonso A, Nicolson M, Steward M, Anthesly A, Greenhalh W, Shaw V, Costello E, Naisbitt D, Rawcliffe C, Nanson G, Neoptolemos J. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeleVac): an open-label, randomised, phase 3 trial. \textit{Lancet Oncol} 2014; \textbf{15}: 829-840 [PMID: 24954781 DOI: 10.1016/s1470-2045(14)70236-0]

122 Monetti M, Bukhari A, Ahsaadi A, Arbuthn M, Brenton JD, Curtis S, Morris T, Duntali M, Hu Z, McGranahan N, Miller ML, Santaana-Gonzalez L, Seymour LW, Shi T, Van Loo P, Yau C, White H, Wietek N, Church DN, Wedge DC, Ahmed AA. Promises and challenges of adoptive T-cell therapies for solid tumours. \textit{Nat Rev Cancer} 2014; \textbf{14}: 224-239 [PMID: 24872209 DOI: 10.1038/nrc3836]

123 Muscarella P, Bekaii-Saab T, McIntyre K, Rosemurgy A, Ross SB, Richards DA, Fisher WE, Flynn JI, Mattson A, Coeshott C, Roder H, Roder J, Harrell FE, Cohn A, Rodell TC, Apelian D. A Phase 2 Randomized Placebo-Controlled Adjuvant Trial of GI-4000, a Recombinant Yeast Expressing Mutated Ras Proteins in Patients with Resected Pancreas Cancer. \textit{J Pancreat Cancer} 2021; 7: 8-19 [PMID: 33768412 DOI: 10.1089/panc.2020.0021]

124 Suedara N, Mizumoto K, Muta T, Tominaga Y, Shimura H, Kitajima S, Hamasaki N, Tsuchiyoshi M, Tanaka M. Telomerase elevation in pancreatic ductal carcinoma compared to nonmalignant pathological states. \textit{Clin Cancer Res} 1997; 3: 993-998 [PMID: 9815776]

125 Bernhardt SL, Gjertsen MK, Trachsel S, Møller S, Erikson JA, Meo M, Buunes T, Gaudernack G. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/I study. \textit{Br J Cancer} 2006; \textbf{95}: 1474-1482 [PMID: 17060934 DOI: 10.1038/sj.bjc.6603437]

126 Muscarella P, Bekaii-Saab T, McIntyre K, Rosemurgy A, Ross SB, Richards DA, Fisher WE, Flynn JI, Mattson A, Coeshott C, Roder H, Roder J, Harrell FE, Cohn A, Rodell TC, Apelian D. A Phase 2 Randomized Placebo-Controlled Adjuvant Trial of GI-4000, a Recombinant Yeast Expressing Mutated Ras Proteins in Patients with Resected Pancreas Cancer. \textit{J Pancreat Cancer} 2021; 7: 8-19 [PMID: 33768412 DOI: 10.1089/panc.2020.0021]

127 Suedara N, Mizumoto K, Muta T, Tominaga Y, Shimura H, Kitajima S, Hamasaki N, Tsuchiyoshi M, Tanaka M. Telomerase elevation in pancreatic ductal carcinoma compared to nonmalignant pathological states. \textit{Clin Cancer Res} 1997; 3: 993-998 [PMID: 9815776]

128 Bernhardt SL, Gjertsen MK, Trachsel S, Møller S, Erikson JA, Meo M, Buunes T, Gaudernack G. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/I study. \textit{Br J Cancer} 2006; \textbf{95}: 1474-1482 [PMID: 17060934 DOI: 10.1038/sj.bjc.6603437]

129 Muscarella P, Bekaii-Saab T, McIntyre K, Rosemurgy A, Ross SB, Richards DA, Fisher WE, Flynn JI, Mattson A, Coeshott C, Roder H, Roder J, Harrell FE, Cohn A, Rodell TC, Apelian D. A Phase 2 Randomized Placebo-Controlled Adjuvant Trial of GI-4000, a Recombinant Yeast Expressing Mutated Ras Proteins in Patients with Resected Pancreas Cancer. \textit{J Pancreat Cancer} 2021; 7: 8-19 [PMID: 33768412 DOI: 10.1089/panc.2020.0021]

130 Suedara N, Mizumoto K, Muta T, Tominaga Y, Shimura H, Kitajima S, Hamasaki N, Tsuchiyoshi M, Tanaka M. Telomerase elevation in pancreatic ductal carcinoma compared to nonmalignant pathological states. \textit{Clin Cancer Res} 1997; 3: 993-998 [PMID: 9815776]

131 Bernhardt SL, Gjertsen MK, Trachsel S, Møller S, Erikson JA, Meo M, Buunes T, Gaudernack G. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/I study. \textit{Br J Cancer} 2006; \textbf{95}: 1474-1482 [PMID: 17060934 DOI: 10.1038/sj.bjc.6603437]

132 Muscarella P, Bekaii-Saab T, McIntyre K, Rosemurgy A, Ross SB, Richards DA, Fisher WE, Flynn JI, Mattson A, Coeshott C, Roder H, Roder J, Harrell FE, Cohn A, Rodell TC, Apelian D. A Phase 2 Randomized Placebo-Controlled Adjuvant Trial of GI-4000, a Recombinant Yeast Expressing Mutated Ras Proteins in Patients with Resected Pancreas Cancer. \textit{J Pancreat Cancer} 2021; 7: 8-19 [PMID: 33768412 DOI: 10.1089/panc.2020.0021]

133 Suedara N, Mizumoto K, Muta T, Tominaga Y, Shimura H, Kitajima S, Hamasaki N, Tsuchiyoshi M, Tanaka M. Telomerase elevation in pancreatic ductal carcinoma compared to nonmalignant pathological states. \textit{Clin Cancer Res} 1997; 3: 993-998 [PMID: 9815776]

134 Bernhardt SL, Gjertsen MK, Trachsel S, Møller S, Erikson JA, Meo M, Buunes T, Gaudernack G. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/I study. \textit{Br J Cancer} 2006; \textbf{95}: 1474-1482 [PMID: 17060934 DOI: 10.1038/sj.bjc.6603437]
DT, Pachter JA, Wang-Gillam A, DeNardo DG. Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy. *Nat Med* 2016; 22: 851-860 [PMID: 27376576 DOI: 10.1038/nm.4123]
