Current Oncology Reports (2019) 21: 60
https://doi.org/10.1007/s11912-019-0811-5

GASTROINTESTINAL CANCERS (J MEYER, SECTION EDITOR)

Current Status of Immunotherapies for Treating Pancreatic Cancer

Annie A. Wu[1] · Elizabeth Jaffee[1] · Valerie Lee[1]

Published online: 17 May 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract
Purpose of Review Despite all efforts, pancreatic ductal adenocarcinoma (PDAC) remains a disease that causes substantial morbidity and mortality, with a 5-year survival rate of 7%. Innovative paradigms for treating PDAC are urgently needed.
Recent Findings We discuss the advances and difficulties in using immunotherapy and developing immunotherapeutic vaccines for PDAC. Current excitement about antigen-specific immunotherapy has been propelled by advances in multiple areas, such as next-generation sequencing to identify neoantigens and manufacturing to produce immunotherapeutic vaccines. Antigen-specific immunotherapy is being actively explored in clinical trials.
Summary As the field of immunotherapy matures and as our understanding of the complex interactions between tumor and host develops, we hope to identify new methods for treating and managing PDAC.

Keywords Pancreatic adenocarcinoma · Immunotherapy · Cancer vaccines

Introduction

Pancreatic ductal adenocarcinoma (PDAC) has a dismal prognosis. Affecting over 55,000 people in the USA each year, incidence continues to rise, while a 5-year survival rate remains at only 7% [1, 2]. High mortality is attributable in part to the inability to detect this cancer before the occurrence of metastatic disease and to the lack of effective therapies. These statistics demonstrate the urgent need for innovative therapies for treating PDAC. However, attempts to improve these data with targeted and immunotherapies have failed thus far. A primary reason for resistance likely lies in the highly immunosuppressive tumor microenvironment and stroma [3, 4], along with low neoantigen burden, both of which inhibit infiltration and recognition by effector T cells. For this reason, recent trials have focused on evaluating vaccines that can induce effector T cells that have the ability to recognize cancer antigens, given in combination with immune checkpoint inhibitors (ICIs) that can allow T cell trafficking and optimal function within the tumor. In this review, we will discuss the trials evaluating use of immunotherapy in PDAC and focus on vaccines designed to recruit and activate the host's T cells against tumor-specific antigens.

This article is part of the Topical Collection on *Gastrointestinal Cancers*

✉ Valerie Lee
Vlee32@jhmi.edu

[1] Johns Hopkins University School of Medicine, 1650 Orleans Street, Room 488, Baltimore, MD 21287, USA

Standard Management of Pancreas Cancer

With only 10–20% initially eligible for surgery [5, 6], the management of PDAC is traditionally focused on systemic management.

For patients with resectable PDAC, traditional management is comprised of surgical resection followed by adjuvant therapy, although the role of neoadjuvant chemotherapy is being evaluated. Following the data from CONKO-001, adjuvant gemcitabine was shown to improve a 10-year overall survival from 7.7% with surgery alone to 12.2% [7]. The 2017 ESPAC-4 study showed incremental change with gemcitabine/capecitabine (improving median OS 25.5 months with gemcitabine alone versus 28 months with gemcitabine and capecitabine) [8]. More recently, the 2018 PRODIGE-24 trial demonstrated greater progress with a median OS of 54.4 months with adjuvant FOLFIRINOX versus 35 months with gemcitabine [9••].

For those with borderline resectable and locally advanced disease, a combination of chemotherapy, radiation therapy, and/or surgery is pursued. However, the optimal systemic regimen, length of treatment, and type of radiation are still to be determined.

For those with metastatic disease, however, treatment options remain limited. Few regimens with promise have been assessed on a large scale since the 2011 publication of FOLFIRINOX data improving median OS to 11.1 months over 6.8 months with gemcitabine alone [10] and the 2013 publication of gemcitabine with nab-paclitaxel improving median OS 8.5 months with combination versus 6.7 months with gemcitabine alone [11]. These data suggest the continued need for improvement for all stages of PDAC.

The Immune Desert of Pancreas Cancer

Pathologically, primary PDAC appears as atypical neoplastic glands within a stroma-rich environment (desmoplasia). This stroma consists of an extensive extracellular matrix of proteins (including collagen, fibronectin, hyaluronic acid, and proteoglycans) with low vascularity, creating a hypoxic environment that supports tumor invasion and metastasis, and functions as a physical barrier against treatments [12, 13]. The small (5–20%) cellular component of the stroma is comprised primarily of activated pancreatic stellate cells which inhibit cancer cell apoptosis, and interact with other cells (including endothelial, neuronal, and immune cells) to stimulate cancer proliferation and migration [14, 15], creating an environment that supports carcinogenesis and metastasis. Tumor development and progression is further promoted by the immune cell population characterized by regulatory CD4+ T cells, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and mast cells, to the exclusion of cytotoxic lymphocytes. This overall regulatory immune population of cells creates a "non-immunogenic" tumor that is often resistant to immune recognition and killing [16]. The makeup of the primary PDAC TME is highly inductive to angiogenesis, lymphangitic spread, and the epithelial-mesenchymal transition [17, 18]. The specific mechanisms are still being explored, for example preclinical data revealing intercellular communication with PDAC-derived exosomes can lead to the establishment of "pre-metastatic" niches characterized by TGF beta activation and fibronectin accumulation [19]. The liver metastatic environment is additionally characterized by fibrosis and an immunosuppressive environment exclusive of cytotoxic T cells [20].

PDAC is also characterized by a low tumor mutational burden (TMB) ranging from 10 to 60 approximately 60 encoded neoantigens, in contrast to the majority of tumors that respond to immunotherapy that express 100–1500 mutations per MB [21]. Higher TMB tumors express increased numbers of neoantigens leading to increased immune surveillance [22]. This is most notably demonstrated in the population of cancer patients with mismatch repair deficiency (dMMR) or microsatellite instability high (MSI-H) tumors, whose tumors demonstrated marked genomic instability, with TMB in the range of the 1000s, and significant response to single agent and dual checkpoint inhibition [23–25]. In PDAC, whole exome sequencing showed a median TMB of 1.8 mutations/Mb, with only 1% of patients having TMB > 20 mutations/Mb [26]. However, the TMB of PDAC is likely to be higher with the development of newer assays for measuring TMB. More commonly, PDAC molecular profiles show driver mutations in KRAS (> 90% seen in both TCGA and MSK-IMPACT studies), TP53, SMAD4, and CDKN2A/B [27•, 14] with a subset (5–10%) showing germline mutations in BRCA2, ATM, PALBB2, and PRSS1; but hypermutated phenotypes are rarely seen (1–2% of PDAC) [26].

The combination of an immune desert tumor microenvironment together with the low mutational burden not only has made PDAC traditionally resistant to immunotherapies, but also simultaneously suggests potential targets for treatment.

Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICIs) are upregulated on T cells that are functionally "exhausted" in the context of chronic antigen exposure. While immune checkpoints enable self-tolerance and prevent autoimmunity under normal physiological conditions, they often become co-opted by tumors to prevent the immune system from mounting effective antitumor responses. The first checkpoints discovered were cytotoxic T lymphocyte–associated protein 4 (CTLA-4) [28] and its ligands B7.1 and B7.2, followed by programmed cell death receptor 1 (PD-1) [29] and its ligands PD-L1 and PD-L2 [30]. These discoveries led to the understanding that blocking CTLA-4 and PD-1 can override T cell desensitization to tumor antigens, with the subsequent production and approval for a number of antagonist antibodies to CTLA-4 [31, 32], PD-1, and PD-L1. ICIs have clinically benefited patients with a wide range of cancers [33–38] and have achieved FDA approvals in melanoma, renal cell carcinoma, lung, bladder, and others (Table 1); however, they have shown limited success in patients with PDAC [39, 40].

The first attempt at testing a single-agent ICI for treatment of PDAC was in a phase II trial of ipilimumab published in 2010. The CTLA-4 inhibitor was administered to 20 patients with metastatic PDAC and 7 patients with locally advanced disease. Unfortunately, 0/27 responses were seen, leading to early discontinuation of the trial [40]. Similar results were seen in the phase I KEYNOTE-001 study of the safety of pembrolizumab, an anti-PD-1 inhibitor, in patients with advanced solid tumors. Only 1/30 patients included in the trial had PDAC and this patient did not respond [41]. A larger phase I trial has evaluated the safety of the first anti-PD-L1 antibody in 207 patients, including 14 with PDAC. While responses were seen in those with melanoma, NSCLC, ovarian, and RCC, none of the patients with metastatic PDAC responded [39].

Table 1 FDA-approved cancer immunotherapy drugs. In the past decade, there has been accelerated progress in cancer immunotherapy starting with the first FDA–approved cancer immunotherapy in 2010

Drug name	Cancer population and date approved
PD-1 blockade	
Keytruda (pembrolizumab)	*2018*: Dec. 19: Merkel cell carcinoma
	Nov. 9: Hepatocellular carcinoma
	June 13: Primary mediastinal large B cell lymphoma
	June 12: PD-L1-positive cervical cancer
	2017: Sept. 22: Gastroesophogeal and gastric cancer
	May 22: MSI-H/dMMR unresectable or metastatic solid tumors
	May 17: Advanced bladder cancer
	May 9: Non-small cell lung cancer
	Mar. 14: Classical Hodgkin's lymphoma
	2016: Oct. 23: PD-L1-positive metastatic non-small cell lung cancer
	Aug. 4: Recurrent or metastatic head and neck squamous cell carcinoma
	2015: Oct. 2: Squamous and non-squamous non-small cell lung cancer
	2014: Sept. 4: Advanced or unresectable melanoma
Libtayo (cemiplimab)	*2018*: Sept. 28: Cutaneous squamous cell carcinoma
Opdivo (nivolumab)	*2018*: Aug. 17: Small cell lung cancer (SCLC)
	2017: Sept. 22: Hepatocellular carcinoma
	Aug. 1: MSI-H colorectal cancer
	Feb. 1: Advanced bladder cancer
	2016: Nov. 9: Head and neck squamous cell carcinoma
	May 16: Classical Hodgkin's lymphoma
	2015: Nov. 23: Metastatic renal cell carcinoma
	Nov. 13: Metastatic squamous non-small cell lung cancer
	Oct. 9: Non-squamous non-small cell lung cancer
	2014: Nov. 13: Metastatic melanoma
CTLA-4 blockade	
Yervoy (ipilimumab)	*2011*: Nov. 13: Metastatic melanoma
PD-1 and CTLA-4 blockade	
Opdivo (nivolumab) and Yervoy (ipilimumab)	*2018*: July 10: MSI-H/dMMR colorectal cancer
	Apr. 16: Advanced renal cell carcinoma
	2015: Oct. 1: Advanced melanoma
PD-L1 blockade	
Imfinzi (durvalumab)	*2018*: Feb. 16: Unresectable stage III non-small cell lung cancer
	2017: Apr. 30: Advanced bladder cancer
Bavencio (avelumab)	*2017*: May 8: Advanced bladder cancer
	Mar. 22: Merkel cell carcinoma
Tecentriq (atezolizumab)	*2016*: Oct. 17: Non-small cell lung cancer
	May 17: Bladder cancer
CAR T cells targeting CD19	
Kymriah (tisagenlecleucel)	*2018*: May 1: Diffuse large B cell lymphoma
	2017: Aug. 30: Children and young adult B cell acute lymphoblastic leukemia
Yescarta (axicabtagene ciloleucel)	*2017*: Oct. 18: Non-Hodgkin large B cell lymphoma
Oncolytic virus	
Imlygic (talimogene laherparepvec)	*2015*: Oct. 27: Advanced melanoma
Cell-based vaccine	
Provenge (sipuleucel-T)	*2010*: Apr.: Prostate cancer

Combination ICIs have also shown only modest activity in refractory metastatic PDAC. The largest study was a phase II trial comparing durvalumab (a PD-L1 inhibitor) alone versus durvalumab with tremilimumab (a CTLA-4 inhibitor), which yielded a median OS of 3.6 months versus 3.1 months, respectively, in the combined 65 patients, with one confirmed partial response in the combination arm [42].

ICIs have shown success for one small group of PDAC patients, those with MSI-H tumors, which are estimated to comprise about 1–3% of the PDAC population [43••]. While rare, due to the significant response rates and duration of responses, NCCN guidelines now recommends consideration of MSI or MMR testing in patients with locally advanced or metastatic PDAC [44]. In a phase II trial, 86 patients with colorectal and non-colorectal cancers (including 8 patients with pancreatic cancer) with MMR deficiency were treated with pembrolizumab. Of the 78 patients who could be evaluated for response, objective responses were seen in 53% of patients overall, and 62% of those with pancreas cancer [23].

The disappointing data for the vast majority of PDAC patients has resulted in the recommendation against further testing with single-agent ICIs. Tumor-infiltrating T cells that display an exhausted phenotype often co-express multiple inhibitory checkpoints, such as TIGIT, PD-1, TIM-3, and LAG-3 [45]. Additionally, agonists to co-stimulatory checkpoint pathways, such as those in the TNF family (OX-40 and CD137), are being developed that can provide additional activating signals for T cells and prevent exhaustion and apoptosis. However, ICIs alone are unlikely to provide long-term success because most PDAC do not have enough quality T cells, which are meaningful in evaluating antitumor responses. "Quality" T cells refer to activated T cells expressing CD69 and CD154 that are markers of re-invigorated T cells [46]. They also express poly-functional cytokines including interferon-gamma, interleukin-2, and tumor necrosis factor alpha; and lack expression of exhaustion markers. Finally, they also exhibit $EOMES^{+}PD\text{-}1^{-}$ surface expression profiles [47, 48]. Thus, alternative combination regimens that activate T cells prevent inhibitory signals and enhance activating signals should be considered.

Therapeutic Vaccines

One method to potentially transition PDAC into more immunogenic tumors is to develop cancer-targeted vaccines that can activate a high-quality T cell with the capability of trafficking into PDAC tumors [49]. T cells can recognize cancer antigens in a similar way to how they recognize virally infected cells. Specifically, they express T cell receptors (TCRs) capable of recognizing intracellular antigenic peptides uniquely expressed on the surface of major histocompatibility complex (MHC) molecules. The diversity of TCRs allows the immune system to recognize foreign antigens such as viral proteins or altered antigens such as the products of mutated cancer genes. While targeted cancer therapies have sought to block specific driver mutations of carcinogenesis, both driver and passenger mutations may lead to non-synonymous mutations and subsequent changes in amino acid sequences. These mutant proteins are expressed by tumors, processed into short peptides (epitopes), and presented on the cell surface by MHC or human leukocyte antigen (HLA) in humans, and become recognizable to T cells as foreign antigens. This distinction between malignant and non-malignant cells provides a method of immune cell targeting tumor cells directly while avoiding damage to normal cells.

Antigens are categorized into two groups: tumor-associated antigens (TAA), antigens mostly restricted to malignant cells with limited expression on normal cells, and tumor-specific antigens (TSA), which are expressed solely on malignant cells [50]. Common TAAs expressed by PDACs include epidermal growth factor receptor (HER/EGFR/ERBB) family proteins, carcinoembryonic antigen (CEA), mesothelin, vascular endothelial growth factor (VEGF, VEGFR) family proteins, and Wilms' tumor 1 (WT-1) [51]. As these TAAs are prominent among PDAC, they serve as a more easily targetable class, allowing for premade therapies that can be administered to a multitude of patients. However, due to variability in presence of TAAs between tumor types and presence on normal cells, efficacy and off-target toxicity remains a clinical concern [52, 53].

In contrast, TSAs are appealing targets for immunotherapy due to their expression solely in individual PDACs. These TSAs or neoantigens can be generated via a diversity of tumor-specific alterations including single nucleotide substitutions, frame shifts, and chromosomal translocations, as well as posttranslational modifications such as phosphorylation and deamidation, and alternative splicing of peptides. Due to the generation of novel epitopes encoded by TSAs, TCRs involved in the recognition of neoantigens are likely to escape deletion by central tolerance. However, since neoantigens are not shared between patients, personalized vaccines are required to target these TSAs, the production of which is more time consuming and costly. Exceptions may include TSAs that are seen with increasing frequency, such cancer-associated MUC1 that is aberrantly glycosylated or mutant KRAS which is expressed by more than 90% of PDAC. Interestingly, only four mutations cover > 99% of KRAS mutations expressed by PDACs, including 45% represented by KRAS G12D alone [54]. There is evidence that mutant KRAS G12D is expressed on murine MHC [55] and human HLA [56], and that cytotoxic T cells can be induced by vaccines to target multiple mutant Kras G12D epitopes present on murine MHC [55] and HLA-A*11:01 or HLA-C*8:02 [56–58]. It is possible, however, that pancreatic tumor cells can evade T cell immune recognition and destruction by downregulating

expression of MHC I [59]. However, to date, such evidence does not exist.

Multiple Forms of Antigen-Specific Vaccines

Various TAA- or TSA-specific vaccine platforms used to treat PDAC have been evaluated throughout the years. While autologous vaccines, which use an individual's own tumor as an antigen source, are patient specific, they are not feasible due to a number of technical challenges, such as low cellularity of PDACs leading to suboptimal antigen concentration and the difficulty in quantifying and reliably reproducing the optimal cellular composition. In contrast, allogeneic vaccines are less specific, but offer the advantages of being more convenient, standardized, and faster to give off-the-shelf to patients. We will discuss a number of platforms that have been tested for patients with PDAC that include shared antigens and patient-specific antigen approaches.

Whole-Cell Vaccines

Cellular vaccines are one approach for delivering combinations of TAAs and TSAs. Two such vaccine platforms that have been tested in PDAC patients are GVAX and algenpantucel-L.

GVAX is a whole-cell vaccine consisting of two human allogeneic pancreatic tumor cell lines (Panc 10.05, Panc 6.03) irradiated to release antigen and transfected with DNA to release GM-CSF at the vaccination site. GM-CSF attracts dendritic cells that phagocytose released antigens from apoptotic PDAC, migrate to draining lymph nodes, and activate effector T cells that target tumors with antigens found in vaccine PDAC cell lines. GVAX has been extensively studied in multiple phase I/II trials as adjuvant therapy for patients with resected PDAC [60]. Patients treated were found to have induction of mesothelin-specific CD8+ T cells, which correlated with DFS. Similarly, when given in a neoadjuvant setting, patients with PDAC who received neoadjuvant cyclophosphamide and GVAX prior to pancreaticoduodenectomy were noted in their surgical resection samples to develop novel vaccine–induced intratumoral tertiary lymphoid aggregates with upregulation of PD-1/PD-L1 pathways [49].

GVAX has also been studied in metastatic PDAC patients in a randomized trial testing ipilimumab ± GVAX for patients refractory to gemcitabine-based therapy. Fifteen patients were enrolled to each arm, and median OS was 3.6 months versus 5.7 months ($p = 0.072$) for single-agent ipilimumab versus combination therapy, respectively. Peripheral mesothelin–specific T cell responses were noted to be enhanced and increased in those patients with a survival rate of more than 4.3 months [61]. In addition, 27% of patients receiving the combination had a 1-year survival rate as compared to only 7% in the ipilimumab alone arm. GVAX has since been combined with a number of other therapies in ongoing trials, including combination with other vaccines (CRS-207 discussed below), radiation, and other checkpoint inhibitors (NCT02648282, NCT03161379, NCT02451982, NCT03153410, Table 2) for patients with all stages of PDAC.

Algenpantucel-L is another whole-cell vaccine consisting of two human allogeneic irradiated pancreatic tumor cell lines (HAPa-1, HAPa-2), which synthesize α-galactosyl (α-Gal) epitopes, a carbohydrate present on the cell surface and not produced by human cells [62]. Normal gastrointestinal flora expressing α-Gal results in the constitutive mass production of α-Gal antibodies, thus algenpantucel-L administration stimulates a hyperacute rejection against α-Gal epitopes from the vaccine. Unfortunately, the phase III IMPRESS trial showed no improvement in survival after treatment of algenpantucel-L with gemcitabine or 5-FU chemoradiotherapy and no further trials are ongoing at this time [63].

Bacterial-Based Vaccines

Bacterial vectors (including *Listeria monocytogenes*, salmonella, *Lactococcus lactis*, *Lactobacillus plantarum*, and Bacillus Calmette-Guerin) have also been studied as they can stimulate a strong cell–mediated adaptive immune response to TAAs. These bacterial delivery systems have the added benefit of providing adjuvant activity since they naturally activate innate immunity as well.

L. monocytogenes is an intracellular bacterium that enters macrophages by phagocytosis and naturally escapes the phagosome and enters the cytosol. The bacterial vector can be modified to express specific antigens and through its natural biology delivers antigen to both MHC I and II processing pathways, eliciting potent CD8+ and CD4+ T cell responses. CRS-207, a live-attenuated listeria vaccine, is engineered to secrete mesothelin, which is highly expressed in PDAC [64, 65]. Unfortunately, in a phase II trial, CRS-207 and GVAX showed no survival advantage over chemotherapy in patients with metastatic PDAC [66]. However, trials studying combinations of CRS-207 with IDO inhibition and other ICIs are ongoing (NCT03006302, NCT03190265, Table 2).

Yeast-Based Vaccines

Yeast can generate significant cytotoxic T cell responses, and thus can be genetically modified to elicit responses against specific tumor antigens. One such vaccine that has undergone testing is GI-4000 a series of four vaccines, composed of different heat-inactivated *S. cerevisiae* expressing three different Ras mutations, covering seven of the most frequent Ras mutations in human cancers. In a randomized phase II trial, GI-4000 given with gemcitabine was tested for resected PDAC and was well tolerated with a modest survival benefit (524 days versus 444 days) [67]. Further phase I/II trials evaluating treatment in the metastatic setting are being analyzed at

Table 2 Actively recruiting PDAC immunotherapy clinical trials in the USA. Current phase II/III trials evaluating immunotherapy combinations for patients with PDAC in the USA [clinicaltrials.gov; PanCan.org]

Phase	Stage of cancer	Trial	Target	NCT number
II	Resectable	Hydroxychloroquine, gemcitabine, nab-paclitaxel ± avelumab	TLR, cytotoxics, PD-L1	NCT03344172
I/II	Resectable	GVAX ± nivolumab ± urelumab	Whole-cell vaccine, PD-1, CD137	NCT02451982
Ib/II	Resectable/borderline resectable	Chemoradiation ± pembrolizumab	Radiation, PD-1	NCT02305186
II	Borderline resectable	GVAX, nivolumab, stereotactic body radiation	Whole-cell vaccine, PD-1, radiation	NCT03161379
II	Borderline resectable or LAPC	Losartan, FOLFIRINOX, nivolumab, stereotactic body radiation	ARB, cytotoxics, PD-1, radiation	NCT03563248
II	LAPC	Cabiralizumab, nivolumab, stereotactic body radiation	CSF1R, PD-1, radiation	NCT03599362
II	LAPC	GVAX, Pembrolizumab, and stereotactic body radiation	Whole-cell vaccine, PD-1, radiation	NCT02648282
Ib/II	LAPC or Metastatic (not progressed on platinum)	Niraparib, nivolumab v ipilimumab	PARP inhibitor, PD-1, CTLA-4	NCT03404960
I/II	Metastatic (maintenance)	CV301, chemotherapy, Durvalumab	CEA/MUC-1 (vaccine), cytotoxics, PD-L1	NCT03376659
II	Metastatic (1st line)	Nab-paclitaxel, Cisplatin, Gemcitabine, nivolumab, paricalcitol,	Cytotoxics, PD-1, Vitamin D,	NCT02754726
Ib/II	Metastatic (1st line)	APX005M, gemcitabine, nab-paclitaxel ± nivolumab	CD40, cytotoxics, PD-1	NCT03214250
Ib/II	Metastatic (1st line +)	BMS-813169, chemo v nivolumab	CCR2/5 inh, cytotoxics, PD-1	NCT03184870
Ib/II	Metastatic (1st line +)	Morpheus (multiple immunotherapy combinations)	PD-L1, MEK inhibitor, stromal (HA), CXCR4, CD40, VEGF, CSF-1R, FAP	NCT03193190
II	LAPC or metastatic (1st line +)	Nivolumab, entinostat	PD-1, HDAC inhibitor	NCT03250273
II	LAPC or metastatic (2nd line +)	Pembrolizumab, azacitidine	PD-1, DNMT inhibitor	NCT03264404
Ib/II	LAPC or metastatic (2nd line +)	MCS110, PDR001	PD-1, M-CSF	NCT02807844
Ib/II	LAPC or metastatic (2nd line +)	LMB-100 ± nab-paclitaxel	Mesothelin-targeted immunotoxin, cytotoxics	NCT02810418
II	LAPC or metastatic (2nd line +)	Cabiralizumab, nivolumab ± chemotherapy	CSF14, PD-1, cytotoxics	NCT03336216
II	LAPC or metastatic (2nd line +)	AZD9150, durvalumab	STAT3, PD-L1	NCT02983578
III	Metastatic (2nd line +)	FOLFOX ± AM0010	Cytotoxics, IL-10	NCT02923921
Ib/II	Metastatic (2nd line +)	NANT PDAC vaccine in combination with a number of therapies	NK cell vaccine	NCT03586869
I/II	Metastatic (2nd line +)	Anti-Kras G12V mTCR peripheral blood lymphocytes	mTCR	NCT03190941
II	Metastatic (2nd line +)	Autologous tumor-infiltrating lymphocytes following a lymphocyte-depleting regimen + pembrolizumab upon progression	TILs, PD-1	NCT01174121
II	Metastatic (2nd line+)	Dendritic cell vaccine against neoantigens expressed by autologous cancer	DC vaccine	NCT03300843
II	Metastatic (2nd line +)	CRS-207, nivolumab, ipilimumab ± GVAX	Mesothelin vaccine (listeria), PD-1, CTLA-4, whole-cell vaccine	NCT03190265
II	Metastatic (2nd line +)	CRS-207, pembrolizumab, epocadostat ± GVAX	Mesothelin vaccine (listeria), PD-1, IDO inh, whole-cell vaccine	NCT03006302
II	Metastatic (2nd line +)	Nivolumab, ipilimumab, radiation	PD-1, CTLA-4, radiation	NCT03104439

this time (NCT03387098, NCT03329248, NCT03136406, NCT03586869), and other personalized neoepitope yeast–based vaccines (YE-NEO-001) are undergoing testing (NCT03552718).

Viral Vector–Based Vaccines

Viral vectors used for vaccines include vaccinia virus (VV), adenovirus (AdV), adeno-associated virus (AVV), alphavirus,

and its derivative vectors. Recombinant VV can be modified to encode TAAs/TSAs. Unfortunately, despite encouraging phase I results, phase III testing has not shown success, including one of PANVAC-V (targeting CEA and MUC-1 and co-stimulatory B7.1, ICAM-1, and LFA-3) vaccinia and PANVAC-F fowlpox viruses with GM-CSF, which did not show improved overall survival compared to palliative chemotherapy or best supportive care [68]. A VV vaccine expressing p53 (MVA-p53) combined with pembrolizumab for PDAC and solid tumors is under evaluation (NCT02432963).

Peptide- and DNA-Based Vaccines

Antigenic peptide and DNA plasmid vaccines are taken up by dendritic cells and processed and presented on MHC to trigger T cell responses. These vaccines are safe and easy to produce, but require identification of immunogenic epitopes and adjuvants to enhance vaccine potency given poor immunogenicity of peptides and DNA plasmids alone [69, 70].

Several vaccines have previously been tested, including targeting mutant RAS peptides with various adjuvants, such as QS21 [71] or GM-CSF, and in combination with gemcitabine [72]. Peptide vaccines have targeted other TSA and TAAs, including WT-1 [73], MUC-1 [74], VEGFR1, and VEGFR2 [75]. Significant clinical benefits have yet to be seen at this time.

Despite their limitations, peptide- and DNA-based vaccines represent ideal vectors for personalizing vaccines against TSAs, especially as next-generation sequencing has become faster and more commercially available, along with improved selection of immunogenic epitopes. A personalized peptide vaccine with adjuvant polyICLC has been shown to be feasible and beneficial in patients for adjuvant treatment of high-risk melanoma [76•]. Personalized peptide vaccines are also being developed and studied as monotherapies (NCT02795650), in combination with GM-CSF (NCT03645148), or with checkpoint inhibitors (NCT02600949) for patients with metastatic PDAC. An ongoing personalized DNA–based vaccine is being tested in the adjuvant setting (NCT03122106).

Dendritic Cell–Based Vaccines

Dendritic cells (DCs) are specialized APCs that capture, process, and present antigens to prime CD4+ and CD8+ T cells. Isolated DCs from peripheral blood can be loaded ex vivo with tumor antigens and administered into patients [77, 78]. In multiple phase I and II trials tested internationally, MUC1-pulsed DCs have been given in the metastatic and adjuvant setting as monotherapies and in combination with chemotherapies [79–82]. Additional phase I trials with WT1 and hTERT-pulsed DCs have also been evaluated and found to be safe.

Ongoing DC–based vaccines include a DC vaccine pulsed with mutant KRAS peptides corresponding to a patient's specific tumor mutation and HLA type (mDC3/8) in a prime-boost regimen will be tested in a phase I study in resected PDAC patients (NCT03592888). Additionally, DC vaccines against defined neoantigens expressed by autologous cancers are being tested in a phase II trial in patients with epithelial cancers including PDAC (NCT03300843).

CAR T Cells

Chimeric antigen receptor (CAR) T cells are genetically engineered to express CARs specific for a tumor antigen [83]. Their receptors consist of fusion proteins combining antibody variable regions with T cell signaling chains in tandem with cytoplasmic signaling domains of CD28, 4-1BB, and other co-stimulatory molecules. CAR T cells are expanded ex vivo for local infusion back into patients with cancer. Upon antigen recognition, CAR T cells directly lyse tumor cells or secrete cytokines. CAR T cells have been developed primarily as autologous options, but also with "off-the-shelf" approaches being evaluated for almost all of the TSAs and TAAs described above [84, 85]. Preclinical and phase I trials have studied CAR T cells targeting HER2, MUC1, CEA, and mesothelin (NCT02713984, NCT02587689, NCT02850536, NCT02349724, NCT02706782, NCT02580747, NCT03323944), but there has been limited data presented. A second generation of CAR T cells against mesothelin (CART-meso) using patients' own T cells to express anti-mesothelin receptors fused to TCRζ and 4-1BB as a co-stimulator for metastatic PDAC is being investigated (NCT03638193, NCT02465983). Additional TSA–targeted CAR T cells are under development and include an ongoing phase I/II trial testing an "off-the-shelf" anti-KRAS G12V and G12D murine TCR–transduced peripheral blood lymphocyte therapy in patients with PDAC who harbor the mutant RAS variants presented on HLA-A*1101 (NCT03190941, NCT03745326). However, although these approaches have the potential to overcome the challenge of a lack of effective PDAC–specific T cells, CAR T cells may still have difficulty trafficking into the tumors and maintaining their activity if they do gain access.

Prospective

While landmark preclinical and clinical immunotherapeutic discoveries and FDA approvals for immunotherapeutic drugs (Table 1) have ramped up dramatically in the past several years, PDAC has remained resistant to these new interventions. Several strategies should be considered for the future.

Immunotherapy is likely most effective when given closer in time to tumor initiation, when immune suppressive mechanisms are fewer and easier to bypass. It takes years from initiating mutation to PDAC establishment, which is driven by accumulating genetic, inflammatory, and stromal changes

within the pancreas [86–89]. Thus, better technology that can detect early stages or even precancerous lesions may provide improved targets for treatment and stimulation of immune surveillance [90].

Additionally, the PDAC microenvironment is more complex and suppressive than in other immunogenic cancers. Thus, a better understanding of the role of immunologic contributors to PDAC growth should allow for the design of multi-agent immunotherapies that can target these immunosuppressive pathways. As discussed, stroma and immune cells have established an integrated suppressive network that contributes to PDAC development. A combination of immunotherapy and targeted therapy against stromal elements may be necessary to improve patient outcome. One stromal target is focal adhesion kinase-1 (FAK1), which is a tyrosine kinase expressed on PDAC cells and stroma that drives stromal fibrosis. FAK inhibitors are being studied in a number of trials in combination with PD-1 blockade and MEK inhibitors (NCT02758587, NCT03727880, NCT02546531, NCT02428270). Alternatively, a phase III trial examining hyaluronic acid (HA) depletion with pegylated recombinant human hyaluronidase (PEGPH20) plus chemotherapy for metastatic PDAC (NCT01959139) was recently completed, but opens the doorway for potential combinations with immunotherapy. TGF beta regulation is also frequently altered throughout the development of PDAC, and thus may provide an additional target for treatment.

Immune suppressive pathways will need to be modulated with novel combination immunotherapies in order to convert the immune hostile PDAC environment into one that is susceptible to immunotherapies. PDAC contains numerous immunosuppressive cell populations [4, 91], including Tregs, tolerogenic dendritic cells, immature macrophages, and MDSCs, each of which express a number of T cell suppressing signals. Attractive therapeutic targets expressed by these cells include IDO (expressed by tolerogenic DCs and Tregs) [92, 93], CCR2 (a chemokine that recruits immunosuppressive macrophages) [94], and colony-stimulating factor-1 receptor (CSF1R, which recruits tumor-infiltrating macrophages) [95] pathways. A number of combination trials are ongoing at this time (Table 2). In addition, chemotherapies and epigenetic therapies have been reported to modulate a number of these suppressive inflammatory cells [96, 97] and when given in combination with ICIs have shown antitumor activity. Thus, attempts to combine immunotherapy with epigenetic modulators including HDAC inhibitors and hypomethylating agents are ongoing [98, 99] (NCT03250273, NCT03264404).

Finally, emerging data suggests that poorly immune-responsive cancers including PDAC require novel combinations of chemotherapy, radiation, vaccines, and novel vaccine agonists, along with ICIs, to fully activate antitumor T cell responses. In preclinical studies from our group, neoepitope peptide vaccines mixed with STING adjuvant along with anti-PD-1 and anti-OX40 agonist in PANC02-bearing mice showed greater response than those treated with vaccine alone or with single-agent checkpoint inhibitors [100]. While translating preclinical successes into true benefit in the clinical setting has been difficult, more data is needed and clinical trial participation should be encouraged for these patients with limited alternate options.

Conclusions

Pancreatic cancer remains difficult to treat with few regimens exhibiting substantial improvement in survival. Overall, treatment with immunotherapy has shown little success to date, but many promising combination therapies remain to be explored in ongoing and upcoming clinical trials.

Compliance with Ethical Standards

Conflict of Interest Annie A. Wu declares that she has no conflict of interest.

Elizabeth Jaffee has received research funding from Adaptive Biotech, IM Core (Genentech), Lustgarten Foundation, Bristol-Myers Squibb, Breast Cancer Research Foundation, Amgen, and Aduro BioTech; has received travel reimbursement and an honorarium from Adaptive Biotech; has received travel reimbursement and honoraria for participating on scientific advisory boards from Dragonfly Therapeutics, CStone Pharmaceuticals, Genocea Biosciences, Parker Institute, and Lustgarten Foundation; and GVAX and *Listeria monocytogenes*—Mesothelin are both licensed to Aduro BioTech with the potential to receive royalties.

Valerie Lee declares that she has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 2014;74(11):2913–21.
2. Society, A.C., Cancer Facts & Figures 2019.
3. Laheru D, Jaffee EM. Immunotherapy for pancreatic cancer - science driving clinical progress. Nat Rev Cancer. 2005;5(6):459–67.
4. Zheng L, Xue J, Jaffee EM, Habtezion A. Role of immune cells and immune-based therapies in pancreatitis and pancreatic ductal adenocarcinoma. Gastroenterology. 2013;144(6):1230–40.
5. Bond-Smith G, Banga N, Hammond TM, Imber CJ. Pancreatic adenocarcinoma. BMJ. 2012;344:e2476.

6. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. *Pancreatic cancer*. Lancet. 2011;378(9791):607–20.
7. Oettle H, Neuhaus P, Hochhaus A, Hartmann JT, Gellert K, Ridwelski K, et al. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. JAMA. 2013;310(14): 1473–81.
8. Neoptolemos JP, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM, et al. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. Lancet. 2017;389(10073):1011–24.
9.•• Conroy T, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul JL, et al. FOLFIRINOX or gemcitabine as adjuvant therapy for pancreatic cancer. N Engl J Med. 2018;379(25):2395–406 **A randomized clinical trial of adjuvant therapy for patients with resected pancreas cancer. DFS at 3 years was 39.7% in FOLFIRINOX group versus 21.4% in gemcitabine group, and median OS was 54.4 months in FOLFIRINOX group versus 35.0 months in gemcitabine group. This shows significant improvement in survival for patients with pancreas cancer who were previously advised a median 28-month survival even with surgical intervention.**
10. Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med. 2011;364(19):1817–25.
11. Von Hoff DD, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med. 2013;369(18): 1691–703.
12. Yuen A, Diaz B. The impact of hypoxia in pancreatic cancer invasion and metastasis. Hypoxia (Auckl). 2014;2:91–106.
13. Feig C, et al. The pancreas cancer microenvironment. Clin Cancer Res. 2012;18(16):4266–76.
14. Kleeff J, Korc M, Apte M, la Vecchia C, Johnson CD, Biankin AV, et al. Pancreatic cancer. Nat Rev Dis Primers. 2016;2:16022.
15. Wood LD, Hruban RH. Pathology and molecular genetics of pancreatic neoplasms. Cancer J. 2012;18(6):492–501.
16. Binnewies M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med. 2018;24(5):541–50.
17. Ren B, et al. Tumor microenvironment participates in metastasis of pancreatic cancer. Mol Cancer. 2018;17(1):108.
18. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19(11):1423–37.
19. Costa-Silva B, et al. Pancreatic cancer exosomes initiate premetastatic niche formation in the liver. Nat Cell Biol. 2015;17(6):816–26.
20. Houg DS, Bijlsma MF. The hepatic pre-metastatic niche in pancreatic ductal adenocarcinoma. Mol Cancer. 2018;17(1):95.
21. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. N Engl J Med. 2017;377(25): 2500–1.
22. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348(6230):69–74.
23. Le DT, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509–20.
24. Andre T, Lonardi S, Wong KYM, Morse M, McDermott RS, Hill AG, et al. Combination of nivolumab (nivo) + ipilimumab (ipi) in the treatment of patients (pts) with deficient DNA mismatch repair (dMMR)/high microsatellite instability (MSI-H) metastatic colorectal cancer (mCRC): CheckMate 142 study. J Clin Oncol. 2017;35:3531.
25. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncol. 2017;18(9):1182–91.
26. Chalmers ZR, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med. 2017;9(1):34.
27.• Cancer Genome Atlas Research Network. Electronic address: andrew_aguirre@dfci.harvard.edu; and N. Cancer Genome Atlas Research, Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. Cancer Cell, 2017. 32(2): p. 185–203 e13. **The TCGA molecular characterizations of cancers have led to better understanding of the different classifications of tumors, and their heterogeneity. This allows for better targeted therapies for patients over time.**
28. Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, et al. A new member of the immunoglobulin superfamily–CTLA-4. Nature. 1987;328(6127):267–70.
29. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11(11):3887–95.
30. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nat Rev Immunol. 2008;8(6):467–77.
31. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271(5256):1734–6.
32. Hodi FS, O'Day SJ, McDermott D, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
33. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28(19):3167–75.
34. Cha E, Klinger M, Hou Y, Cummings C, Ribas A, Faham M, et al. Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. Sci Transl Med. 2014;6(238): 238ra70.
35. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med. 2015;373(2):123–35.
36. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2015;16(4):375–84.
37. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015;373(17):1627–39.
38. Motzer RJ, Escudier B, McDermott D, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373(19):1803–13.
39. Brahmer JR, Tykodi SS, Chow LQM, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455–65.
40. Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, et al. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother. 2010;33(8):828–33.
41. Patnaik A, et al. Phase I study of pembrolizumab (MK-3475; anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. Clin Cancer Res. 2015;21(19):4286–93.
42. O'Reilly EM, Oh D-Y, Dhani N, Renouf DJ, Lee MA, Sun W, et al. A randomized phase 2 study of durvalumab monotherapy and in combination with tremelimumab in patients with metastatic pancreatic ductal adenocarcinoma (mPDAC): ALPS study. J Clin Oncol. 2018;26(4_suppl):217.

43.•• Le DT, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357(6349):409–13 **In this landmark study, patients with refractory, metastatic cancers with mismatch repair deficiency were treated with PD-1 blockade with pembrolizumab. Fifty-three percent of patients had an objective response. This study led to the first FDA approval of tumor-agnostic therapy for pembrolizumab for these patients.**

44. *NCCN Guidelines for Patients: Pancreatic Cancer, Version 1.* National Comprehensive Cancer Network, 2017.

45. Marin-Acevedo JA, et al. Next generation of immune checkpoint therapy in cancer: new developments and challenges. J Hematol Oncol. 2018;11(1):39.

46. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature. 2017;545(7652):60–5.

47. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15(8):486–99.

48. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. Trends Immunol. 2015;36(4):265–76.

49. Lutz ER, Wu AA, Bigelow E, Sharma R, Mo G, Soares K, et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. Cancer Immunol Res. 2014;2(7):616–31.

50. Ward JP, Gubin MM, Schreiber RD. The role of neoantigens in naturally occurring and therapeutically induced immune responses to cancer. Adv Immunol. 2016;130:25–74.

51. Johnson BA 3rd, et al. Strategies for increasing pancreatic tumor immunogenicity. Clin Cancer Res. 2017;23(7):1656–69.

52. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther. 2010;18(4):843–51.

53. Lamers CH, Sleijfer S, van Steenbergen S, van Elzakker P, van Krimpen B, Groot C, et al. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol Ther. 2013;21(4):904–12.

54. Bryant KL, Mancias JD, Kimmelman AC, der CJ. KRAS: feeding pancreatic cancer proliferation. Trends Biochem Sci. 2014;39(2):91–100.

55. Keenan BP, Saenger Y, Kafrouni MI, Leubner A, Lauer P, Maitra A, et al. A Listeria vaccine and depletion of T-regulatory cells activate immunity against early stage pancreatic intraepithelial neoplasms and prolong survival of mice. Gastroenterology. 2014;146(7):1784–94 e6.

56. Tran E, Robbins PF, Lu YC, Prickett TD, Gartner JJ, Jia L, et al. T-cell transfer therapy targeting mutant KRAS in Cancer. N Engl J Med. 2016;375(23):2255–62.

57. Tran E, et al. Immunogenicity of somatic mutations in human gastrointestinal cancers. Science. 2015;350(6266):1387–90.

58. Wang QJ, Yu Z, Griffith K, Hanada K, Restifo NP, Yang JC. Identification of T-cell receptors targeting KRAS-mutated human tumors. Cancer Immunol Res. 2016;4(3):204–14.

59. Pandha H, Rigg A, John J, Lemoine N. Loss of expression of antigen-presenting molecules in human pancreatic cancer and pancreatic cancer cell lines. Clin Exp Immunol. 2007;148(1):127–35.

60. Jaffee EM, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol. 2001;19(1):145–56.

61. Le DT, et al. 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(7):382–9.

62. Macher BA, Galili U. The Galalpha1,3Galbeta1,4GlcNAc-R (alpha-gal) epitope: a carbohydrate of unique evolution and clinical relevance. Biochim Biophys Acta. 2008;1780(2):75–88.

63. Corporation NG, NewLink genetics announces results from phase 3 IMPRESS trial of algenpantucel-L for patients with resected pancreatic cancer. Acquire Media, 2016.

64. Lauer P, Chow MYN, Loessner MJ, Portnoy DA, Calendar R. Construction, characterization, and use of two Listeria monocytogenes site-specific phage integration vectors. J Bacteriol. 2002;184(15):4177–86.

65. Brockstedt DG, Giedlin MA, Leong ML, Bahjat KS, Gao Y, Luckett W, et al. Listeria-based cancer vaccines that segregate immunogenicity from toxicity. Proc Natl Acad Sci U S A. 2004;101(38):13832–7.

66. Le DT, Ko AH, Wainberg ZA, Picozzi VJ, Kindler HL, Wang-Gillam A, et al. Results from a phase 2b, randomized, multicenter study of GVAX pancreas and CRS-207 compared to chemotherapy in adults with previously-treated metastatic pancreatic adenocarcinoma (ECLIPSE Study). J Clin Oncol. 2017;35(4_suppl):345.

67. Muscarella P, Wilfong LS, Ross SB, Richards DA, Raynov J, Fisher WE, et al. A randomized, placebo-controlled, double blind, multicenter phase II adjuvant trial of the efficacy, immunogenicity, and safety of GI-4000 plus gem versus gem alone in patients with resected pancreas cancer with activating RAS mutations/survival and immunology analysis of the R1 subgroup. J Clin Oncol. 2012;30:e14501.

68. Finke LH, et al. Lessons from randomized phase III studies with active cancer immunotherapies–outcomes from the 2006 meeting of the Cancer Vaccine Consortium (CVC). Vaccine. 2007;25(Suppl 2):B97–B109.

69. Khong H, Overwijk WW. Adjuvants for peptide-based cancer vaccines. J Immunother Cancer. 2016;4:56.

70. Lynn GM, et al. In vivo characterization of the physicochemical properties of polymer-linked TLR agonists that enhance vaccine immunogenicity. Nat Biotechnol. 2015;33(11):1201–10.

71. Ramanathan RK, Lee KM, McKolanis J, Hitbold E, Schraut W, Moser AJ, et al. Phase I study of a MUC1 vaccine composed of different doses of MUC1 peptide with SB-AS2 adjuvant in resected and locally advanced pancreatic cancer. Cancer Immunol Immunother. 2005;54(3):254–64.

72. Palmer D, Dueland S, Juan V, Aksnes A-K. A phase I/II trial of TG01/GM-CSF and gemcitabine as adjuvant therapy for treating patients with resected RAS-mutant adenocarcinoma of the pancreas. J Clin Oncol. 2017;35(15 supplement):4119.

73. Nishida S, et al. Wilms tumor gene (WT1) peptide-based cancer vaccine combined with gemcitabine for patients with advanced pancreatic cancer. J Immunother. 2014;37(2):105–14.

74. Yamamoto K, et al. MUC1 peptide vaccination in patients with advanced pancreas or biliary tract cancer. Anticancer Res. 2005;25(5):3575–9.

75. Suzuki N, Hazama S, Iguchi H, Uesugi K, Tanaka H, Hirakawa K, et al. Phase II clinical trial of peptide cocktail therapy for patients with advanced pancreatic cancer: VENUS-PC study. Cancer Sci. 2017;108(1):73–80.

76.• Ott PA, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature. 2017;547(7662):217–21 **A main barrier to development of personalized vaccines has been due to cost and timeliness of sequencing and development. This trial demonstrated the feasibility of developing an immunogenic vaccine in a reasonable time period to administer to high-risk melanoma patients in the adjuvant setting.**

77. Carreno BM, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science. 2015;348(6236):803–8.

78. Tanyi JL, Bobisse S, Ophir E, Tuyaerts S, Roberti A, Genolet R, et al. Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. Sci Transl Med. 2018;10(436): eaao5931.
79. Kondo H, Hazama S, Kawaoka T, Yoshino S, Yoshida S, Tokuno K, et al. adoptive immunotherapy for pancreatic cancer using MUC1 peptide-pulsed dendritic cells and activated T lymphocytes. Anticancer Res. 2008;28(1B):379–87.
80. Shindo Y, Hazama S, Maeda Y, Matsui H, Iida M, Suzuki N, et al. Adoptive immunotherapy with MUC1-mRNA transfected dendritic cells and cytotoxic lymphocytes plus gemcitabine for unresectable pancreatic cancer. J Transl Med. 2014;12:175.
81. Kimura Y, Tsukada J, Tomoda T, Takahashi H, Imai K, Shimamura K, et al. Clinical and immunologic evaluation of dendritic cell-based immunotherapy in combination with gemcitabine and/or S-1 in patients with advanced pancreatic carcinoma. Pancreas. 2012;41(2):195–205.
82. Lepisto AJ, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis J, et al. 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(B):955–64.
83. Fesnak AD, June CH, Levine BL. Engineered T cells: the promise and challenges of cancer immunotherapy. Nat Rev Cancer. 2016;16(9):566–81.
84. Rosenberg SA, Tran E, Robbins PF. T-cell transfer therapy targeting mutant KRAS. N Engl J Med. 2017;376(7):e11.
85. June CH, et al. CAR T cell immunotherapy for human cancer. Science. 2018;359(6382):1361–5.
86. Iacobuzio-Donahue CA. Genetic evolution of pancreatic cancer: lessons learnt from the pancreatic cancer genome sequencing project. Gut. 2012;61(7):1085–94.
87. Makohon-Moore AP, Zhang M, Reiter JG, Bozic I, Allen B, Kundu D, et al. Limited heterogeneity of known driver gene mutations among the metastases of individual patients with pancreatic cancer. Nat Genet. 2017;49(3):358–66.
88. Jones S, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008;321(5897):1801–6.
89. Morris JPT, Wang SC, Hebrok M. KRAS, hedgehog, Wnt and the twisted developmental biology of pancreatic ductal adenocarcinoma. Nat Rev Cancer. 2010;10(10):683–95.
90. Haugk B. Pancreatic intraepithelial neoplasia-can we detect early pancreatic cancer? Histopathology. 2010;57(4):503–14.
91. 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(18):5423–34.
92. Witkiewicz A, Williams TK, Cozzitorto J, Durkan B, Showalter SL, Yeo CJ, et al. Expression of indoleamine 2,3-dioxygenase in metastatic pancreatic ductal adenocarcinoma recruits regulatory T cells to avoid immune detection. J Am Coll Surg. 2008;206(5): 849–54 discussion 854-6.
93. Munn DH, Mellor AL. IDO in the tumor microenvironment: inflammation, counter-regulation, and tolerance. Trends Immunol. 2016;37(3):193–207.
94. Sanford DE, et al. Inflammatory monocyte mobilization decreases patient survival in pancreatic cancer: a role for targeting the CCL2/CCR2 axis. Clin Cancer Res. 2013;19(13):3404–15.
95. Zhu Y, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res. 2014;74(18): 5057–69.
96. Jones SF, Bendell JC, Infante JR, Spigel DR, Thompson DS, Yardley DA, et al. A phase I study of panobinostat in combination with gemcitabine in the treatment of solid tumors. Clin Adv Hematol Oncol. 2011;9(3):225–30.
97. Jones SF, Infante JR, Spigel DR, Peacock NW, Thompson DS, Greco FA, et al. Phase 1 results from a study of romidepsin in combination with gemcitabine in patients with advanced solid tumors. Cancer Investig. 2012;30(6):481–6.
98. Kim K, Skora AD, Li Z, Liu Q, Tam AJ, Blosser RL, et al. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. Proc Natl Acad Sci U S A. 2014;111(32):11774–9.
99. Christmas BJ, Rafie CI, Hopkins AC, Scott BA, Ma HS, Cruz KA, et al. Entinostat converts immune-resistant breast and pancreatic cancers into checkpoint-responsive tumors by reprogramming tumor-infiltrating MDSCs. Cancer Immunol Res. 2018;6(12): 1561–77.
100. Kinkead HL, Hopkins A, Lutz E, Wu AA, Yarchoan M, Cruz K, et al. Combining STING-based neoantigen-targeted vaccine with checkpoint modulators enhances antitumor immunity in murine pancreatic cancer. JCI Insight. 2018;3(20):122857.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.