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**Understanding the immune response and the current landscape of immunotherapy in pancreatic cancer**

Ostios-Garcia *et al*. Immunotherapy in pancreatic cancer

Lorena Ostios-Garcia, Julia Villamayor, Esther Garcia-Lorenzo, David Vinal, Jaime Feliu

**Lorena Ostios-Garcia, Julia Villamayor, Esther Garcia-Lorenzo, David Vinal, Jaime Feliu,** Department of Oncology, La Paz University Hospital, IDIPAZ, CIBERONC, Cátedra UAM-AMGEN, Madrid 28046, Spain

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**Corresponding author: Jaime Feliu, MD, PhD, Associate Professor, Chief Physician,** Department of Oncology, La Paz University Hospital, IDIPAZ, CIBERONC, Cátedra UAM-AMGEN, Paseo de la Castellana, 261, Madrid 28046, Spain. jaimefeliu@hotmail.com

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**Abstract**

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive tumor with high lethality. Even with surgery, radiotherapy, chemotherapy, and other locoregional or systemic therapies, the survival rates for PDAC are low and have not significantly changed in the past decades. The special characteristics of the PDAC’s microenvironment and its complex immune escape mechanism need to be considered when designing novel therapeutic approaches in this disease. PDAC is characterized by chronic inflammation with a high rate of tumor-associated macrophages and myeloid-derived suppressor cells and a low rate of natural killer and effector T cells. The pancreatic microenvironment is a fibrotic, microvascularized stroma that isolates the tumor from systemic vascularization. Immunotherapy, a novel approach that has demonstrated effectiveness in certain solid tumors, has failed to show any practice-changing results in pancreatic cancer, with the exception of PDACs with mismatch repair deficiency and high tumor mutational burden, which show prolonged survival rates with immunotherapy. Currently, numerous clinical trials are attempting to assess the efficacy of immunotherapeutic strategies in PDAC, including immune checkpoint inhibitors, cancer vaccines, and adoptive cell transfer, alone or in combination with other immunotherapeutic agents, chemoradiotherapy, and other targeted therapies. A deep understanding of the immune response will help in the development of new therapeutic strategies leading to improved clinical outcomes for patients with PDAC.

**Key Words:** Pancreatic cancer; Immunity; Immune evasion; Tumor microenvironment; Immunotherapy; Cancer vaccines

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**Core Tip:** Immunotherapy has demonstrated effectiveness in treating several solid tumors and has become a major revolution in oncology. In pancreatic ductal adenocarcinoma, however, the outcomes continue to be poor due to its special immune response and microenvironmental characteristics. In this review, we summarize the most important concepts of the immune system and the current landscape of immunotherapy in pancreatic cancer.

**INTRODUCTION**

Pancreatic ductal adenocarcinoma cancer (PDAC) derives from pancreatic glandular tissue and is considered the fourth most lethal malignant tumor worldwide, with high mortality and poor prognosis[1]. The aggressive nature of PDAC is the result of a multifactorial condition related to its desmoplastic stroma and characteristic tumor microenvironment (TME), the ability to evade the immune response, the low tumor mutational burden (TMB) and the lack of effective treatments[2].

Despite the use of various approaches and therapies such as surgical resection, radiotherapy, systemic chemotherapy and the combination of these therapies[3], 80% of such patients will die within a year of the diagnosis[4]. Even for patients with resectable disease who undergo R0 pancreatic surgery, the 5-year survival rate is less than 20%[5,6], and the 5-year overall survival (OS) is lower than 7% for those with metastatic PDAC[7]. Novel therapeutic strategies such as adjuvant modified FOLFIRINOX (folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin) have improved postoperative survival rates[8]. For advanced and metastatic PDAC, chemotherapy is the only treatment option. Despite the new chemotherapy regimens developed over the past few years (FOLFIRINOX and gemcitabine plus nab-paclitaxel), however, the median OS is still under 12 mo[1,8]. Most of the driver mutations identified in PDAC lack targeted therapies. Nevertheless, patients might benefit from platinum-based chemotherapy and poly (ADP-ribose) polymerase (PARP) inhibitors in the case of BRCA1/2 mutation[9], immune checkpoint inhibitors in microsatellite instability[10] and tyrosine-kinase inhibitors for patients harboring neurotrophic-tropomyosin receptor kinase (NTRK) gene fusions[11], although the improvement in survival is not as successful as in other tumors. Even with all these advances, the OS for PDAC has hardly improved in recent decades.

Immunotherapy is a classic treatment approach and has been recognized since 2017 as the fourth pillar of treatment for many solid tumors[12]. A high number of preclinical and clinical studies are evaluating the efficacy of immunotherapeutic strategies in PDAC, including immune checkpoint inhibitors, cancer vaccines, and adoptive cell transfer, combined with other immunotherapeutic agents, chemoradiotherapy or other molecularly targeted agents. However, this new strategy still fails to meet the clinical needs of patients with PDAC, a consequence of the complex immune escape mechanisms, which need to be understood to improve the efficacy of new therapies.

In this review, we summarize the immune response mechanisms, the current landscape and the limitations of immunotherapy in PDAC.

**IMMUNE RESPONSE IN PANCREATIC CANCER**

The immune system and inflammatory state involve numerous interactions with premalignant and malignant lesions. While chronic inflammation induced by the immune system is associated with tumor growth[13], tumors cause tissue destruction and trigger inflammatory signals leading to the recruitment of cells of the innate immune system such as natural killer (NK), T cells, macrophages, and dendritic cells (DCs)[13]. Inflammation is characterized by supporting hallmarks capabilities that contribute to tumor progression by providing cytokines, chemokines, proteases, and growth and proangiogenic factors; encouraging angiogenesis and tissue remodeling, assisting in the invasion and metastasis and ultimately driving local and systemic immune suppression[13].

In an effort to understand the dynamics of the immune response from preinvasive pancreatic intraepithelial neoplasia (PanIN) to invasive and metastatic PDAC, a study with genetically engineered *KRAS* and p53 mutant mice showed that immune system cells with suppressive properties are involved from the earliest stages of tumorigenesis. The *RAS* oncogene induces inflammation and immune suppression in the microenvironment, and both carry immune privilege in the tumor[14]. The predominant cells in the tumor stroma are immunosuppressive leukocytes that weakens the antitumor functions of lymphocytes infiltrating the pancreas and promote disease progression[15]. In contrast, many other solid tumors harbor a larger infiltration of effector T cells, which has been associated with enhanced clinical outcomes[14].

***Immune system cells in pancreatic ductal adenocarcinoma***

The most common immune cells in the tumor microenvironment (from PanIN to PDAC) include tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), neutrophils, regulatory T cells (Treg), a smaller number of effector T cells and, rarely, NK cells[13–15].

TAMs and MDSCs are innate immune cells that play a dual role in PDAC and are involved in cancer cell recognition and antitumor response but also lead to chronic inflammation[16,17]. Macrophages are predominant in the initial stages of the disease, but the ratio of TAMs and MDSCs becomes similar as the disease progresses[15]. There are very few lymphocytes in the tumor; CD4+ T cells, along with a high portion of Tregs, are the most frequent. NK and CD8+ T cells are extremely rare at any stage[15].

***Tumor-associated macrophages***

Two types of TAMs have been identified: M1 and M2 macrophages. M1 macrophages secrete proinflammatory cytokines with antineoplastic effects, while M2 macrophages release cytokines that lead to tumor progression[18,19]. Macrophages are the most common immune cells in the tumor microenvironment at the early stage. Several reports have shown an inverse correlation between the prognosis and M2 TAM infiltration in vairous tumors, including PDAC[20,21].

TAMs produce cytokines, proteases and metabolites, such as indoleamine dioxygenase and reactive oxygen species, which inhibit T-cell activity against tumor and attract Tregs to the tumor site[22]. Moreover, TAMs facilitate tumor angiogenesis by releasing pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and participate in matrix remodeling to facilitate invasion and metastasis[23].

***Myeloid derived suppressor cells***

MDSCs are a heterogeneous group of immature cells divided into polymorphonuclear (PMN-MDSC) and monocytic (M-MDSC), resembling neutrophils and monocytes, respectively[24,25]. Myeloid cells are more frequent in the advanced stages of PDAC, when the number of these cells is comparable to TAMs’. MDSCs inhibit the innate antitumor immunity[26].

MDSCs have a variety of mechanisms to attenuate immune response. They inhibit the antigen-specific response of CD4+ and CD8+ T cells. Some of these mechanisms occur through direct cell-to-cell contact between MDSCs and lymphocytes with the participation of programmed death-ligand 1 (PD-L1) and programmed death-1 (PD-1). MDSCS therefore induce the suppression of T-cell activation and self-tolerance[26]. In addition, MDSCs suppress the proliferation, cytokine production, and cytotoxic function of T cells[27]; decrease T-cell receptor signaling[28]; and induce apoptosis of intratumor T cells[28]. Furthermore, MDSCs induce Treg expansion through interleukin (IL)-10[29], resulting in additional suppression of T-cell function. As with TAMs, an increase in MDSCs in patients with cancer is related to high levels of VEGF[30].

***Natural Killer cells***

NK cells are an important immune component in controlling the antitumor immune response. These immune cells can interact with DCs, macrophages, T cells and endothelial cells through cell-to-cell contact and secreting cytokines[31]. A number of studies have found that natural killer (NK) cells are positively correlated with survival in patients with PDAC[32]. However, PDAC is characterized by a very low count of NK cells in the tumor site (< 0.5%)[33]. Furthermore, the antitumor effect of NK cells decreases by the action of pancreatic stellate cells[34] and the expression of IL-10, transforming growth factor beta (TGF-b), indoleamine 2,3-dioxygenase (IDO), and matrix metalloproteinases (MMPs)[35].

***Tumor associated Neutrophils***

Tumor associated neutrophils (TANs) are typically localized at the periphery of the tumor site in early stages of the disease and infiltrate the center of the tumor in advanced stages. The neutrophil-to-lymphocyte ratio (NLR) has been studied as a predictor, and high TAN counts are correlated with poor prognoses[36–38]. However, the value of the NLR with regard to prognosis and survival prediction requires further validation.

There are two types of TANs: N1 and N2 which have different functions[39]. N1 TANs are proinflammatory and releases IL-12, tumor necrosis factor (TNF)-a and other immunostimulatory cytokines involved in the migration and activation of CD8+ T cells[40]. N2 TANs are immunosuppressive and promote angiogenesis, invasion and metastases. These tumor-promoting activities are mediated by hepatocyte growth factor (HGF), oncostatin M, reactive oxygen species (ROS), reactive nitrogen species (RNS), matrix metalloproteinase (MMPS), and neutrophil elastase (NE)[39].

***T cells***

PDAC presents a variable number of T cells, encompassing several T-cell subpopulations. The immunosuppressive microenvironment, compounded by fibroblast and desmoplastic stroma, prevents T-cell infiltration and influences their spatial distribution in the tumor site[41]. CD4+ and CD8+ T cells have specific regulation, produce several cytokines, and have different functions in immunity[42]. Consequently, the effects of T cells in PDAC depend on the subpopulation type, their spatial distribution, and macrophage infiltration. A high infiltration of CD4+ and CD8+ T cells, low infiltration of Treg cells, and high ratio of M1/M2 macrophages are therefore associated with greater survival in PDAC[43,44].

CD4+ lymphocytes are differentiated into T helper (Th) 1, Th2, Th17 and Treg subpopulations. Th1 produces interferon gamma (IFN-γ) and cytotoxic molecules that support cellular type I immunity (priming, activation and recruitment of cytotoxic T lymphocytes, M1 macrophages and NK cells) that attack intracellular pathogens and tumors immunity[45]. Th2 produces IL-4, IL-5 and IL-3, participates in humoral type II immunity (inducing M2 macrophages) and contributes to allergy and asthma[45]. Th2 is predominant in PDAC and is associated with disease progression[46] and shorter OS[47]. Th17 produces IL-17, IL-21 and IL-22 and protects against extracellular bacterial and fungal infections[48]. Oncogenic *KRAS,* expressed in pancreatic cells*,* induces the recruitment of Th17 cells into the TME[49]. Th17 and IL-17 are involved in PDAC progression[49]. A high proportion of Th17 cells in tumor site is correlated with shorter OS and distant disease.

Regulatory T cells or suppressor T cells are a subpopulation of CD4+ lymphocytes that infiltrate thetumor. The main function of Treg in healthy patients is to maintain tolerance to self-antigen and prevent autoimmune disease[50]. Treg counts increases from PanIN to advanced PDAC[15]. In case of PDAC, Tregs have differing roles depending on the tumor stage. Tregs promote tumorigenesis by suppressing the cytotoxic function of T cells, IFN- γ and IL-2, induced by tumor antigens[15]. In contrast, Tregs can delay tumor progression by assessing the suppressive myeloid cells[51]. Tregs are associated with worse prognosis and decreased survival[52].

CD8+ T cells, also Known as cytotoxic T lymphocytes and effector T lymphocytes, produce IFN-γ, TNF and cytotoxic molecules against tumor cells and protect from cancer recurrence[53]. CD8+ T cells are scarce in PDAC.

***T cell evasion***

The most well-known mechanism of immune tolerance in cancer is the exhaustion of effector T cells (CD8+ T lymphocytes), produced by the binding of cytotoxic T lymphocyte antigen 4 (CTLA-4) to CD80 and of PD-L1 (CD86) to PD-1. However, effector T lymphocytes are scarce in pancreatic cancer, and the inflammatory immune reaction develops early, suppressing the development of an adaptative immune response.

T cell evasion in PDAC has been describe as a 4-step process[14,15]: 1) Induction: mutations in oncogenes (i.e., *KRAS*) and tumor suppressor genes initiate the process of tumorigenesis from normal pancreatic cells to PDAC. 2) Inflammation: PanIN, a preinvasive lesion, produces soluble factors inducing local inflammation and recruitment of immune cells in the tumor site. 3) Immunosuppression: chronic inflammatory reaction and enrollment of TAMs, MDSCs and Treg cells produce immunosuppressive cytokines and suppressive response with cell-to-cell contact. Macrophages and suppressive T cells prevent effector T cells from entering the TME[54], while cytokines such as IL-10 and TGF inactivate the few effector T cells present in TME[55]. This condition prevents an efficient adaptive response against the tumor. 4) Immune Privilege: in the advanced stage of the disease, immune evasion persists, and the TME becomes a site of immune privilege for pancreatic tumor cells.

***Tumor microenvironment: A barrier for immunotherapy***

The PDAC microenvironment is a classical barrier involved in tumor progression[56], and low microvascular density hinders the diffusion of therapeutic agents[57,58]. This fibroinflammatory structure is a mixture of immune cells, malignant cells, and a dense desmoplastic stroma, which includes fibroblasts, blood vessels, and pancreatic stellate cells[59,60]. The stroma is a traditional characteristic of PDAC that participates in tumor growth, vascularization, drug diffusion, resistance to treatment, and metastasis[61]. Tumor cells and stromal cells undergo into a continuous change throughout the transformation process from a healthy pancreatic tissue to an invasive malignance[13]. TME plays a key role in this immunosuppression and presents similar characteristics in both primary and metastatic lesions[62].

Based on the biological, prognostic, and predictive characteristics, it is possible to differentiate two molecular subtypes (classical and basal-like)[63–65] and two stromal subtypes of PDAC (normal and activated)[66]. The basal-like subtype is associated with an activated stroma and poor prognosis[64,65]. Patients with the activated stromal PDAC subtype have a poorer prognosis compared with the normal stromal subtype, presenting a median OS of 15 *vs* 24 mo, respectively[66]. Activated stroma is associated with a greater presence of macrophages and activated fibroblasts, both responsible for poor clinical outcomes[66]. Modulating the TME would possibly redirect the immune system to eliminate the tumor, achieving greater efficiency in antitumor therapies.

***Tumor mutational burden and mismatch repair deficiency: assistance for immunotherapy***

TMB is defined as the total number of nonsynonymous mutations per coding area of a tumor gene[67]. A high number of somatic mutations produces and releases neoantigens in the TME which lead to increase in inflammatory cytokines and effector T cells[2,10,68]. A high TMB therefore stimulates the efficacy of immunotherapy[67,69]. PDAC is characterized by low immunogenicity and low TMB (1 mutation/megabase) compared with other tumors such as melanoma (10 mutations/megabase)[67] and lung or bladder cancer (just under 10 mutations/megabase)[70].

There is a known association between mismatch repair deficiency (dMMR) and high TMB, and PDAC is not an exception[71]. dMMR represents a loss of function of the mismatch repair (MMR) pathway, a DNA repair pathway that plays a key role in maintaining genomic stability. Random mutations occurring in small repetitive elements define microsatellite instability (MSI)[72]. dMMR is defined as the loss of expression of one or more of the MLH1, MSH2, MSH6, and PMS2 proteins. Characteristically, dMMR is correlated with high TMB[72] and increased effector T cells in tumor site[63]. A high MSI status, an emerging predictor of immunotherapy response in PDAC, has a prevalence of 0.3-1.3% in this tumor type[73]. Patients with this condition present prolonged survival rates[71,74]. Neoantigens must be presented by antigen-presenting cells (APCs) to achieve a T-cell response and, in the case of PDAC, DCs (the major APCs), are usually immature or scarce, causing weakened T-cell activation[75].

**LANDSCAPE OF IMMUNOTHERAPY IN PANCREATIC DUCTAL ADENOCARCINOMA**

Our understanding of the immune response in cancer is still incomplete and continues to be investigated, with the reported relationships between the various cell populations and microenvironment composition[59,60] the basis for the development of immune-based therapies in PDAC. The combination of several immune strategies, such as checkpoints inhibitors, immune checkpoints inhibitors or vaccines plus chemotherapy, and the use of adoptive cellular therapy are some of these new approaches[76].

***Immune checkpoints agents***

Whereas immune checkpoint inhibitors have proven their efficacy in many tumors, they have falied to do so in PDAC due to its low immunogenicity and low TMB[67]. These inhibitors help to interrupt the intratumoral T-cell dysfunction and provide the block of the overexpressed receptors involved in its exhaustion such as PD-1 and CTLA-4[77]. The union between PD-L1 or PD-L2 and PD-1 induces the cessation of T-cell effector functions causing a suppression in T-cell motility[78], an antagonization of T cell receptor (TCR) signaling[79] and a suppression in gene transcription[80]. PD-L1 expression is therefore inversely correlated with survival. Thus, anti-PD-L1 and anti-PD-1 therapies have been shown to reduce tumor volume in mice that were subcutaneously injected with a murine PDAC cell line[81].

***Antibody anticytotoxic T lymphocyte antigen-4***

Several phase II clinical trials in PDAC have been developed using antibodies against CTLA-4, such as Ipilimumab[82] and Tremelimumab[83] as single agents; however, these studies have not achieved an impact in OS[82,83]. Ipilimumab in a phase II trial (NCT00112580) of pretreated patients with locally advanced and metastatic pancreatic cancer showed a median OS of 4.5 mo, with no responders except for one patient who had a delayed objective response. A phase II trial of Tremelimumab (NCT02527434) in metastatic pancreatic cancer had a median OS of 4 mo (95%CI 2.83-5.42), with 18 out 20 patients with progressive disease.

***Antibody anti-programmed death-1***

The efficacy of pembrolizumab in MSI-H PDAC has been reported in the phase II NTC02628067 study. The OS and progression-free survival (PFS) were 4 mo and 2.1 mo, respectively[76], highly disappointing results compared with those obtained in other tumors such as colorectal cancer in which a PFS of 16.5 mo was reported in the KEYNOTE-177 trial[84]. We do not have results for nivolumab or pembrolizumab as single agents in microsatellite stable tumors.

***Antibody anti-programmed death-ligand 1***

A phase I/II clinical trial (NCT03829501) assessing the efficacy of atezolizumab in advanced PDAC and other tumors refractory to first-line treatment is currently ongoing.

***Combination of immune checkpoints agents***

The combination of anti–PD-1/anti–PD-L1 and anti–CTLA-4 might have additive or synergistic activity[85]. In fact, the combination has shown enhanced activity in certain tumor types such as melanoma and non-small cell-lung cancer[85]. Several clinical trials have therefore sought to assess the efficacy of this combination.

Durvalumab alone or in combination with tremelimumab for patients with previously treated metastatic PDAC (NCT 02558894) did not achieved any improvement in OS. The combination achieved a median of 3.1 mo, while durvalumab alone achieved 3.6 mo[85]. However, the evaluation of the combination of other immunotherapy agents such as nivolumab and ipilimumab (NCT01928394) is still ongoing, with no results posted yet[86]. Table 1 Lists other immunotherapy agents that have been employed in treating PDAC.

***Combination of immune checkpoints and chemotherapy***

It has been suggested that the efficacy of immune checkpoint inhibitors could improve when combined with chemotherapy, the latter acting by activating the intratumoral immune response when inducing immunogenic cell death. This type of cell death is characterized by a necrolytic release of danger signals that can modify the stroma, change cytokine rates, reduce the presence of suppressive cells such as MDSCs and Tregs, promote the expression of molecules of the major histocompatibility complex (MHC) in cancerous cells and stimulate DC maturation[87]. In animal models, a synergetic effect has been observed between the combination of gemcitabine and anti-PDL1[88].

Several clinical trials have investigated the combination of immune checkpoint inhibitors and chemotherapy in PDAC. Table 2 Lists the main clinical trials in this field.

A phase I/II clinical trial (NCT02331251) of metastatic PDAC naïve chemotherapy, tested the combination of pembrolizumab (anti-PDL1), nab-pacliatxel and gemcitabine[89], achieving a median PFS and OS of 9,1 and 15.0 mo, respectively[89].

Other combinations such as ipilimumab plus gemcitabine (NCT01473940)[90] or CD40 agonist plus gemcitabine[91] in a phase I clinical trial in first-line treatment have also improved OS in the experimental arm , with an OS of 8.5 mo and 8.4 mo, respectively. CD40 is a member of the tumor necrosis factor receptor superfamily. The binding of CD40 by its ligand or by an agonistic monoclonal antibody activates the receptor and results in APC activation, including DCs, B cells and monocytes. In mouse pancreatic cancer models, the combination of agonistic CD40 monoclonal antibody with gemcitabine plus nab-paclitaxel triggers T-cell-dependent tumor regressions and improves survival benefit, which are further augmented by the addition of an anti-PD-1 monoclonal antibody. The preliminary results from the combination of a CD40 monoclonal antibody agonist combined with gemcitabine plus nab-paclitaxel and PD1 inhibitor have been published, showing a 58% response rate from 24 evaluable patients[92].

The COMBAT/Keynote-2020 clinical trial deserves special mention. This trial is based on CXC chemokine receptor 4 (CXCR4) blockades, that promotes T-cell tumor infiltration and is synergistic with anti-PD-1 therapy in PDAC mouse models. One cohort included 22 patients and combined the CXCR4 antagonist BL-8040 (motixafortide) with pembrolizumab-nanoliposomal irinotecan-fluorouracil-folinic acid in PDAC second-line treatment. The preliminary results showed an objective response rate, disease control rate and median duration of response of 32%, 77% and 7.8 mo, respectively, suggesting that it is indeed a promising strategy in treating PDAC[93].

According to trial results, patients, who received only immunotherapy, experienced 9%-11% of grade ≥ 3 immune related adverse events[76], while patients receiving immunotherapy in combination with chemotherapy presented grade ≥ 3 adverse effects in up to 53%. In these cases the most common toxicities were hematologic[2].

***Vaccines treatment as a single agent or in combination***

Several types of vaccines have been tested as single agents (Table 3) or in combination with chemotherapy (Table 4) or immunotherapy (Table 5), including whole-cell vaccines, DCs, DNA and peptide vaccines that entail the presentation of immunogenic cancer antigens to the immune system, resulting in the activation of cancer antigen-specific cytotoxic T lymphocytes *in vivo* and the subsequent anticancer immune response[94].

***GVAX***

The mechanism of actions of GVAX vaccines is the stimulation of APC antigen uptake and T-cell priming through PDAC cell modification to express granulocyte-macrophage colony-stimulating factor (GM-CSF)[95].

A number of phase I and II clinical trials have been conducted on localized PDAC. A phase II clinical trial (NCT00084383) with 60 randomized patients evaluated the effectiveness of the GVAX vaccine administrated in two times: 1) after surgery and before 5-fluoracil based chemotherapy and 2) after chemotherapy had finished. The median PFS was 17.3 mo, and the median OS of 24.8 mo[96].

The GVAX vaccine has also been tested in metastatic PDAC. A phase II clinical trial (NCT01417000) in which 93 patients were randomized to the combination of GVAX plus cyclophosphamide with or without CRS-207 reported an OS of 6.1 mo in the experimental arm *vs* 3.9 mo in the control arm[97]. However, a phase IIb clinical trial (NCT02004262) showed no benefit in its primary endpoint of OS[98].

***Algenpantucel***

Algenpantucel is a whole cell vaccine that works by harnessing a natural robust immune response against pancreatic cancer[99]. The vaccine has been tested in combination with gemcitabine plus 5-Fluoracil chemoraditherapy in patients with resected PDAC, achieving a 12-months PFS of 65% and a 12-months OS of 83% (vs. 45% and 63% in the control arm, respectively)[99]. This trial is still on-going for patients with borderline and locally advanced PDAC[99].

***KIF20A-66 and survivin-2B 80-88 peptides***

KIF20A and survivin are two up-regulated HLA-A24-restricted peptides that have been employed as epitopes in vaccines development[100,101]. KIF20A-66 was tested in a phase I/II clinical trial for patients with metastatic HLA-2402-positive PDAC who had progressed to first-line gemcitabine chemotherapy (UMIN000004919). The OS was 4.7 mo in the treated arm *vs* 2.7 mo in the best supportive care arm. The PFS was 1.8 mo in the vaccine arm[100].

Survivine-2B (SVN-2B) is an HLA-A24 restricted peptide that has been investigated since 2003 with inconspicuous results. A clinical trial (UMIN000000905) with 6 HLA-A2402-positive patients was conducted, resulting in a clinical y immunogenic response in 50% of patients[101].

***Other vaccines targeting KRAS and MUC-1***

These vaccines target distinct tumor antigens based on the identification of mutated oncogenes, such as *KRAS*, altered tumor suppressor genes, such as *TP53*, *CDKN2A*, *DPC4*, *BRCA2*, and *ERBB2*, as well as the overexpression of tumor-associated antigens, such as *CEA* and *MUC-1*, in pancreatic carcinoma cells[102]. A phase I clinical trial was conducted using MUC-1 vaccines in advanced PDAC, the results of which confirmed that a specific T-cell immune response was obtained, achieving a significant improvement in OS (15.3 mo vs. 3.9 mo)[103].

A phase I/II clinical trial was conducted in the adjuvant setting, the results of which revealed that 4 out of 12 patients survived without disease recurrence[104].

Clinical trials using *KRAS* against vaccines have also been developed. There is a phase I/II study that combines mutant RAS peptides and GM-CSF in patients with resected or locally advanced PDAC, with an OS of 25.6 mo and 10.2 mo, respectively[105].

GV1001 is another tested vaccine. A phase I/II trial combining this vaccine with GM-CSF for unresectable PDAC[106], and observed an immune response in 63% of patients, with an OS of 7.2 mo *vs* 2.9 mo in no-immune responders. After these encouraging results, a phase III study for locally advanced PDAC was conducted[107]. However, the combination of GV1001 plus gemcitabine plus capecitabine did not improve OS compared with chemotherapy alone (6.9 mo vs. 7.9 mo).

***Combination of vaccines treatment with immune checkpoints***

Preclinical reports have supported the concept of synergy between cancer vaccines and immune checkpoint blockade in non-immunogenic tumors. Based on these results, several clinical trials have been conducted to assess the efficacy of this combination in PDAC therapy[108].

In clinical trial NCT00836407, 30 patients with previously treated metastatic PDAC were randomized to a high dose of Ipilimumab (10mg/kg) as the single agent or in combination with the GVAX vaccine. Five patients showed stable disease, and an OS of 5.7 mo was achieved in the experimental arm compared with 3.6 mo in the control arm[108].

Other studies that included patients with metastatic PDAC combined with nivolumab and ipilimumab, with or without GVAX and CRS-207, showed enhanced T-cell responses. Studies are currently evaluating the role of immune checkpoint inhibitors in combination with cyclophosphamide/GVAX and CRS-207 vaccines (NCT02243371, NCT02451982), and the results have yet to be published[99].

Pembrolizumab combined with the modified p53-expressing Ankara virus (p53MVA) vaccine has been studied in patients with several malignancies included PDAC, with clinical responses observed in 3 of 11 patients and disease stabilization for 30, 32 and 49 wk[109].

Vaccines were well tolerated. Most common toxicities were grade 1-2 induration/erythema or pain/soreness at the vaccine sites, pyrexia, chills, fatigue and nausea, with < 5% of patients reporting serious adverse events[96,98-99,106]. There were not reported clinical signs of auto-immune disease, abnormal biochemical or haematological parameters related with the vaccinations[105]. A grade 5 fatal myocarditis was reported with Pembrolizumab combined with the p53MVA vaccine[109].

***Adoptive cell transfer***

The most clinically important form of adoptive cell transfer therapy is chimeric antigen receptor (CAR) T-cell therapy (Table 6). CAR-T cells can target any extracellular molecular structure recognizable by an antibody, thereby avoiding the MHC restriction. CAR consists of an extracellular domain of a single-chain variable fragment (scFv) of an antibody that recognizes a specific tumor antigen and an intracellular domain that contains the T cell receptor signal transduction sequence[39]**.** To generate the appropriate cell therapy product for adaptive transfer, T cells are collected from patients *via* leukapheresis, manipulated to target the specific antigen, expanded, and then reinfused[110]

The ideal target antigen is one that is selectively expressed in tumor cells; however, most targets of CAR-T cells are also expressed in normal tissues such as the mesothelin, CD24, carcinoembryonic antigen (CEA) and human epidermal growth factor receptor 2 (HER2), and have been considered as targets in PDAC[110].

Several clinical trials have followed this approach; however, the use of CAR-T cell therapy in PDAC is scarce, and the clinical results are moderate. For example, a phase I study of Her2-specific CAR-T cells (NCT01935843) showed that 5 out of the 11 treated patients achieved stable disease, while 2 patients achieved partial response with a median PFS of 4.8 mo[111].

However, CAR-T cell therapy might trigger a cytokine release syndrome, a severe complication that consists of the release of cytokines that cause several symptoms including fever and hypotension[110].

The most characteristic toxicity associated with CAR-T therapy is the cytokine release syndrome. If the antigen selected for CAR-T cell therapy is expressed on normal tissues, toxicity and autoimmunity might appear[2].

**CONCLUSION**

The immune response in cancer is a complex process that involves the balance between tumor-promoting innate immune responses and tumor-suppressing adaptive immune responses. In PDAC, immune evasion is an early event during tumorigenesis and is associated with proinflammatory signals, infiltration of immunosuppressive cells (Tregs and MDSCs), and a sophisticated TME where numerous interactions occur between stromal signals, the immune system and tumor cells. As a consequence, PDAC is characterized by low immunogenicity and antigenicity, a critical concept when developing novel immunotherapeutic approaches for treating PDAC.

The emergence of immunotherapy as a new treatment approach for solid tumors has become a revolution in modern oncology. Immune checkpoint inhibitors improve survival in several tumors, such as melanoma, lung cancer, head and neck, and genitourinary. These tumors, known as “hot tumors”, present numerous mutations that create a high number of neoantigens recognized by effector T cells that are released to fight the cancerous cells. However, in the case of PDAC, these results have not been as spectacular as other tumors’ due to the intrinsic characteristics of PDAC, which enable it to self-isolate through a complex stroma, thereby evading the immune system.

Immune checkpoint inhibitors as single agents do not provide a benefit in PDAC. However, their combination with chemotherapy may transform PDAC into a “hot tumor” that is more susceptible to immunotherapy. The outcomes in phase I/II clinical trials using this approach are encouraging, with a benefit in OS in several combinations. Cancer vaccines employed as single-agent treatments or combined with classical chemotherapy, chemoradiotherapy or immunotherapy have promising results in the early phases of clinical trials. However, these outcomes have not been confirmed in more advanced studies. Lastly, adoptive cell transfer, specifically the CART-T cell approach, is still in very early phase of development.

Despite advances in treating PDAC, this tumor continues to be associated with extremely poor outcomes and high mortality. Systemic therapies and new strategies therefore need to be developed to improve patient prognoses, which will depend on an ever-increasing understanding of basic immunity and its role in PDAC. They keys to achieving significant changes in the treatment of PDAC include research into safe novel inhibitors (such as immunosuppressive factor, TAMs, MDSCs, and Tregs), increasing the prophylactic efforts in the early stages of carcinogenesis, deepening the understanding of molecular subtyping in PDAC and conducting appropriate patient selection.

**REFERENCES**

1 **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]

2 **Young K**, Hughes DJ, Cunningham D, Starling N. Immunotherapy and pancreatic cancer: unique challenges and potential opportunities. *Ther Adv Med Oncol* 2018; **10**: 1758835918816281 [PMID: 30574212 DOI: 10.1177/1758835918816281]

3 **Ilic M**, Ilic I. Epidemiology of pancreatic cancer. *World J Gastroenterol* 2016; **22**: 9694-9705 [PMID: 27956793 DOI: 10.3748/wjg.v22.i44.9694]

4 **Seufferlein T**, Bachet JB, Van Cutsem E, Rougier P; ESMO Guidelines Working Group. Pancreatic adenocarcinoma: ESMO-ESDO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23 Suppl 7**: vii33-vii40 [PMID: 22997452 DOI: 10.1093/annonc/mds224]

5 **Mayo SC**, Nathan H, Cameron JL, Olino K, Edil BH, Herman JM, Hirose K, Schulick RD, Choti MA, Wolfgang CL, Pawlik TM. Conditional survival in patients with pancreatic ductal adenocarcinoma resected with curative intent. *Cancer* 2012; **118**: 2674-2681 [PMID: 21935914 DOI: 10.1002/cncr.26553]

6 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617 [PMID: 20427809 DOI: 10.1056/NEJMra0901557]

7 **Kamisawa T**, Wood LD, Itoi T, Takaori K. Pancreatic cancer. *Lancet* 2016; **388**: 73-85 [PMID: 26830752 DOI: 10.1016/S0140-6736(16)00141-0]

8 **Conroy T**, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul JL, Choné L, Francois E, Artru P, Biagi JJ, Lecomte T, Assenat E, Faroux R, Ychou M, Volet J, Sauvanet A, Breysacher G, Di Fiore F, Cripps C, Kavan P, Texereau P, Bouhier-Leporrier K, Khemissa-Akouz F, Legoux JL, Juzyna B, Gourgou S, O'Callaghan CJ, Jouffroy-Zeller C, Rat P, Malka D, Castan F, Bachet JB; Canadian Cancer Trials Group and the Unicancer-GI–PRODIGE Group. FOLFIRINOX or Gemcitabine as Adjuvant Therapy for Pancreatic Cancer. *N Engl J Med* 2018; **379**: 2395-2406 [PMID: 30575490 DOI: 10.1056/NEJMoa1809775]

9 **Golan T**, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, Park JO, Hochhauser D, Arnold D, Oh DY, Reinacher-Schick A, Tortora G, Algül H, O'Reilly EM, McGuinness D, Cui KY, Schlienger K, Locker GY, Kindler HL. Maintenance Olaparib for Germline *BRCA*-Mutated Metastatic Pancreatic Cancer. *N Engl J Med* 2019; **381**: 317-327 [PMID: 31157963 DOI: 10.1056/NEJMoa1903387]

10 **Le DT**, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, Wong F, Azad NS, Rucki AA, Laheru D, Donehower R, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Greten TF, Duffy AG, Ciombor KK, Eyring AD, Lam BH, Joe A, Kang SP, Holdhoff M, Danilova L, Cope L, Meyer C, Zhou S, Goldberg RM, Armstrong DK, Bever KM, Fader AN, Taube J, Housseau F, Spetzler D, Xiao N, Pardoll DM, Papadopoulos N, Kinzler KW, Eshleman JR, Vogelstein B, Anders RA, Diaz LA Jr. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017; **357**: 409-413 [PMID: 28596308 DOI: 10.1126/science.aan6733]

11 **O'Reilly EM**, Hechtman JF. Tumour response to TRK inhibition in a patient with pancreatic adenocarcinoma harbouring an NTRK gene fusion. *Ann Oncol* 2019; **30**: viii36-viii40 [PMID: 31605106 DOI: 10.1093/annonc/mdz385]

12 **Emens LA**, Ascierto PA, Darcy PK, Demaria S, Eggermont AMM, Redmond WL, Seliger B, Marincola FM. Cancer immunotherapy: Opportunities and challenges in the rapidly evolving clinical landscape. *Eur J Cancer* 2017; **81**: 116-129 [PMID: 28623775 DOI: 10.1016/j.ejca.2017.01.035]

13 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]

14 **Vonderheide RH**, Bayne LJ. Inflammatory networks and immune surveillance of pancreatic carcinoma. *Curr Opin Immunol* 2013; **25**: 200-205 [PMID: 23422836 DOI: 10.1016/j.coi.2013.01.006]

15 **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]

16 **de Visser KE**, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer* 2006; **6**: 24-37 [PMID: 16397525 DOI: 10.1038/nrc1782]

17 **Hinshaw DC**, Shevde LA. The Tumor Microenvironment Innately Modulates Cancer Progression. *Cancer Res* 2019; **79**: 4557-4566 [PMID: 31350295 DOI: 10.1158/0008-5472.CAN-18-3962]

18 **Mosser DM**, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008; **8**: 958-969 [PMID: 19029990 DOI: 10.1038/nri2448]

19 **Biswas SK**, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010; **11**: 889-896 [PMID: 20856220 DOI: 10.1038/ni.1937]

20 **Zhang R**, Liu Q, Peng J, Wang M, Li T, Liu J, Cui M, Zhang X, Gao X, Liao Q, Zhao Y. CXCL5 overexpression predicts a poor prognosis in pancreatic ductal adenocarcinoma and is correlated with immune cell infiltration. *J Cancer* 2020; **11**: 2371-2381 [PMID: 32201508 DOI: 10.7150/jca.40517]

21 **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]

22 **Pollard JW**. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 2004; **4**: 71-78 [PMID: 14708027 DOI: 10.1038/nrc1256]

23 **Wyckoff J**, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J, Condeelis J. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* 2004; **64**: 7022-7029 [PMID: 15466195 DOI: 10.1158/0008-5472.CAN-04-1449]

24 **Thyagarajan A**, Alshehri MSA, Miller KLR, Sherwin CM, Travers JB, Sahu RP. Myeloid-Derived Suppressor Cells and Pancreatic Cancer: Implications in Novel Therapeutic Approaches. *Cancers (Basel)* 2019; **11** [PMID: 31652904 DOI: 10.3390/cancers11111627]

25 **Trovato R**, Fiore A, Sartori S, Canè S, Giugno R, Cascione L, Paiella S, Salvia R, De Sanctis F, Poffe O, Anselmi C, Hofer F, Sartoris S, Piro G, Carbone C, Corbo V, Lawlor R, Solito S, Pinton L, Mandruzzato S, Bassi C, Scarpa A, Bronte V, Ugel S. Immunosuppression by monocytic myeloid-derived suppressor cells in patients with pancreatic ductal carcinoma is orchestrated by STAT3. *J Immunother Cancer* 2019; **7**: 255 [PMID: 31533831 DOI: 10.1186/s40425-019-0734-6]

26 **Pinton L**, Solito S, Damuzzo V, Francescato S, Pozzuoli A, Berizzi A, Mocellin S, Rossi CR, Bronte V, Mandruzzato S. Activated T cells sustain myeloid-derived suppressor cell-mediated immune suppression. *Oncotarget* 2016; **7**: 1168-1184 [PMID: 26700461 DOI: 10.18632/oncotarget.6662]

27 **Gabrilovich DI**, Velders MP, Sotomayor EM, Kast WM. Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. *J Immunol* 2001; **166**: 5398-5406 [PMID: 11313376 DOI: 10.4049/jimmunol.166.9.5398]

28 **Otsuji M**, Kimura Y, Aoe T, Okamoto Y, Saito T. Oxidative stress by tumor-derived macrophages suppresses the expression of CD3 zeta chain of T-cell receptor complex and antigen-specific T-cell responses. *Proc Natl Acad Sci U S A* 1996; **93**: 13119-13124 [PMID: 8917554 DOI: 10.1073/pnas.93.23.13119]

29 **Huang B**, Pan PY, Li Q, Sato AI, Levy DE, Bromberg J, Divino CM, Chen SH. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Cancer Res* 2006; **66**: 1123-1131 [PMID: 16424049 DOI: 10.1158/0008-5472.CAN-05-1299]

30 **Melani C**, Chiodoni C, Forni G, Colombo MP. Myeloid cell expansion elicited by the progression of spontaneous mammary carcinomas in c-erbB-2 transgenic BALB/c mice suppresses immune reactivity. *Blood* 2003; **102**: 2138-2145 [PMID: 12750171 DOI: 10.1182/blood-2003-01-0190]

31 **Chiossone L**, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. *Nat Rev Immunol* 2018; **18**: 671-688 [PMID: 30209347 DOI: 10.1038/s41577-018-0061-z]

32 **Davis M**, Conlon K, Bohac GC, Barcenas J, Leslie W, Watkins L, Lamzabi I, Deng Y, Li Y, Plate JM. Effect of pemetrexed on innate immune killer cells and adaptive immune T cells in subjects with adenocarcinoma of the pancreas. *J Immunother* 2012; **35**: 629-640 [PMID: 22996369 DOI: 10.1097/CJI.0b013e31826c8a4f]

33 **Lim SA**, Kim J, Jeon S, Shin MH, Kwon J, Kim TJ, Im K, Han Y, Kwon W, Kim SW, Yee C, Kim SJ, Jang JY, Lee KM. Defective Localization With Impaired Tumor Cytotoxicity Contributes to the Immune Escape of NK Cells in Pancreatic Cancer Patients. *Front Immunol* 2019; **10**: 496 [PMID: 31024520 DOI: 10.3389/fimmu.2019.00496]

34 **Huang Q**, Huang M, Meng F, Sun R. Activated pancreatic stellate cells inhibit NK cell function in the human pancreatic cancer microenvironment. *Cell Mol Immunol* 2019; **16**: 87-89 [PMID: 29628497 DOI: 10.1038/s41423-018-0014-2]

35 **Peng YP**, Zhang JJ, Liang WB, Tu M, Lu ZP, Wei JS, Jiang KR, Gao WT, Wu JL, Xu ZK, Miao Y, Zhu Y. Elevation of MMP-9 and IDO induced by pancreatic cancer cells mediates natural killer cell dysfunction. *BMC Cancer* 2014; **14**: 738 [PMID: 25274283 DOI: 10.1186/1471-2407-14-738]

36 **Arima K**, Okabe H, Hashimoto D, Chikamoto A, Tsuji A, Yamamura K, Kitano Y, Inoue R, Kaida T, Higashi T, Taki K, Imai K, Komohara Y, Beppu T, Takeya M, Baba H. The diagnostic role of the neutrophil-to-lymphocyte ratio in predicting pancreatic ductal adenocarcinoma in patients with pancreatic diseases. *Int J Clin Oncol* 2016; **21**: 940-945 [PMID: 27023215 DOI: 10.1007/s10147-016-0975-z]

37 **Tao L**, Zhang L, Peng Y, Tao M, Li G, Xiu D, Yuan C, Ma C, Jiang B. Preoperative neutrophil-to-lymphocyte ratio and tumor-related factors to predict lymph node metastasis in patients with pancreatic ductal adenocarcinoma (PDAC). *Oncotarget* 2016; **7**: 74314-74324 [PMID: 27494847 DOI: 10.18632/oncotarget.11031]

38 **Pu N**, Yin H, Zhao G, Nuerxiati A, Wang D, Xu X, Kuang T, Jin D, Lou W, Wu W. Independent effect of postoperative neutrophil-to-lymphocyte ratio on the survival of pancreatic ductal adenocarcinoma with open distal pancreatosplenectomy and its nomogram-based prediction. *J Cancer* 2019; **10**: 5935-5943 [PMID: 31762803 DOI: 10.7150/jca.35856]

39 **Huber M**, Brehm CU, Gress TM, Buchholz M, Alashkar Alhamwe B, von Strandmann EP, Slater EP, Bartsch JW, Bauer C, Lauth M. The Immune Microenvironment in Pancreatic Cancer. *Int J Mol Sci* 2020; **21** [PMID: 33022971 DOI: 10.3390/ijms21197307]

40 **Mollinedo F**. Neutrophil Degranulation, Plasticity, and Cancer Metastasis. *Trends Immunol* 2019; **40**: 228-242 [PMID: 30777721 DOI: 10.1016/j.it.2019.01.006]

41 **Balli D**, Rech AJ, Stanger BZ, Vonderheide RH. Immune Cytolytic Activity Stratifies Molecular Subsets of Human Pancreatic Cancer. *Clin Cancer Res* 2017; **23**: 3129-3138 [PMID: 28007776 DOI: 10.1158/1078-0432.CCR-16-2128]

42 **Ino Y**, Yamazaki-Itoh R, Shimada K, Iwasaki M, Kosuge T, Kanai Y, Hiraoka N. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. *Br J Cancer* 2013; **108**: 914-923 [PMID: 23385730 DOI: 10.1038/bjc.2013.32]

43 **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 U S A* 2013; **110**: 20212-20217 [PMID: 24277834 DOI: 10.1073/pnas.1320318110]

44 **Hiraoka N**, Onozato K, Kosuge T, Hirohashi S. Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. *Clin Cancer Res* 2006; **12**: 5423-5434 [PMID: 17000676 DOI: 10.1158/1078-0432.CCR-06-0369]

45 **Huber M**, Lohoff M. IRF4 at the crossroads of effector T-cell fate decision. *Eur J Immunol* 2014; **44**: 1886-1895 [PMID: 24782159 DOI: 10.1002/eji.201344279]

46 **De Monte L**, Reni M, Tassi E, Clavenna D, Papa I, Recalde H, Braga M, Di Carlo V, Doglioni C, Protti MP. Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. *J Exp Med* 2011; **208**: 469-478 [PMID: 21339327 DOI: 10.1084/jem.20101876]

47 **Galon J**, Bruni D. Tumor Immunology and Tumor Evolution: Intertwined Histories. *Immunity* 2020; **52**: 55-81 [PMID: 31940273 DOI: 10.1016/j.immuni.2019.12.018]

48 **Dobrzanski MJ**. Expanding roles for CD4 T cells and their subpopulations in tumor immunity and therapy. *Front Oncol* 2013; **3**: 63 [PMID: 23533029 DOI: 10.3389/fonc.2013.00063]

49 **Vizio B**, Novarino A, Giacobino A, Cristiano C, Prati A, Ciuffreda L, Montrucchio G, Bellone G. Potential plasticity of T regulatory cells in pancreatic carcinoma in relation to disease progression and outcome. *Exp Ther Med* 2012; **4**: 70-78 [PMID: 23060925 DOI: 10.3892/etm.2012.553]

50 **Wing K**, Fehérvári Z, Sakaguchi S. Emerging possibilities in the development and function of regulatory T cells. *Int Immunol* 2006; **18**: 991-1000 [PMID: 16720616 DOI: 10.1093/intimm/dxl044]

51 **Chellappa S**, Hugenschmidt H, Hagness M, Line PD, Labori KJ, Wiedswang G, Taskén K, Aandahl EM. Regulatory T cells that co-express RORγt and FOXP3 are pro-inflammatory and immunosuppressive and expand in human pancreatic cancer. *Oncoimmunology* 2016; **5**: e1102828 [PMID: 27141387 DOI: 10.1080/2162402X.2015.1102828]

52 **Curiel TJ**, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004; **10**: 942-949 [PMID: 15322536 DOI: 10.1038/nm1093]

53 **Tassi E**, Gavazzi F, Albarello L, Senyukov V, Longhi R, Dellabona P, Doglioni C, Braga M, Di Carlo V, Protti MP. Carcinoembryonic antigen-specific but not antiviral CD4+ T cell immunity is impaired in pancreatic carcinoma patients. *J Immunol* 2008; **181**: 6595-6603 [PMID: 18941250 DOI: 10.4049/jimmunol.181.9.6595]

54 **Blazquez JL**, Benyamine A, Pasero C, Olive D. New Insights Into the Regulation of γδ T Cells by BTN3A and Other BTN/BTNL in Tumor Immunity. *Front Immunol* 2018; **9**: 1601 [PMID: 30050536 DOI: 10.3389/fimmu.2018.01601]

55 **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]

56 **Torphy RJ**, Zhu Y, Schulick RD. Immunotherapy for pancreatic cancer: Barriers and breakthroughs. *Ann Gastroenterol Surg* 2018; **2**: 274-281 [PMID: 30003190 DOI: 10.1002/ags3.12176]

57 **Heinemann V**, Reni M, Ychou M, Richel DJ, Macarulla T, Ducreux M. Tumour-stroma interactions in pancreatic ductal adenocarcinoma: rationale and current evidence for new therapeutic strategies. *Cancer Treat Rev* 2014; **40**: 118-128 [PMID: 23849556 DOI: 10.1016/j.ctrv.2013.04.004]

58 **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]

59 **Watt J**, Kocher HM. The desmoplastic stroma of pancreatic cancer is a barrier to immune cell infiltration. *Oncoimmunology* 2013; **2**: e26788 [PMID: 24498555 DOI: 10.4161/onci.26788]

60 **Neesse A**, Algül H, Tuveson DA, Gress TM. Stromal biology and therapy in pancreatic cancer: a changing paradigm. *Gut* 2015; **64**: 1476-1484 [PMID: 25994217 DOI: 10.1136/gutjnl-2015-309304]

61 **Kleeff J**, Beckhove P, Esposito I, Herzig S, Huber PE, Löhr JM, Friess H. Pancreatic cancer microenvironment. *Int J Cancer* 2007; **121**: 699-705 [PMID: 17534898 DOI: 10.1002/ijc.22871]

62 **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: 25695692 DOI: 10.1158/1078-0432.CCR-14-1051]

63 **Bailey P**, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, Idrisoglu S, Manning S, Nourbakhsh E, Wani S, Fink L, Holmes O, Chin V, Anderson MJ, Kazakoff S, Leonard C, Newell F, Waddell N, Wood S, Xu Q, Wilson PJ, Cloonan N, Kassahn KS, Taylor D, Quek K, Robertson A, Pantano L, Mincarelli L, Sanchez LN, Evers L, Wu J, Pinese M, Cowley MJ, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chantrill LA, Mawson A, Humphris J, Chou A, Pajic M, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Lovell JA, Merrett ND, Toon CW, Epari K, Nguyen NQ, Barbour A, Zeps N, Moran-Jones K, Jamieson NB, Graham JS, Duthie F, Oien K, Hair J, Grützmann R, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Rusev B, Capelli P, Salvia R, Tortora G, Mukhopadhyay D, Petersen GM; Australian Pancreatic Cancer Genome Initiative, Munzy DM, Fisher WE, Karim SA, Eshleman JR, Hruban RH, Pilarsky C, Morton JP, Sansom OJ, Scarpa A, Musgrove EA, Bailey UM, Hofmann O, Sutherland RL, Wheeler DA, Gill AJ, Gibbs RA, Pearson JV, Waddell N, Biankin AV, Grimmond SM. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* 2016; **531**: 47-52 [PMID: 26909576 DOI: 10.1038/nature16965]

64 **Aguirre AJ**, Nowak JA, Camarda ND, Moffitt RA, Ghazani AA, Hazar-Rethinam M, Raghavan S, Kim J, Brais LK, Ragon D, Welch MW, Reilly E, McCabe D, Marini L, Anderka K, Helvie K, Oliver N, Babic A, Da Silva A, Nadres B, Van Seventer EE, Shahzade HA, St Pierre JP, Burke KP, Clancy T, Cleary JM, Doyle LA, Jajoo K, McCleary NJ, Meyerhardt JA, Murphy JE, Ng K, Patel AK, Perez K, Rosenthal MH, Rubinson DA, Ryou M, Shapiro GI, Sicinska E, Silverman SG, Nagy RJ, Lanman RB, Knoerzer D, Welsch DJ, Yurgelun MB, Fuchs CS, Garraway LA, Getz G, Hornick JL, Johnson BE, Kulke MH, Mayer RJ, Miller JW, Shyn PB, Tuveson DA, Wagle N, Yeh JJ, Hahn WC, Corcoran RB, Carter SL, Wolpin BM. Real-time Genomic Characterization of Advanced Pancreatic Cancer to Enable Precision Medicine. *Cancer Discov* 2018; **8**: 1096-1111 [PMID: 29903880 DOI: 10.1158/2159-8290.CD-18-0275]

65 **Aung KL**, Fischer SE, Denroche RE, Jang GH, Dodd A, Creighton S, Southwood B, Liang SB, Chadwick D, Zhang A, O'Kane GM, Albaba H, Moura S, Grant RC, Miller JK, Mbabaali F, Pasternack D, Lungu IM, Bartlett JMS, Ghai S, Lemire M, Holter S, Connor AA, Moffitt RA, Yeh JJ, Timms L, Krzyzanowski PM, Dhani N, Hedley D, Notta F, Wilson JM, Moore MJ, Gallinger S, Knox JJ. Genomics-Driven Precision Medicine for Advanced Pancreatic Cancer: Early Results from the COMPASS Trial. *Clin Cancer Res* 2018; **24**: 1344-1354 [PMID: 29288237 DOI: 10.1158/1078-0432.CCR-17-2994]

66 **Moffitt RA**, Marayati R, Flate EL, Volmar KE, Loeza SG, Hoadley KA, Rashid NU, Williams LA, Eaton SC, Chung AH, Smyla JK, Anderson JM, Kim HJ, Bentrem DJ, Talamonti MS, Iacobuzio-Donahue CA, Hollingsworth MA, Yeh JJ. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. *Nat Genet* 2015; **47**: 1168-1178 [PMID: 26343385 DOI: 10.1038/ng.3398]

67 **Champiat S**, Ferté C, Lebel-Binay S, Eggermont A, Soria JC. Exomics and immunogenics: Bridging mutational load and immune checkpoints efficacy. *Oncoimmunology* 2014; **3**: e27817 [PMID: 24605269 DOI: 10.4161/onci.27817]

68 **Upadhrasta S**, Zheng L. Strategies in Developing Immunotherapy for Pancreatic Cancer: Recognizing and Correcting Multiple Immune "Defects" in the Tumor Microenvironment. *J Clin Med* 2019; **8** [PMID: 31527414 DOI: 10.3390/jcm8091472]

69 **Kleeff J**, Korc M, Apte M, La Vecchia C, Johnson CD, Biankin AV, Neale RE, Tempero M, Tuveson DA, Hruban RH, Neoptolemos JP. Pancreatic cancer. *Nat Rev Dis Primers* 2016; **2**: 16022 [PMID: 27158978 DOI: 10.1038/nrdp.2016.22]

70 **Alexandrov LB**, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Børresen-Dale AL, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjörd JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Ilicic T, Imbeaud S, Imielinski M, Jäger N, Jones DT, Jones D, Knappskog S, Kool M, Lakhani SR, López-Otín C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenstiel P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt AN, Valdés-Mas R, van Buuren MM, van 't Veer L, Vincent-Salomon A, Waddell N, Yates LR; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MMML-Seq Consortium; ICGC PedBrain, Zucman-Rossi J, Futreal PA, McDermott U, Lichter P, Meyerson M, Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ, Stratton MR. Signatures of mutational processes in human cancer. *Nature* 2013; **500**: 415-421 [PMID: 23945592 DOI: 10.1038/nature12477]

71 **Nakata B**, Wang YQ, Yashiro M, Nishioka N, Tanaka H, Ohira M, Ishikawa T, Nishino H, Hirakawa K. Prognostic value of microsatellite instability in resectable pancreatic cancer. *Clin Cancer Res* 2002; **8**: 2536-2540 [PMID: 12171881]

72 **Salem ME**, Puccini A, Grothey A, Raghavan D, Goldberg RM, Xiu J, Korn WM, Weinberg BA, Hwang JJ, Shields AF, Marshall JL, Philip PA, Lenz HJ. Landscape of Tumor Mutation Load, Mismatch Repair Deficiency, and PD-L1 Expression in a Large Patient Cohort of Gastrointestinal Cancers. *Mol Cancer Res* 2018; **16**: 805-812 [PMID: 29523759 DOI: 10.1158/1541-7786.MCR-17-0735]

73 **Hu ZI**, Shia J, Stadler ZK, Varghese AM, Capanu M, Salo-Mullen E, Lowery MA, Diaz LA Jr, Mandelker D, Yu KH, Zervoudakis A, Kelsen DP, Iacobuzio-Donahue CA, Klimstra DS, Saltz LB, Sahin IH, O'Reilly EM. Evaluating Mismatch Repair Deficiency in Pancreatic Adenocarcinoma: Challenges and Recommendations. *Clin Cancer Res* 2018; **24**: 1326-1336 [PMID: 29367431 DOI: 10.1158/1078-0432.CCR-17-3099]

74 **Fraune C**, Burandt E, Simon R, Hube-Magg C, Makrypidi-Fraune G, Kluth M, Büscheck F, Höflmayer D, Blessin NC, Mandelkow T, Li W, Perez D, Izbicki JR, Wilczak W, Sauter G, Schrader J, Neipp M, Mofid H, Daniels T, Isbert C, Clauditz TS, Steurer S. MMR Deficiency is Homogeneous in Pancreatic Carcinoma and Associated with High Density of Cd8-Positive Lymphocytes. *Ann Surg Oncol* 2020; **27**: 3997-4006 [PMID: 32108923 DOI: 10.1245/s10434-020-08209-y]

75 **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 DOI: 10.1097/00006676-200401000-00023]

76 **Schizas D**, Charalampakis N, Kole C, Economopoulou P, Koustas E, Gkotsis E, Ziogas D, Psyrri A, Karamouzis MV. Immunotherapy for pancreatic cancer: A 2020 update. *Cancer Treat Rev* 2020; **86**: 102016 [PMID: 32247999 DOI: 10.1016/j.ctrv.2020.102016]

77 **Hodi FS**, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363**: 711-723 [PMID: 20525992 DOI: 10.1056/NEJMoa1003466]

78 **Freeman GJ**, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000; **192**: 1027-1034 [PMID: 11015443 DOI: 10.1084/jem.192.7.1027]

79 **Zinselmeyer BH**, Heydari S, Sacristán C, Nayak D, Cammer M, Herz J, Cheng X, Davis SJ, Dustin ML, McGavern DB. PD-1 promotes immune exhaustion by inducing antiviral T cell motility paralysis. *J Exp Med* 2013; **210**: 757-774 [PMID: 23530125 DOI: 10.1084/jem.20121416]

80 **Patsoukis N**, Brown J, Petkova V, Liu F, Li L, Boussiotis VA. Selective effects of PD-1 on Akt and Ras pathways regulate molecular components of the cell cycle and inhibit T cell proliferation. *Sci Signal* 2012; **5**: ra46 [PMID: 22740686 DOI: 10.1126/scisignal.2002796]

81 **Pauken KE**, Sammons MA, Odorizzi PM, Manne S, Godec J, Khan O, Drake AM, Chen Z, Sen DR, Kurachi M, Barnitz RA, Bartman C, Bengsch B, Huang AC, Schenkel JM, Vahedi G, Haining WN, Berger SL, Wherry EJ. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science* 2016; **354**: 1160-1165 [PMID: 27789795 DOI: 10.1126/science.aaf2807]

82 **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]

83 Study of Tremelimumab in Patients With Advanced Solid Tumors - Study Results - ClinicalTrials.gov. [cited 20 January 2021]. Available from: https://clinicaltrials.gov/ct2/show/results/NCT02527434

84 Pembrolizumab in Microsatellite-Instability–High Advanced Colorectal Cancer | NEJM. [cited 20 January 2021]. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa2017699

85 **O’Reilly EM,** Oh D-Y, Dhani N, Renouf DJ, Lee MA, Sun W, Fisher G, Hezel A, Chang S-C, Vlahovic G, Takahashi O, Yang Y, Fitts D, Philip PA. Durvalumab With or Without Tremelimumab for Patients With Metastatic Pancreatic Ductal Adenocarcinoma: A Phase 2 Randomized Clinical Trial. *JAMA Oncol* (e-pub ahead of print 18 July 2019 [DOI:10.1001/jamaoncol.2019.1588]

86 **Bristol-Myers Squibb. A Phase 1/2,** Open-label Study of Nivolumab Monotherapy or Nivolumab Combined With Ipilimumab in Subjects With Advanced or Metastatic Solid Tumors. ClinicalTrials.gov. [cited 20 January 2021]. Available from: https://clinicaltrials.gov/ct2/show/NCT01928394

87 **Beatty GL**, Gladney WL. Immune escape mechanisms as a guide for cancer immunotherapy. *Clin Cancer Res* 2015; **21**: 687-692 [PMID: 25501578 DOI: 10.1158/1078-0432.CCR-14-1860]

88 **Ho TTB**, Nasti A, Seki A, Komura T, Inui H, Kozaka T, Kitamura Y, Shiba K, Yamashita T, Yamashita T, Mizukoshi E, Kawaguchi K, Wada T, Honda M, Kaneko S, Sakai Y. Combination of gemcitabine and anti-PD-1 antibody enhances the anticancer effect of M1 macrophages and the Th1 response in a murine model of pancreatic cancer liver metastasis. *J Immunother Cancer* 2020; **8** [PMID: 33188035 DOI: 10.1136/jitc-2020-001367]

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

90 **Mohindra NA,** Kircher SM, Nimeiri HS, Benson AB, Rademaker A, Alonso E, Blatner N, Khazaie K, Mulcahy MF. Results of the phase Ib study of ipilimumab and gemcitabine for advanced pancreas cancer. *J Clin Oncol* 2015; **33**: e15281-e15281 [DOI: 10.1200/jco.2015.33.15\_suppl.e15281]

91 **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 in chemotherapy-naive patients with metastatic pancreatic cancer. *Ann Oncol* 2014; **25**: 1750-1755 [PMID: 24907635 DOI: 10.1093/annonc/mdu205]

92 **O'Hara MH**, O'Reilly EM, Varadhachary G, Wolff RA, Wainberg ZA, Ko AH, Fisher G, Rahma O, Lyman JP, Cabanski CR, Mick R, Gherardini PF, Kitch LJ, Xu J, Samuel T, Karakunnel J, Fairchild J, Bucktrout S, LaVallee TM, Selinsky C, Till JE, Carpenter EL, Alanio C, Byrne KT, Chen RO, Trifan OC, Dugan U, Horak C, Hubbard-Lucey VM, Wherry EJ, Ibrahim R, Vonderheide RH. CD40 agonistic monoclonal antibody APX005M (sotigalimab) and chemotherapy, with or without nivolumab, for the treatment of metastatic pancreatic adenocarcinoma: an open-label, multicentre, phase 1b study. *Lancet Oncol* 2021; **22**: 118-131 [PMID: 33387490 DOI: 10.1016/S1470-2045(20)30532-5]

93 **Bockorny B**, Semenisty V, Macarulla T, Borazanci E, Wolpin BM, Stemmer SM, Golan T, Geva R, Borad MJ, Pedersen KS, Park JO, Ramirez RA, Abad DG, Feliu J, Muñoz A, Ponz-Sarvise M, Peled A, Lustig TM, Bohana-Kashtan O, Shaw SM, Sorani E, Chaney M, Kadosh S, Vainstein Haras A, Von Hoff DD, Hidalgo M. BL-8040, a CXCR4 antagonist, in combination with pembrolizumab and chemotherapy for pancreatic cancer: the COMBAT trial. *Nat Med* 2020; **26**: 878-885 [PMID: 32451495 DOI: 10.1038/s41591-020-0880-x]

94 **Janikashvili N**, Larmonier N, Katsanis E. Personalized dendritic cell-based tumor immunotherapy. *Immunotherapy* 2010; **2**: 57-68 [PMID: 20161666 DOI: 10.2217/imt.09.78]

95 **Le DT**, Pardoll DM, Jaffee EM. Cellular vaccine approaches. *Cancer J* 2010; **16**: 304-310 [PMID: 20693840 DOI: 10.1097/PPO.0b013e3181eb33d7]

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]

97 **Whiting C,** Lutz E, Nair N, Chang S, Lemmens E, Chen S-Y, Solt S, Ferber S, Maecker H, Murphy A, Brockstedt DG, Jaffee EM, Le DT. Phase II, randomized study of GVAX pancreas and CRS-207 immunotherapy in patients with metastatic pancreatic cancer: Clinical update on long term survival and biomarker correlates to overall survival. *J Clin Oncol* 2015; **33**: 261–261 [DOI: 10.1200/jco.2015.33.3\_suppl.261]

98 **Le DT**, Picozzi VJ, Ko AH, Wainberg ZA, Kindler H, Wang-Gillam A, Oberstein P, Morse MA, Zeh HJ 3rd, Weekes C, Reid T, Borazanci E, Crocenzi T, LoConte NK, Musher B, Laheru D, Murphy A, Whiting C, Nair N, Enstrom A, Ferber S, Brockstedt DG, Jaffee EM. Results from a Phase IIb, Randomized, Multicenter Study of GVAX Pancreas and CRS-207 Compared with Chemotherapy in Adults with Previously Treated Metastatic Pancreatic Adenocarcinoma (ECLIPSE Study). *Clin Cancer Res* 2019; **25**: 5493-5502 [PMID: 31126960 DOI: 10.1158/1078-0432.CCR-18-2992]

99 **McCormick KA**, Coveler AL, Rossi GR, Vahanian NN, Link C, Chiorean EG. Pancreatic cancer: Update on immunotherapies and algenpantucel-L. *Hum Vaccin Immunother* 2016; **12**: 563-575 [PMID: 26619245 DOI: 10.1080/21645515.2015.1093264]

100 **Asahara S**, Takeda K, Yamao K, Maguchi H, Yamaue H. Phase I/II clinical trial using HLA-A24-restricted peptide vaccine derived from KIF20A for patients with advanced pancreatic cancer. *J Transl Med* 2013; **11**: 291 [PMID: 24237633 DOI: 10.1186/1479-5876-11-291]

101 **Kameshima H**, Tsuruma T, Kutomi G, Shima H, Iwayama Y, Kimura Y, Imamura M, Torigoe T, Takahashi A, Hirohashi Y, Tamura Y, Tsukahara T, Kanaseki T, Sato N, Hirata K. Immunotherapeutic benefit of α-interferon (IFNα) in survivin2B-derived peptide vaccination for advanced pancreatic cancer patients. *Cancer Sci* 2013; **104**: 124-129 [PMID: 23078230 DOI: 10.1111/cas.12046]

102 **Laheru D**, Jaffee EM. Immunotherapy for pancreatic cancer - science driving clinical progress. *Nat Rev Cancer* 2005; **5**: 459-467 [PMID: 15905855 DOI: 10.1038/nrc1630]

103 **Kaufman HL**, Kim-Schulze S, Manson K, DeRaffele G, Mitcham J, Seo KS, Kim DW, Marshall J. Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. *J Transl Med* 2007; **5**: 60 [PMID: 18039393 DOI: 10.1186/1479-5876-5-60]

104 **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/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. *Cancer Ther* 2008; **6**: 955-964 [PMID: 19129927]

105 **Gjertsen MK**, Buanes T, Rosseland AR, Bakka A, Gladhaug I, Søreide O, Eriksen JA, Møller M, Baksaas I, Lothe RA, Saeterdal I, Gaudernack G. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant: Clinical and immunological responses in patients with pancreatic adenocarcinoma. *Int J Cancer* 2001; **92**: 441-450 [PMID: 11291084 DOI: 10.1002/ijc.1205]

106 **Bernhardt SL**, Gjertsen MK, Trachsel S, Møller M, Eriksen JA, Meo M, Buanes T, Gaudernack G. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/II study. *Br J Cancer* 2006; **95**: 1474-1482 [PMID: 17060934 DOI: 10.1038/sj.bjc.6603437]

107 **Middleton G**, Silcocks P, Cox T, Valle J, Wadsley J, Propper D, Coxon F, Ross P, Madhusudan S, Roques T, Cunningham D, Falk S, Wadd N, Harrison M, Corrie P, Iveson T, Robinson A, McAdam K, Eatock M, Evans J, Archer C, Hickish T, Garcia-Alonso A, Nicolson M, Steward W, Anthoney A, Greenhalf 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 (TeloVac): an open-label, randomised, phase 3 trial. *Lancet Oncol* 2014; **15**: 829-840 [PMID: 24954781 DOI: 10.1016/S1470-2045(14)70236-0]

108 **Le DT**, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, Zheng L, Diaz 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.0b013e31829fb7a2]

109 **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 p53MVA 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]

110 **Sunami Y,** Kleeff J. Immunotherapy of pancreatic cancer. In: Progress in Molecular Biology and Translational Science. Elsevier, 2019: 189–216

111 **Feng K**, Liu Y, Guo Y, Qiu J, Wu Z, Dai H, Yang Q, Wang Y, Han W. Phase I study of chimeric antigen receptor modified T cells in treating HER2-positive advanced biliary tract cancers and pancreatic cancers. *Protein Cell* 2018; **9**: 838-847 [PMID: 28710747 DOI: 10.1007/s13238-017-0440-4]

**Footnotes**

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**Table 1 Clinical trials on pancreatic ductal adenocarcinoma using immunotherapy**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **National clinical trial number** | **Sample** | **Phase** | **Settings** | **Drug** | **Results** |
| NCT00112580 | 82 | II | Advanced; Metastatic; Any line | Ipilimumab | No improvement in survival rate |
| NCT02527434 | 64 | II | Advanced; Metastatic; ≥ Second line | Tremelimumab | OS 4 m (95%CI: 2.83-5.42) |
| NCT02558894 | 65 | II | Metastatic; ≥ Second line | Tremelimumab + Durvalumab *vs* Durvalumab alone | OS 3.1 m (95%CI: 2.2-6.1) Combination therapy; OS 3.6 m (95%CI: 2.7-6.1) Durvalumab alone |
| NCT00112580 | 27 | II | Advanced; Metastatic; ≥ Second line | Ipilimumab | No improvement in survival rate |
| NCT03379259 | 14 | I | Advanced (PDAC and other tumors); ≥ Second line | Anti-PD-L1 (BMS-936559) | No improvement in survival rate |
| NCT01928394 | 1131 | I/II | Advanced (PDAC and other tumors) Any line | Nivolumab ± Ipilimumab | Ongoing |
| NCT03829501 | 412 | I/II | Advanced (PDAC and other tumors); ≥ Second line | Atezolizumab | Ongoing |
| NCT03080974 | 10 | I | Advanced (stage III) Any line | Nivolumab +  Irreversible electroporation | PFS 6.3 m (95%CI: 3.5-10.0); OS 18 m (95%CI: 9.2-26.8) |

OS: Overall survival; PDAC: Pancreatic ductal adenocarcinoma; PFS: Progression-free survival.

**Table 2** **Clinical trials on pancreatic ductal adenocarcinoma combining immunotherapy and chemotherapy**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **National clinical trial number** | **Sample** | **Phase** | **Settings** | **Drug** | **Results** |
| NCT01473940 | 21 | I | Advanced; Metastatic; Any line | Ipilimumab; Gemcitabine | OS 8,5 m (95%CI: 2.2-10.3) |
| NCT00556023 | 34 | I | Metastatic Chemotherapy naïve | Tremelimumab Gemcitabine | OS 7.4 m (95%CI: 5.8-9.4) |
| NCT02331251 | 81 | I/II | Metastatic (PDAC and other tumors) Chemotherapy naïve | Pembrolizumab; Nab-Paclitaxel Gemcitabine | OS 15 m (95%CI: 6.8-22.6) |
| Beatty *et al, Clin Cancer Res* 2013; **19**: 6286–6295 | 22 | I | Advanced; First line | CD40 agonist (CP-870893); Gemcitabine | PFS 5.2 m (95%CI: 1.9-7.4); OS 8.4 m (95%CI: 5.3-11.8); 1-y OS 28.6% |
| [NCT01413022](http://clinicaltrials.gov/show/NCT01413022) | 47 | I | Borderline; Locally advanced Chemotherapy naïve | CCR2 inhibitor (PF-04136309); FOLFIRINOX | Combination arm: 49% OR Chemotherapy arm: 0% OR |
| NCT02268825 | 39 | I | Advanced  Metastatic (gastrointestinal malignancies)  Any line | Pembrolizumab mFOLFOX | No results posted |
| NCT02309177 | 138 | I | Advanced; Metastatic (PDAC and other tumors); Any treatment naive | Nivolumab; Nab-Paclitaxel Gemcitabine | No results posted |
| NCT02077881 | 98 | I/II | Metastatic; First line | IDO inhibitor (indoximod); Nab-Paclitaxel; Gemcitabine | No results posted |
| NCT04045730 | 17 | I/II | Metastatic  First line | Gemcitabine; Nab-Pacliatxel; Pembrolizumab | PFS 9.1 m (95%CI: 4.9-13.3); OS 15 m (95%CI: 6.8-23) |
| [NCT03214250](http://clinicaltrials.gov/show/NCT03214250) | 30 | I | Metastatic; First line | Gemcitabine; Nab-Paclitaxel; Nivolumab; CD40 (agonistic monoclonal antibody) APX005M (sotigalimab) | Ongoing |
| NCT02826486 | 80 | II | Metastatic; Any line | BL-8040; Pembrolizumab; Pegylated liposomal Irinotecan + 5FU | Disease Control Rate 34.5%; OS: 3.3 m Patients receiving study drugs as second-line therapy: 7.5 m |

OS: Overall survival; PDAC: Pancreatic ductal adenocarcinoma; PFS: Progression-free survival; OR: Objective response.

**Table 3 Clinical trials on pancreatic ductal adenocarcinoma using vaccines**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **National clinical trial number** | **Sample** | **Phase** | **Settings** | **Drug** | **Results** |
| UMIN000004919 | 31 | I/II | Metastatic ≥ Second line | KIF20A-66 | KIF20A-66 vaccine: OS 4.7 m ± 0.8; Best supportive care: OS 2.7 m ± 1.1 |
| UMIN000000905 | 6 | I | Advanced (gastrointestinal and endocrine malignancies)  Any line | SVN-2B; IFA; INFa | > 50% of the patients had positive clinical and immunological responses |
| NCT00569387 | 73 | II | Adjuvant treatment | Algenpantucel-L | 12-m OS: 86% |
| Kaufman *et al, J Transl Med* 2007; **5**: 60 | 10 | I | Advanced; Metastatic; Any line | MUC1; HLA-A2; ICAM-1; LFA-3; CM-CSF | antiCEA/MUC-1 positive: OS 15.1 m; antiCEA/MUC-1 negative: OS 3.9 m |
| Lepisto *et al*, *Cancer Ther* 2008; **6**: 955–964 | 12 | I/II | Adjuvant treatment | MUC1 peptide-loaded DC vaccine | Four of twelve patients are still alive without disease recurrence |
| NCT01410968 | 12 | I | Advanced; Metastatic; Any line | w/Poly-ICLC peptide-pulsed DC-CIK | OS 7.7 m |
| Gjertsen *et al, Int J Cancer* 2001; **92**: 441–50 | 48 | I/II | Surgically resected; Advanced; Any line | K-Ras vaccine GM-CSF | Resected: OS 25.6 m (95%CI: 10-39); Unresectable: OS 10.2 m (95%CI: 3-28) |
| Abou-Alfa *et al, Am J Clin Oncol* 2011; **34**: 321–5 | 24 | I | Adjuvant (KRAS mutant) | Ras-peptide GM-CSF | OS 20.3 (95%CI: 11.6-45.3) |
| Bernhardt *et al, Br J Cancer* 2006; **95**: 1474–1482 | 48 | I/II | Advanced Treatment naive | GV1001; GM-CSF | Responders: OS 7.2 m (95%CI: 4.8-10.7); Non-responders: OS 2.9 m (95%CI: 1.7-6.30) |
| Shima *et al*, *Cancer Sci* 2019; **110**: 2378-2385 | 83 | II | Unresectable ≥ Second line | Survivin 2B peptide (SVN‐2B); Interferon‐β | SVN-2B + IFNβ: OS 312 d (95%CI: 43‐460); IFNβ: OS 39 d (95%CI: 13‐153) |

CIK: cytokine-induced killer **;** DC: dendritic cells; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon;m: months; OS: overall survival; PDAC: pancreatic ductal adenocarcinoma; PFS: progression-free survival.

**Table 4 Clinical trials on pancreatic ductal adenocarcinoma combining vaccines and chemotherapy**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **National clinical trial number** | **Sample** | **Phase** | **Settings** | **Drug** | **Results** |
| Jaffee *et al, J Clin Oncol* 2001; **19**: 145–56 | 14 | I | Adjuvant (stage I/II/III) | CVAX, Chemorradiation | Effective anti-tumor immunty |
| NCT00084383 | 60 | II | Adjuvant (stage I/II) | GVAX; 5FU; Chemorradiation | Combination: OS 24.8 m (95%CI: 21.2-31.6); 5FU/Chemorradiation: OS 20.3 m (95%CI: 18-23.9) |
| NCT01417000 | 93 | IIa | Metastatic ≥ Second line | GVAX/Cy CRS-207 | Combination: OS 6.28 m (95%CI: 4.47-9.40); GVAX/Cy: OS 4.07 m (95%CI: 3.32-5.42) |
| NCT02004262 | 303 | IIb | Metastatic ≥ Second line | GVAX/Cy CRS-207 Chemotherapy | Combination: OS 3.7 m (95%CI: 2.9-5.3); CRS-207 alone: OS 5.4 m (95%CI: 4.2-6.4); Chemotherapy: OS 4.6 m (95%CI: 4.2-5.7) |
| UMIN000008082 | 60 | II | Advanced  First line | KIF20A; VEGFR1/2; Gemcitabine | OS 9 m HLA matched; OS 10 m HLA unmatched |
| NCT01072981 | 722 | III | Adjuvant treatment | Algenpantucel-L; Gemcitabine-5FU | 1-y DFS 86% algenpantucel-L *vs* 63% Gemcitabine-5FU; 1-y OS 65% algenpantucel-L *vs* 45% Gencitabine-5FU |
| NCT01836432 | 302 | III | Borderline resectable; Locally advanced usresectable; First line | Algenpantucel-L; FOLFIRINOX; Gemcitabine; Nab-Paclitaxel; Capecitabine; 5FU | No results posted |
| NCT01781520 | 47 | I/II | Unresectable locally advanced  Metastatic Chemotherapy naïve | DC-CIK Chemotherapy S-1 | DC-CIK+Chemotherapy S-1: OS 7 m; DC-CIK alone: OS 4.2 m; Chemotherapy S-1 alone: OS 4.7 m; Supportive care only: OS 1.73 m |
| Muscarella *et al, J Clin Oncol* 2012; **30**: e14501-e. | 176 | II | Resected (KRAS mutant) adjuvant | GI-4000 Gemcitabine | GI-4000+Gemcitabine OS 19.8 m; Placebo-gemcitabine: OS 14.8 m |
| Middleton *et al, Lancet Oncol* 2014; **15**: 829–840 | 1062 | III | Advanced  Metastatic Chemotherapy naïve | GV1001 Gemcitabine Capecitabine | Treated group: OS 6.9 m (95%CI: 6.4-7.6); Chemotherapy alone: OS 7.9 m (95% 7.1-8.8) |
| Yanagisawa *et al*, *Anticancer Res* 2018; **38**: 2217-2225 | 8 | I | Adjuvant (I, II, III) | WT1-DC Vaccine  S-1 Chemotherapy Gemcitabine | No results posted |
| Suzuki *et al,* *Cancer Sci* 2017; **108**: 73-80 | 66 | II | Advanced  First line | Antiangiogenic cancer vaccines targeting VEGFR1 and VEGFR2 in addition to the KIF20A peptide; Gemcitabine | PFS HLA matched: 4.7 m; PFS HLA unmatched: 5.2 m |

OS: overall survival; PDAC: pancreatic ductal adenocarcinoma: PFS: progression-free survival; Cy: Cyclophosphamide; VEGFR: vascular endothelial growth factor receptor.

**Table 5 Clinical trials on pancreatic ductal adenocarcinoma combining vaccines and immunotherapy**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **National clinical trial number** | **Sample** | **Phase** | **Settings** | **Drug** | **Results** |
| NCT00836407 | 30 | I | Metastatic ≥ Second line | Ipilimumab GVAX | Combination: OS 5.7 m (95%CI: 4.3-14.7); Ipilimumab: OS 3.6 m (95%CI: 2.5-9.2) |
| NCT02243371 | 93 | II | Metastatic  ≥ Second line | Nivolumab  Cy; GVAX; CRS-207 | No results posted |
| NCT02451982 | 62 | I/II | Neoadjuvant Adjuvant | Nivolumab Cy GVAX Urelumab | No results posted |
| NCT04627246 | 3 | I/II | Adjuvant | DC vaccine loaded with personalized peptides (PEP-DC); Nivolumab  SOC | No results posted |
| NCT02432963 | 11 | I | Advanced (solid malignancies) ≥ Second line | Pembrolizumab p53MVA | Clinical responses in three out of eleven patients |

OS: overall survival; Cy: Cyclophosphamide; SOC: Standard of Care Chemotherapy; p53MVA: Modified Vaccinia Virus Ankara Vaccine Expressing p53.

**Table 6 Clinical trials on pancreatic ductal adenocarcinoma using adoptive cell transfer**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **National clinical trial number** | **Sample** | **Phase** | **Settings** | **Drug** | **Results** |
| NCT00570713 | 155 | II | Unresectable First-line | MORb-009 Gemcitabine | Combination:  OS 6.5 m (95%CI: 4.5-8.10); Placebo plus Gemcitabine: OS 6.9 m (95%CI: 5.4-8.8) |
| NCT01935843 | 10 | I/II | Advanced (PDAC and other tumors Her2-positive) ≥ Second line | Her2-specific CAR-T cells | OS 4.8 m (95%CI: 1.5-8.3) |
| NCT01781520 | 47 | I/II | Advanced; Any line | DC-CIK Chemoterapy S-1 | DC-CIK + Chemotherapy S-1: OS 7 m DC-CIK alone: OS 4.2 m; Chemotherapy S-1 alone: OS 4.7 m; Supportive care only: OS 1.73 m |
| Aoki *et al,* *Cytotherapy* 2017; **19**: 473-485 | 48 | I | Adjuvant | Gemcitabine; Autologous γδ; T-cell transfer | PFS 26 m (no statistical diference); OS No statistical difference |
| NCT01959672 | 11 | I/II | Neoadjuvant | Gemcitabine; Leucovorin-Fluorouracil; Oregovomab; Nelfinavir + SBRT | Prematurely closed  PFS 8.6 m; OS 13 m (95%CI: 7-22) |
| NCT00720785 | 40 | I | Metastatic (PDAC and other tumors) ≥ Second line | Irreversible electroporation (IRE); Allogeneic natural killer cell therapy | No results posted |
| NCT04212026 | 67 | I | Metastatic ≥ Second line | Irreversible electroporation; Allogeneic natural killer cell therapy | Stage III PFS 9,1 m (IRE-NK) *vs* 7.9 m (IRE); Stage III OS 13.6 m (IRE-NK) *vs* 12.2 m (IRE); Stage IV OS 10.2 m (IRE-NK) *vs* 9.1 m (IRE) |
| NCT01583686 | 6 | I | Metastatic  ≥ Second line | Mesothelin-CART | 2 patients stabilized disease  PFS patient 1: 3.8 m  PFS patient 2: 5.4 m |

CAR: chimeric antigen receptor; DC-CIK: dendritic cell-activated cytokine-induced killer cell; IRE: irreversible electroporation; OS: overall survival; NK: natural killer; PFS: progression-free survival; SBRT: stereotactic body radiation therapy.