

Immunotherapy for pancreatic ductal adenocarcinoma: an overview of clinical trials

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Authors' introduction: Dr. Alessandro Paniccia is a surgical resident at the University of Colorado in Denver (USA) and a research fellow in tumor immunology in the Department of Surgery.

Originally a native of Rome (IT), Dr. Paniccia earned his medical degree, graduating magna cum laude, from the University of Rome "Sapienza" [2008]. He then completed 2 years of general surgery residency at The Johns Hopkins Hospital (USA) before being recruited to the University of Colorado to continue his surgical training under the guidance of Dr. Richard Schulick. While at the University of Colorado, Dr. Paniccia enrolled in a 2-year post-doctoral research fellowship in tumor immunology. During this time, his work focused on the identification and characterization of new T-cell immunologic checkpoints.

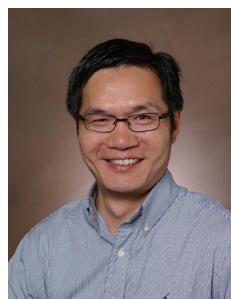
During his residency he was awarded the Ernest E. Moore Award in Basic Science Research from the University of Colorado for outstanding presentation of basic science research at the Annual Department of Surgery Research Symposium. In addition, Dr. Paniccia received training in clinical research design and statistical analysis through the Global Clinical Scholar Research-Training program (GCSRT) at Harvard Medical School.

His primary academic interests are in pancreatic cancer translational research and in particular neoadjuvant treatment for borderline resectable pancreatic adenocarcinoma and cancer immunotherapy.

Dr. Yuwen Zhu obtained his PhD in immunology from the Mayo Clinic, Rochester, Minnesota, USA, and was trained as a postdoctoral fellow and worked as a research faculty member in Dr. Lieping Chen's laboratory at Johns Hopkins University and Yale University. He is now an assistant professor in the Department of Surgery at the University of Colorado Anschutz Medical Campus, Colorado, USA. His research focuses on the discovery of novel immunomodulatory pathways and their potential applications in cancer immunotherapy.



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Abstract: Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death and current therapeutic strategies are often unsatisfactory. Identification and development of more efficacious therapies is urgently needed. Immunotherapy offered encouraging results in preclinical models during the last decades, and several clinical trials have explored its therapeutic application in PDAC. The aim of this review is to summarize the results of clinical trials conducted to evaluate the future perspective of immunotherapy in the treatment of PDAC.

Keywords: Immunotherapy; pancreatic neoplasm; cancer vaccines; clinical trial

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Evolution of tumor immunology

The role of the immune system in the development of neoplastic diseases has been the subject of investigation and controversy for several decades. In 1891, William Coley offered one of the first examples of the efficacy of the immune system in treating cancerous lesions. His strategy consisted of intratumoral injections of live or inactivated *Streptococcus pyogenes* and *Serratia marcescens*, known as “Coley’s toxin”. The injected bacteria were capable of initiating a local inflammatory response resulting in activation of antibacterial phagocytes and potential killing of nearby tumor cells by virtue of profound inflammatory response (1). Data derived from Coley’s work were collected for over 40 years and the results of his studies were published in 1953 (2,3). As a result of his pioneering work, Coley is often credited as the father of cancer immunotherapy.

The current view of immune surveillance suggests that cancerous cells are maintained in check by the immune system, which recognizes and eliminates abnormal cells (4-7). The process of immune-surveillance depends on a series of events that are necessary to mount an effective antitumor response (1). Cancer cells express specific epitopes (i.e., neo-antigens) on their cell surface as a result of cancerous transformation (8,9). These epitopes are also known as tumor-associated antigens (TAAs) and are usually captured, processed and presented by dendritic cells (DCs) (10,11). DCs, which are often recognized as the most potent antigen-presenting cells in the human body, require activation and/or maturation signals to differentiate and eventually migrate to regional lymph nodes (12,13). Once in the lymph nodes, mature DCs present TAAs to naive T cells that then undergo expansion and differentiation to become activated T cells. activated T cells eventually leave

the lymph nodes and infiltrate into the tumor site where they execute their cytotoxic activity to kill tumor cells (1).

Tumor cells, however, can evade immune control through several complex mechanisms, utilizing immunosuppressive and tolerogenic strategies including immunoediting (14,15). Immunoediting is composed primarily of three sequential stages known as elimination, equilibrium, and escape (7,14,16). During the first phase of “elimination”, cancerous cells are identified and appropriately destroyed by the immune system. During the second phase of “equilibrium,” the immune system prevents further tumor outgrowth but it fails to eliminate cancerous cells completely. The third phase, “escape,” is a direct consequence of the previous two phases, and can be seen as the product of selective pressure of the immune system on cancer cells. In this final phase, cancer cells, which evolve from the original cancerous cell, are now capable of evading the immune surveillance and continue to proliferate.

The pancreatic cancer microenvironment

Pancreatic ductal adenocarcinoma (PDAC) presents several challenges that set it apart from those more immunogenic tumors, such as melanoma and renal cell cancer (17,18). A dysregulation of the immune system is one of the facilitating factors for PDAC development, thus legitimizing the role of the immune network in PDAC (19-22).

One of the principal characteristics of PDAC is the abundance of stromal desmoplasia that constitutes the tumor microenvironment in which the components of the immune network are distributed (23,24). This extensive stromal desmoplasia, also known as fibrosis, has been shown to promote tumor development and most importantly to prevent the penetration and uptake of chemotherapeutic

agents (25,26). One of the major players in PDAC desmoplasia is the pancreatic stellate cell (PSC). Stimulated by transforming growth factor β (TGF- β) and platelet-derived growth factor (PDGF), the PSCs initiate a process of synthesis and deposition of extracellular matrix (ECMs) proteins that eventually leads to the extensive desmoplastic reaction seen in PDAC (27,28). Preclinical models have shown that targeting the signaling cascade leading to ECMs protein synthesis could enhance drug penetration in the pancreatic neoplastic tissue (29). However, PDAC clinical trials have yet to show a significant benefit from this approach. In addition, activation of inhibitory T-cell checkpoints (i.e., CTLA-4, PD-1) may have a contributing role as does the particularly hostile tumor microenvironment characterized by abundant stroma that prevents the effector T-cell from functioning in various manners (30).

Several cytokines appear to be dysregulated and contribute to cancer progression in PDAC. In particular, higher levels of circulating interleukin-6 (IL-6) are identified in patients with PDAC and appear to promote cancer progression through enhancement of protumorigenic Stat3 signaling (20,31). Furthermore, members of the IL-1 family [e.g., IL- α , IL- β and IL-1 receptor antagonist (IL-1ra)] seem to play a role in PDAC development (32-34). Immunosuppressive cytokine IL-10 is up regulated in PDAC, which leads to a reduction in effector cell function in the PDAC microenvironment and indicates a worse prognosis (35,36).

Tumor-infiltrating lymphocytes (TILs) have a paramount role in tumor specific cellular adaptive immunity. The main components of this population are CD8+ cytotoxic T cells, CD4+ helper T cells (e.g., Th1, Th2, and Th17), and regulatory T-cells (T_{regs}) (18). CD8+ T-lymphocytes are the dominant subset of T-lymphocytes in the PDAC microenvironment and their presence is associated with prolonged survival (37-39). CD8+ cytotoxic T-cells recognize TAA peptides associated with major histocompatibility complex class I on tumor cells, resulting in cancer cell destruction. In addition to their direct cytotoxic effect on tumor cells, CD8+ T cells are capable of mobilizing and triggering macrophage tumoricidal activity (18,40,41). The presence of Th1 and Th2 lymphocytes in the tumor microenvironment appears to have opposite prognostic significance in the setting of PDAC progression (42,43). In fact, the presence of Th1 is associated with favorable prognosis while a predominant infiltration of Th2 and its related cytokines (IL-4, IL-5 or IL-13) often correlates with disease progression (18). Of interest is the

role of IL-5 and IL-13, these cytokines likely stimulate the desmoplastic reaction increasing ECM deposition and collagen synthesis (44). Furthermore, IL-13 appears to downregulate proinflammatory cytokines (IL-1, IL-6, TNF- α) and chemokines, and effectively inhibits antibody-dependent cellular toxicity (45,46). Nevertheless, IL-13 acts as an autocrine growth factor for PDAC (47,48). Regulatory T-cells (T_{regs}), which are positive for CD4+, CD25+, and Foxp3, are enriched in the tumor microenvironment (49,50). T_{regs} effectively suppress the adaptive immune response and their presence in the tumor microenvironment leads to a decreased presence of CD8+ T-cells and often correlates with poor prognosis (50,51). Other cell types, like myeloid-derived suppressive cells (MDSCs) and neutrophils, also participate in the immune reaction during the development and progression of PDAC resulting in dynamic interactions between the tumor cells, and the immune system.

Strategies of cancer immunotherapy

Different strategies for cancer immunotherapy have been proposed and investigated. These therapeutic strategies can be grouped into active or passive, based on the involvement of the host immune system. Active immunotherapy aims to stimulate the host immune response to recognize TAAs and eventually destroy tumor cells. This often requires administration of cytokines, immunomodulatory agents, or therapeutic vaccines that eventually lead to the expansion of tumor-specific T cells. Passive immunotherapy requires the exogenous administration of activated lymphocytes (e.g., tumor-specific immune effector cells) or antibodies that mediate an immune response (52).

Overview of clinical trials in PDAC immunotherapy

Results from recent clinical trials conducted between 2005 and 2015 are summarized in *Table 1*. In addition, trials conducted between 2010 and 2015 are discussed in the following sections.

Adoptive therapy

In one of the most recent phase II trials, Chung *et al.* evaluated the use of adoptive immunotherapy in patients with advanced pancreatic cancer who experienced disease progression during gemcitabine-based chemotherapy (73). In this study, the authors utilized *ex vivo* expanded, cytokine-induced killer (CIK) cells (i.e., heterogenous cell population

Table 1 Pancreatic cancer immunotherapy: an overview of selected clinical trials

Type of immune therapy	Phase	Population	Outcome	n	Year	References
Vaccine						
GV1001 (TeloVac) (telomerase vaccination)	III	Locally-advanced and/or metastatic PDAC	MST: 7.9 mos with chemotherapy alone, 6.9 mos (sequential chemotherapy) vs. 8.4 mos (concurrent with chemotherapy)	1,062	2014	(30)
Chemotherapy						
Chemotherapy with sequential GV1001						
Chemotherapy with concurrent GV1001						
WT1 peptide-based cancer vaccine	I	Locally-advanced and/or metastatic or recurrent PDAC	MPFS: 4.2 mos MST: 8.1 mos OS: 71% and 29% (6 mos and 1 yr)	32	2014	(53)
Combined with gemcitabine						
KIF20A-derived peptide with gemcitabine (kinesin superfamily motor proteins)	I	Locally-advanced and/or metastatic PDAC	MST: 173 days OS: 11.1% (1 yr)	9	2014	(54)
Personalized peptide vaccination (PPV)	II	Advanced PDAC that failed 1 st -line chemotherapy	MST: 7.9 mos OS: 26.8% (1 yr)	41	2013	(55)
HLA-A24-restricted peptide vaccine from KIF20A	I/II	Locally-advanced and/or metastatic PDAC	MPFS: 56 days MST: 142 days	31	2013	(56)
Algenpantucel-L + chemotherapy/chemoradiotherapy	II	Resected PDAC	DFS: 62% (1 yr) OS: 86% (1 yr)	72	2013	(57)
muRAS-transfected EBV-transformed lymphoblastoid cell lines	I	Relapsed or metastatic, muRas-positive cancer	MPFS: 3.1 mos	7	2012	(58)
MUC1-peptide pulsed dendritic cells	I	Recurrent or metastatic PDAC, MUC1-positive cancer	Diseases progression observed in all patients within 3 mos	7	2012	(59)
GM-CSF secreting vaccine	II	Resected pancreatic cancer	Median DFS: 17.3 mos MST: 24.8 mos	60	2011	(60)
VEGF-receptor 2+ gemcitabine	I	Locally-advanced and/or metastatic PDAC	MPFS: 3.9 mos MST: 7.7 mos	18	2010	(61)
Personalized peptide vaccination (PPV) + gemcitabine	II	Locally-advanced and/or metastatic PDAC	MST: 9 mos OS: 38% (1 yr)	21	2010	(62)
OK432-pulsed DCs + CD3-LAKs + GEM	I	Locally-advanced PDAC	MST: 478 days	5	2009	(63)
Preoperative immunotherapy IL-2	III	Resected PDAC	The arm pre-treated with IL-2 had a longer MPFS and OS compared with surgery only (P<0.01 and P<0.05)	30	2008	(64)
GM-CSF cancer cell line (CG8020/CG2505)	III	Advanced PDAC	Arm 1: MST =2.3 mos Arm 2: MST =4.3 mos	50	2008	(65)
Arm 1: vaccine alone						
Arm 2: vaccine + cyclophosphamide						
Poxvirus-based vaccine targeting CEA and MUC-1	I	Locally-advanced and/or metastatic PDAC	MST: 6.3 mos Patients who generated specific immune response to CEA and/or MUC-1 showed a survival advantage (15.1 vs. 3.9 mos; P=0.002)	10	2007	(66)

Table 1 (continued)

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Type of immune therapy	Phase	Population	Outcome	n	Year	References
Vaccine						
Heat shock protein (HSPPC-96)	I	Resected PDAC	MST: 2.2 yr	10	2007	(67)
Personalized peptide vaccination (PPV) + gemcitabine	I	Locally-advanced and/or metastatic or recurrent PDAC	MPFS: 17 weeks MST: 7.6 mos	13	2007	(68)
Telomerase peptide GV1001 (dose escalating trial)	I/II	Non-resectable PDAC	Low dose group: MST =4.0 mos Intermediate dose group: MST =8.6 mos* High dose group: MST =5.1 mos	48	2006	(69)
Personalized peptide vaccination	I	Locally-advanced and/or metastatic or recurrent PDAC	OS: 80% (6 mos) OS: 20% (12 mos)	11	2005	(70)
MUC1 + SB-AS2 (patients were allowed to receive adjuvant therapy after completing three vaccinations)	I	Resected or locally advanced PDAC	MST: 12 mos	16	2005	(71)
Immunotherapy check-point						
Single agent ipilimumab (anti-CTLA-4)	II	Locally advanced or metastatic PDAC	Significant delayed regression of metastatic pancreatic cancer in 1 out of 27 patients	27	2010	(72)
Passive immunotherapy						
Cytokine-induced killer (CIK) cells	II	Advanced pancreatic cancer with disease progression during gemcitabine-based therapy	MPFS =11.0 weeks MST =26.6 weeks	20	2014	(73)
Combination strategies						
Ipilimumab (anti-CTLA-4) + GM-CSF cell-based vaccine (GVAX)	Ib	Advanced PDAC, previously treated	Arm 1: Ipilimumab alone MST: 3.6 mos OS: 7% (1 yr) Arm 2: Ipilimumab + GVAX MST: 5.7 mos OS: 27% (1 yr)	30	2013	(74)
Survivin 2B-derived peptide vaccination + α -INF	I	Advanced PDAC	No survival information	6	2013	(75)
Cytokine-induced killer (CIK) cells + α -Gal-dendritic cells (DCs)	I	Locally-advanced PDAC	MST: 24.7 months	14	2013	(76)
MUC1-DC + MUC1-CTL	II	Locally advanced or recurrent PDAC	Mean OS: 9.8 mos OS: 20% (1 yr) OS: 10% (2 yr) OS: 5% (3 yr)	20	2008	(3)

* , significantly increased survival in the intermediate group compared to low dose (P=0.006) and high dose (P=0.05). OS, overall survival; MST, median overall survival time; DFS, disease free survival; MPFS, median progression free survival; mos, months; yr, year; PDAC, pancreatic adenocarcinoma; WT-1, Wilms tumor 1; VEGF-R, vascular endothelial growth factor; IL-2, interleukin-2; α -Gal, alpha-galactosyl epitope; α -INF, alpha-interferon; LAK, lymphokine-activated killer; GEM, gemcitabine; MUC1, human-mucin1; CTL, cytotoxic T-lymphocyte; CEA, carcinoembryonic antigen; GVAX, irradiated cancer cell lines (PANC 6.03 and PANC 10.05) that were engineered to express GM-CSF (whole cell vaccine); EBV, Epstein-Barr virus.

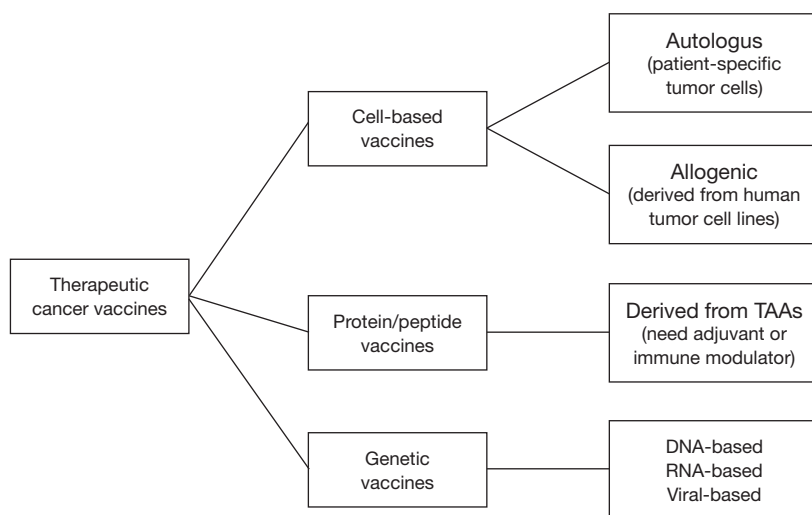


Figure 1 Therapeutic cancer vaccine categories. TAA, tumor associated antigen.

containing >20% of CD3+ CD56+ cells) previously shown to have cytolytic activity in a major histocompatibility complex (MHC)-unrestricted manner (77). Patients enrolled in this study received CIK as the sole cancer therapy. The authors reported a median estimated progression free survival (PFS) of 11.0 weeks and a median estimated overall survival (OS) of 26.6 weeks, which were similar to prior studies using conventional cytotoxic chemotherapy (73,78-80).

Cancer vaccines

Cancer vaccines aim to stimulate the immune system to produce tumor-specific T cells and B cells (81). The primary mechanism of action of therapeutic cancer vaccines is their capacity to increase the presentation of TAAs to the immune system. Generally vaccines can be classified in three major approaches: cell-based vaccines, protein/peptide vaccines, and genetic vaccines. Each strategy has been well-investigated, and each seems to have its own advantages and disadvantages (Figure 1).

Table 2 summarizes the most common cellular targets utilized in recent clinical trials of PDAC cancer vaccines, including: telomerase, Wilms tumor gene, KIF20A, alpha-galactosyl (α -Gal), survivin, mutated Ras protein, human mucin MUC1 protein, and vascular endothelial growth factor receptor 2 (VEGFR2).

The TeloVac study is one of the largest randomized, phase III clinical trials to evaluate the efficacy of cancer vaccine in PDAC (30). This trial was conducted in 51 hospitals in the United Kingdom and enrolled 1,062

subjects. It aimed to assess the efficacy and safety of sequential or simultaneous telomerase vaccination (GV1001) in combination with chemotherapy in patients with locally advanced or metastatic pancreatic cancer. Results showed that adding GV1001 vaccine either simultaneously or sequentially to a standard treatment regimen of gemcitabine and capecitabine did not improve OS. The authors suggest that the lack of response seen in this trial may be due to the characteristic rapid progression of pancreatic cancer to metastatic disease, which could prevent an active immune response from developing.

Active peptide-based immunotherapy utilizing Wilms tumor (WT1) protein has been investigated in combination with gemcitabine for patients with advanced pancreatic cancer (53). In this phase I clinical trial, vaccination with WT1 in combination with gemcitabine was found to be safe. Furthermore, although the trial was not designed to evaluate survival benefit, it appears that the patients in whom a WT1 specific immunity was induced had better clinical outcomes translating to a 12-month or longer survival time and an improved quality of life (QOL).

Suzuki *et al.* conducted the first phase I trial aimed to investigate the use of a vaccine composed of an epitope peptide KIF20A in combination with gemcitabine in patients with advanced pancreatic cancer (unresectable and/or metastatic) who had already received prior conventional chemotherapy and/or radiotherapy (54). The authors reported no adverse events directly attributable to the vaccine and demonstrated enhancement of INF- γ -producing cells in 8 out of the 9 patients enrolled (54).

Table 2 Common cellular targets utilized in recent clinical trials for PDAC cancer vaccine

Cellular targets	Rationale	References
Telomerase	Enzyme that is reactivated during oncogenic transformation Prevents the naturally occurring shortening of the telomeric ends of DNA during replication, which would lead to cell senescence and eventually cell death	(82-84)
Wilms tumor gene (WT1)	Identified on the cell surface of several cancerous cells including pancreatic cancer cells Highly immunogenic, eliciting both humoral and cellular responses	(53,85-91)
KIF20A (RAB6KIFL)	Member of the kinesin superfamily of motor proteins, that has a paramount role in the intracellular trafficking of molecules and organelles during the growth of pancreatic cancer	(54)
Alpha-galactosyl (α -Gal) epitope	Human cells do not present the α -Gal epitope and on the contrary the anti-Gal antibody is abundant in human serum (about 1% of circulating human antibody) Genetically modified tumor cells that express α -Gal in addition to TAAs, in an attempt to induce a complement and antibody-dependent cell-mediated hyperacute rejection that would favor the processing and presentation of TAAs	(76,92-95)
Survivin (also known as baculoviral inhibitor of apoptosis repeat-containing 5; BIRC5)	Member of the inhibitor apoptosis protein (IAP) that is highly expressed in neoplastic tissues but absent in non-neoplastic human cells	(75,96-98)
Mutated Ras protein	Derives from the Ki-Ras p21 oncogene and is expressed in cancer derived from different histologies and in approximately 90% of PDAC cases A point mutation at codon 12 results in specific substitution of a normal glycine (Gly) amino acid with an aspartic acid (Asp), valine (Val), cysteine (Cys), or arginine (Arg) which can easily be targeted by a formulation of four different vaccines	(58,99)
Human mucin MUC1 protein	This protein is specifically expressed on the surface of pancreatic cancer cells and can be used as a specific tumor associated antigen (TAA)	(48,59,71,100)
Vascular endothelial growth factor receptor 2 (VEGFR2)	VEGFR2 is highly expressed on endothelial cells of tissues undergoing a process of tumor-induced neovascularization but it is absent in normal blood vessels VEGFR2 has been identified on PDAC cancer cells Vaccination leads to the generation of CTL able to interfere with the processes associated with PDAC neovascularization. In addition, specific-CTLs have the potential to target PDAC cancer cells directly	(61,101-103)
Mesothelin	Overexpressed in most PDAC Participate in cell adhesion and has a potential role in metastatic progression	(104)
Personalized peptide vaccination (PPV)	Relatively new strategy of peptide-based vaccination The peptide utilized is chosen from a number of different pooled peptides and selected based on the patient's HLA-class IA types and levels of peptide-specific IgG responses prior to vaccination	(55,62,68)

The enthusiasm that followed two trials conducted by Yanagimoto *et al.*, aimed at the evaluation of personalized peptide vaccination (PPV) in combination with gemcitabine (62,68), prompted Yutani *et al.* to test this vaccination strategy in a phase II trial in patients with chemotherapy-resistant advanced pancreatic cancer (55). Patients enrolled in this trial had a median survival time (MST) of 7.9 months with a 1-year survival rate of 26.8%. However the authors

noted that patients who were treated solely with PPV (n=8) had a MST of 3.1 months compared to patients who received PPV vaccination combined with chemotherapy (9.6 months; P=0.0013). Therefore, Yutani *et al.* concluded that PPV offers no advantages as a single therapy in patients with advanced PDAC, although its use combined with chemotherapy could positively influence OS.

Algenpantucel-L (NewLink Genetics Corporation,

Ames, IA, USA) is an allogenic cancer vaccine composed of two human PDAC cell lines (HAPa-1 and HAPa-2) (57). These cells express the α [1,3]-galactosyl epitopes (α -Gal) as a result of genetic engineering processes. Injection of algenpantucel-L generates a hyperacute rejection that ultimately stimulates the patient's immune system to target the existing PDAC lesions (57,105). In the phase II trial conducted by Hardacre *et al.*, algenpantucel-L was administered in combination with standard chemotherapy and chemoradiotherapy (gemcitabine + 5-fluorouracil-based chemoradiotherapy) as adjuvant treatment following surgical resection of a primary PDAC lesion. Results from this trial were encouraging; with a reported 12-month disease free-survival of 62% and 12-month OS of 86% with a median follow-up of 21 months. The authors remarked that the percentage of patients surviving at 12-month was higher than survival predicted by the widely accepted prognostic nomogram described by Brennan *et al.* (86% *vs.* 55-63%) (57). Another positive note was that patients treated with algenpantucel-L experienced minimal side effects, mainly consisting of injection site pain and induration. Although several interesting findings emerged from this study, its results should be interpreted carefully as no definitive conclusion was achieved on the advantage provided by the addition of algenpantucel-L to standard chemotherapy regimens.

Asahara *et al.* conducted a non-randomized, open-label, phase I/II clinical trial utilizing the KIF20A-66 epitope restricted to the HLA-A2402 (the most common HLA-A allele in the Japanese population enrolled in the study). The KIF20A-66 is a member of the kinase superfamily protein (see above) that is highly expressed in pancreatic cancer cells. Patients with advanced PDAC who failed gemcitabine-based therapy comprised the cohort selected for this trial. Median survival time was compared to a historic cohort and patients treated with cancer vaccine therapy showed an overall median survival time of 142 days compared to 83 days ($P=0.0468$) of the historic cohort. Interestingly, the authors reported the case of one patient who experienced complete response with resolution of liver metastatic lesion. This patient was noted to have a strong cytotoxic T-cell (CTL) response to KIF20A-66 epitope that remained detectable even 2 years from the last dose of vaccine administration (56).

Kubuschol *et al.* investigated the use of an autologous lymphoblastoid cell line (LCL)-based vaccine. LCLs are "professional" antigen presenting cells (APCs) characterized by a very high immunostimulatory capacity that are easily

obtained from EBV-positive patients. These cells are a particularly attractive source of APCs because they are characterized by a rapid growth *in vitro* providing an easily accessible cell pool (58). In this trial LCLs were engineered to express a mutated Ras-protein on the cell surface (muRas-LCL). Patients enrolled in the study, received weekly subcutaneous injections with muRac-LCL vaccine. Tumor specific T-cell response (muRas-specific) was observed in six of the seven patients enrolled in the trial (85%). However, despite an initial clinical response observed in 57% of cases, after 4 months from initial vaccination, all patients showed disease progression. One of the most important findings of this study was that the use of tumor antigen-transfected LCL proved to be an efficient alternative to DCs to serve in the role of APCs for future vaccine trials (58).

Rong *et al.* investigated the immunological response induced by the administration of MUC1-peptide-pulsed DCs-based vaccine in a cohort of advanced PDAC patients (59). Patients were selected based on tumor expression of MUC1. Patients' autologous DCs were collected, pulsed with MUC1-peptide and injected intradermally for three to four administrations. Although the vaccination regimen was safe, evidence of a significant immune response was observed in only two of the seven patients enrolled.

Lutz *et al.* conducted a phase II clinical trial enrolling 60 patients with resected pancreatic adenocarcinoma (60). In their trial, the authors utilized an allogenic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine (GM-CSF), based on cancer cell lines PANC 10.05 and PANC 6.03, injected directly into lymph node regions. The initial vaccine dose was followed by 5-FU based chemoradiotherapy and additional vaccine doses were given after chemotherapy completion in patients that remained disease free. Patients that completed all 4 doses of the vaccine therapy received a final vaccine booster 6 months after the administration of the fourth dose. The first observation from the study was that the regimen of vaccination with GM-CSF-secreting tumor cells following adjuvant chemoradiotherapy was well tolerated. In fact, no local or dose-limiting toxicities were observed. Additionally, when the study cohort was compared to a historical cohort treated at the same institution, the authors found no significant difference in the median OS (HR: 0.96, 95% CI, 0.68-1.35, $P=0.8$).

Miyazawa *et al.* investigated the use of a peptide vaccine for human vascular endothelial growth factor receptor 2 (VEGFR-2) in combination with gemcitabine adjuvant therapy (61). In this phase I clinical trial, 21 patients with

advanced pancreatic cancer were enrolled and 18 patients were able to complete the vaccination schedule and were evaluated in their final analysis. Although the treatment was well tolerated, and specific CTL response against the vaccinated peptide was observed in the majority of the treated patients (61%), no correlation of CTL response and overall clinical outcome was appreciated. Following the results of this study a new double-blind, placebo-controlled trial was designed to investigate the role of an oral VEGFR-2 vaccine in patients with stage IV and locally advanced pancreatic cancer. The study is currently ongoing (NCT01486329) (106).

The use of GVAX, a whole-cell vaccine composed of two irradiated cancer cell lines (PANC 6.03 and PANC 10.05) engineered to express GM-CSF has been investigated in multiple phase I and II studies. Early studies showed that vaccination with GVAX leads to induction of CD8+ T-cell responses against multiple mesothelin-specific epitopes that has been shown to correlate with improved survival (60,65,107).

Although designed to evaluate a mixed cohort with advanced solid tumor, the study conducted by Le and colleagues offered interesting results on the use of *Listeria*-based vaccines (108). *Live-Attenuated Listeria vaccines* are used based on the ability of *Listeria monocytogenes (Lm)* to stimulate both innate and adaptive immunity. After administration, *Lm* is phagocytized in the liver and generates a local inflammatory response leading to the activation and recruitment of natural killer (NK) and T cells. Le and colleagues, investigated the use of live-attenuated *Lm*-based vaccines in two cohort of patients with liver metastasis originated from PDAC (108). In the first phase of their study, the safety and efficacy of the use of *Lm*-based vaccine (ANZ-100) was tested and found to be acceptable. Following these initial findings, *Lm* was modified to express human mesothelin (CRS-207), a tumor associated antigen (TAA) known to be expressed by PDAC. The ultimate goal was to induce an immune response that would produce tumor antigen-specific T cells directed toward PDAC expressing human mesothelin protein. Three of the seven patients treated with (CRS-207) survived more than 15 months and showed specific T-cell response to the vaccine component listeriolysin O (LLO), although all three patients had received prior immunotherapy with GM-CSF-based whole-cell vaccine (GVAX) which confounds the overall results. Unfortunately, LLO-response was not evaluated in the remaining patients who survived less than 15 months.

Taken together these results suggest that cancer vaccines are in general well tolerated and able to generate an immune

response directed toward specific cancer targets. However, with the exception of some isolated but remarkable clinical responses, the impact of cancer vaccines on OS in PDAC appears to be minimal for the majority of patients. Several explanations for this lack of efficacy have been proposed. It is worth noting that advanced stages of PDAC are characterized by rapid disease progression that might not allow enough time for the immune system to mount an effective response that often requires weeks to months to develop.

Immune checkpoint blockade

T cell response can be controlled by a few cosignaling receptors with inhibitory functions, now known as immune checkpoints, which include CTLA-4, PD-1 and BTLA. Agents blocking these molecules are able to unleash endogenous anti-tumor T cell responses, so as to limit tumor growth (109). Royal *et al.* investigated the role of single agent Ipilimumab, an anti-CTLA-4 antibody, in a cohort of locally advanced or metastatic pancreatic adenocarcinoma (72). Ipilimumab has been previously effective in the treatment of melanoma, renal cell carcinoma, and prostate cancer (110-112). CTLA-4 is transiently expressed on the T-cell surface following activation and leads to a decrease in T-cell response following its binding to B7-1 or B7-2 on APCs or target tissue (113). In this phase 2 trial, the authors observed a significant delayed regression of metastatic pancreatic cancer in one out of the twenty-seven patients enrolled in the study. The findings of this phase 2 trial were particularly interesting as they underlined the mechanism of action of Ipilimumab represented by immunomodulation rather than direct tumoricidal activity. In fact, the patient who showed a response to Ipilimumab treatment had initially experienced marked progression of the disease. The authors concluded that Ipilimumab alone might not be a valuable treatment for advanced pancreatic cancer, however they laid the basis for future trials of combination therapy with immune checkpoint blockade combined with vaccine or chemotherapy (72).

Combination immunotherapy trials

Cancer vaccine and immune checkpoint blockade

Although the study conducted by Royal *et al.* (phase II trial) showed minimal efficacy of anti-CTLA-4 (Ipilimumab) therapy on advanced pancreatic cancer, one patient enrolled in this initial trial showed a significant delayed response suggesting a possible role for immune checkpoint blockade

in PDAC (72). Several preclinical studies suggest a possible synergistic role of cancer vaccine therapy that stimulates the immune system and the use of immune checkpoint blockade to allow for the unopposed effector function of cytotoxic T-cells (114,115). On this premise, Le *et al.* conducted a phase Ib, open-label, randomized study to determine the safety profile of ipilimumab alone or in combination with GVAX in patients with previously treated PDAC (74). This study showed that the use of Ipilimumab in PDAC patients, with or without GM-CSF-based cell therapy, has an acceptable side effect profile. Induction of immune response was observed as a result of the treatment regimen and correlated with clinical activity, although prolonged treatment appears to be required to obtain a clinical response in the setting of advanced PDAC disease (74). One of the most interesting aspects of this study was the difference in 12-month OS of 27% *vs.* 7% and the median OS of 5.7 *vs.* 3.6 months (HR =0.51; P=0.072) respectively for combination therapy *vs.* monotherapy. Although the trial was not designed to show significant survival differences, the results obtained point to a superiority of the combination therapy over monotherapy (74).

Active immune therapy combined with passive immune therapy

Qiu *et al.* investigated the use of a combination of DC-based and CIK-based therapy (76). In this study, DCs were initially pulsed with patients' primary pancreatic carcinoma cells previously transfected *in vitro* to express α -Gal epitope and opsonized with anti-Gal IgG. This approach enhances the antigenicity of TAAs and facilitates phagocytosis by DCs (76). Subsequently, DCs were co-cultured with CIKs derived from bone marrow stem cells, ultimately generating tumor specific immune responders cells *ex vivo* (76). The generated CIKs and the mature DCs were then injected in 14 patients with inoperable stage III/IV pancreatic adenocarcinoma. The authors reported a significant increase in patients' cellular immunity, especially in the percentage of cytotoxic T cells (CD3+CD8+), activated and memory T cells (CD3+CD45RO+), and activated T and NK cells (CD3+CD56+). Furthermore, no serious side effects were experienced during treatment and the reported median OS was 24.7 months (108.1 \pm 35.1 weeks), higher than the usual survival reported in the literature for unresectable stage III/IV PDAC.

Kameshima *et al.* investigated the use of a vaccination protocol of survivin-2B80-88 plus incomplete Freud's adjuvant (IFA) and α -interferon (INF α) based on favorable

results previously obtained in the treatment of colon cancer (75,116,117). The authors reported that more than 50% of the treated patients showed positive clinical and immunological response.

Immunotherapy combined with chemotherapy

Algenpantucel-L is currently being investigated in an open label, phase III, randomized trial in combination with FOLFIRINOX (oxaliplatin, 5-FU, irinotecan, and leucovorin) in patients with borderline resectable or locally advanced pancreatic cancer (NCT01836432). The estimated primary completion date is September 2015. This is currently the first study that is using a FOLFIRINOX based chemotherapy.

Conclusions and prospective

Traditional treatments for PDAC are limited and ineffective, and novel therapeutic strategies are greatly needed. Despite recent advancements in systemic chemotherapeutic regimens, the median survival time of advanced pancreatic cancer patients remains 4-11 months (118-121). The identification and development of more efficacious therapies is of paramount importance. Immunotherapy offers encouraging results in preclinical models but often fails to show clear benefits in clinical trials for PDAC. Immunotherapy, as a single treatment strategy, might not be sufficient to effectively treat PDAC. For example, evidence suggests that active immunotherapy should be used in combination with traditional chemotherapy and/or radiotherapy or even in combination with other forms of immune therapy (e.g., immune checkpoint blockade or passive immune therapy) (122). This strategy could take advantage of the various effects traditional chemotherapeutic agents and/or radiotherapy exert on the immune system (123,124). Acting through direct killing of cancerous cells, chemotherapeutic agents indirectly lead to the release of pro-inflammatory molecules and TAAs (85). In addition, chemotherapy can suppress the inhibitory mechanism in the tumor microenvironment. In fact, reduction of the number of T_{regs} cells and myeloid derived suppressor cells (MDSC) and their related cytokines (IL-17 and IL-15) are one of the recognized positive effects of chemotherapy on tumor microenvironment. This change in the composition of cells in the tumor microenvironment could facilitate the development of a more efficacious effector immune response against cancer cells (52,122,125). However, the potential synergistic effects of chemotherapy

have to be balanced with its potential immunosuppressive effects. Future studies should focus on identifying appropriate dosing and timing of synergistic chemotherapy administration in order to mitigate its immunosuppressive effects and maximize the effect of immunotherapeutic cancer treatments. Several aspects remain to be clarified in PDAC cancer immunotherapy, including optimal cellular targets, delivery vectors for cancer vaccines, combination with existing treatment strategies, and patient selection. Future clinical trials should be designed to address these unresolved aspects of PDAC immunotherapy.

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Footnote

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