

## Immune checkpoint therapy for pancreatic cancer

Henrik Johansson, Roland Andersson, Monika Bauden, Sarah Hammes, Stefan Holdenrieder, Daniel Ansari

Henrik Johansson, Roland Andersson, Monika Bauden, Daniel Ansari, Department of Surgery, Clinical Sciences Lund, Lund University, and Skåne University Hospital, SE-221 85 Lund, Sweden

Sarah Hammes, Stefan Holdenrieder, Institute of Laboratory Medicine, German Heart Center at the Technical University Munich, 80331 Munich, Germany

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**Correspondence to:** Daniel Ansari, MD, PhD, Department of Surgery, Clinical Sciences Lund, Lund University, and Skåne University Hospital, SE-221 85 Lund, Sweden. [daniel.ansari@med.lu.se](mailto:daniel.ansari@med.lu.se)  
Telephone: + 46-46-2224672

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### Abstract

Novel treatment modalities are necessary for pancreatic

cancer. Immunotherapy with immune checkpoint inhibition has shown effect in other solid tumors, and could have a place in pancreatic cancer treatment. Most available clinical studies on immune checkpoint inhibitors for pancreatic cancer are not yet completed and are still recruiting patients. Among the completed trials, there have been findings of a preliminary nature such as delayed disease progression and enhanced overall survival after treatment with immune checkpoint inhibitors in mono- or combination therapy. However, due to small sample sizes, major results are not yet identifiable. The present article provides a clinical overview of immune checkpoint inhibition in pancreatic cancer. PubMed, ClinicalTrials.gov and American Society of Clinical Oncology's meeting abstracts were systematically searched for relevant clinical studies. Four articles, five abstracts and 25 clinical trials were identified and analyzed in detail.

**Key words:** Pancreatic cancer; Immune checkpoint inhibitors; Clinical trials

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**Core tip:** Immunotherapy is a rapidly expanding field within pancreatic cancer research. Here we summarize the effectiveness of immune checkpoint inhibition in the treatment of pancreatic cancer, focusing on the anti-tumor response and toxicity of drugs targeting cytotoxic T lymphocyte antigen 4, programmed cell death 1 and programmed cell death ligand 1. Based on the results from small series it appears that immune checkpoint inhibitors may be safe and effective, but still little published evidence is available to prove or disprove the clinical benefit of these drugs in patients with pancreatic cancer. Several well-designed clinical trials are ongoing and the results from these trials are eagerly awaited.

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## INTRODUCTION

The human immune system is an elaborative biological system of cellular interactions, structures and processes that have been evolved to protect the body from foreign substances. In diseases such as cancer, understanding the role of the immune system in disease development and progression has resulted in important insights.

Recent research attempts aimed at utilizing the immune system for cancer therapy have shown promising results in cancer elimination. It is now known that the innate and the adaptive immune system recognizes tumor-specific antigens such as neoantigens (derivatives associated with carcinogenesis mutation) and oncogenic virus derivatives, in order to act against cancers in a process referred to as tumor immune surveillance<sup>[1]</sup>. However, the tumor modifies the human immune system to avoid detection, both locally and systematically. A novel insight in addressing the challenge has been found in the concept of immune checkpoints.

An integral function of the immune system is its ability to differentiate between self and non-self. For this purpose the immune system depends on multiple "checkpoints", which are molecules on certain immune cells that need to be activated or inactivated to start an immune reaction. Tumor cells often take advantage of these checkpoints to avoid being detected and attacked by the immune system. Checkpoint inhibitors have been investigated as a novel mode of cancer treatment<sup>[2]</sup>.

Immunotherapy is a treatment modality that encompasses a wide and varied range of techniques. In cancer related cases, these often consist of the use of vaccines, cytokines and monoclonal antibodies that stimulate the human immune system in general or target specific cells. The potential benefits with immunotherapy, compared to other approaches, is its ability to detect specific tumor cells, creating a durable response and a much better survival-prognosis in cancer patients<sup>[3]</sup>. Unfortunately, it has been a therapy with little success in solid tumors, especially in pancreatic ductal adenocarcinoma (PDA)<sup>[4]</sup>.

PDA is a highly aggressive malignancy, characterized by delayed diagnosis and treatment resistance<sup>[5]</sup>. At the time of clinical detection, most cancers are either locally advanced, or metastatic, *i.e.*, ineligible for surgical resection and with a five-year survival in the single digits<sup>[6]</sup>. One of the reasons for the poor effect of treatment is the ability of PDA to evade host immune surveillance<sup>[1,7]</sup>. The tumor microenvironment of PDA is composed of a dense fibrotic stroma of extracellular matrix components and a variety of inflammatory cells<sup>[6]</sup>. On one level, there are infiltrating immunosuppressive

cells, such as regulatory T cells (Tregs), myeloid-derived suppressive cells (MDSCs), tumor-associated macrophages (TAMs), transmitting cancer-inflammatory signals that hinder the immunologic cell activity of cytotoxic T cells (CTLs) and natural killer cells (NKs)<sup>[1,4]</sup>. On another level, the tumor cells avoid detection, in several ways: by the use of immunosuppressive factor secretion, such as interleukin (IL)-10, vascular endothelial growth factor, downregulation of major histocompatibility complex (MHC) class I presentation and using contact-dependent factors, such as immune checkpoint molecules, resulting in immunosuppression and tumor progression<sup>[1,4]</sup>. PDA has been considered a non-immunogenic cancer. However, four subtypes of PDA were recently reported. One subtype, containing upregulated immune networks with tumor infiltrating cells (TILs) including CD4<sup>+</sup> and CD8<sup>+</sup> T-cells and immune checkpoint molecule expression in its tumor microenvironment, was defined as immunogenic<sup>[8]</sup>.

The existing treatment modalities, including surgical resection and conventional chemotherapies, prolong survival but fail to cure the disease. New novel treatment modalities are needed<sup>[4,6]</sup>. Immunotherapy with immune checkpoint inhibition has shown effect in other solid tumors. It could also have a place in PDA treatment. This review aims to discuss the current development status of and future challenges in utilizing immune checkpoint inhibitors for PDA, with focus on cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1), three immune checkpoints with current clinical information.

## LITERATURE SEARCH

A systematic literature search on immune checkpoint therapy of pancreatic ductal adenocarcinoma was conducted. Results of articles, abstracts and clinical trials from the United States National Library of Medicine's PubMed database, from American Society of Clinical Oncology's (ASCO) Gastrointestinal Cancers Symposia and from the United States National Institutes of Health Clinical Trials were obtained. The search was conducted in March 2016. Articles were found through the mesh-term "Carcinoma, Pancreatic Ductal", combined with "immune checkpoint therapy"; the mesh-term "Pancreatic Neoplasms", combined with "immune checkpoint therapy"; "Ipilimumab", "CTLA 4", "PD-L1", "PD-1"; the mesh-term "Carcinoma, Pancreatic Ductal/therapy" combined with the mesh-term "Carcinoma, Pancreatic Ductal/immunology"; the term "pancreatic neoplasm" and "pancreas cancer" were both combined with "Immune therapy"; the term "pancreatic cancer" was combined with "Ipilimumab", "anti PDL1", "anti PD1", "anti CTLA4" and further restricted to studies based on humans and to articles published in English. The Boolean operator "AND" was used throughout the whole search. Additional

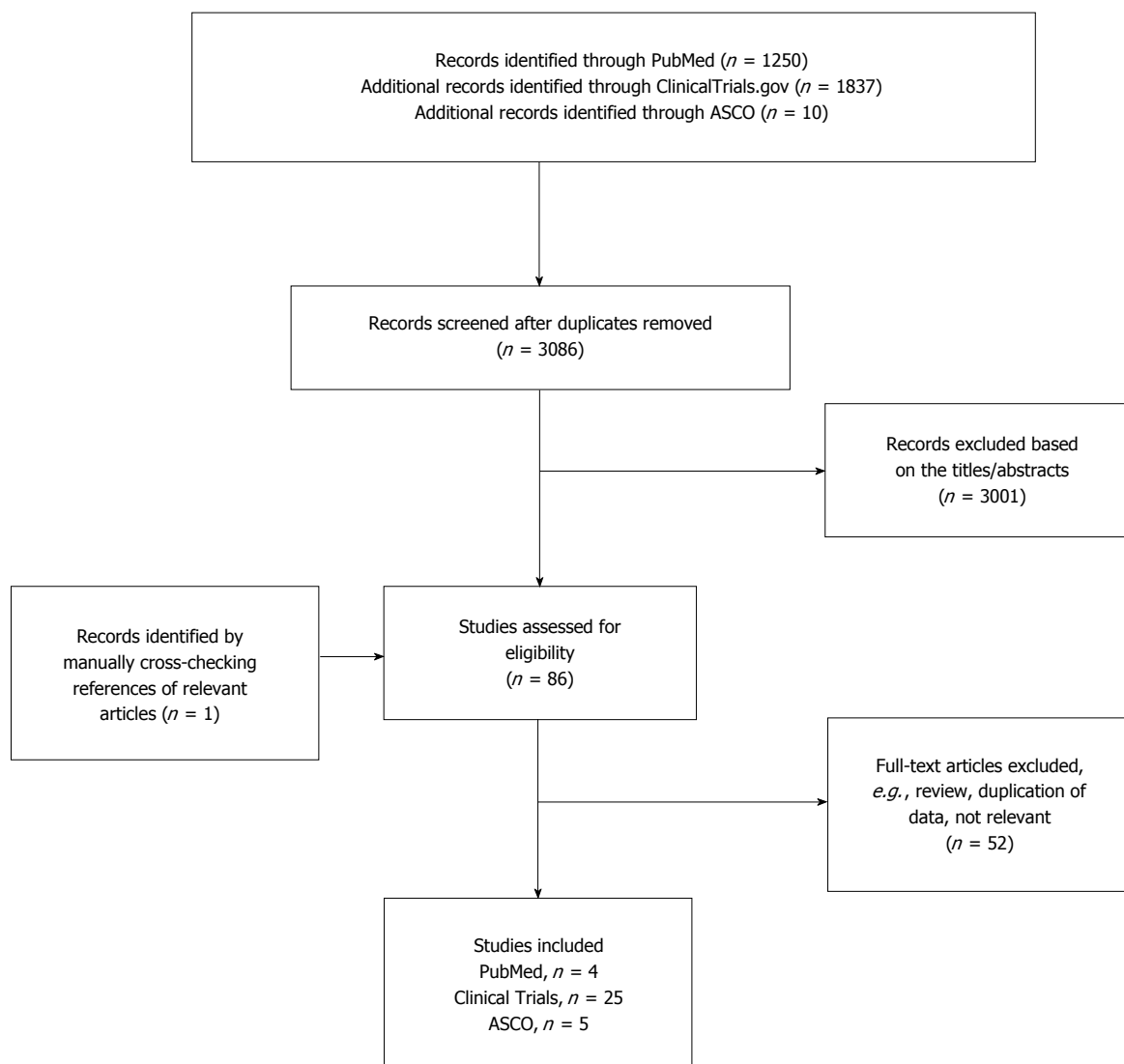


Figure 1 Flow diagram of article selection. ASCO: American Society of Clinical Oncology's.

articles were retrieved by manually cross-checking bibliographies of relevant articles. The search strategy is shown in Figure 1. Abstracts from Multidisciplinary Treatment, Translational Research of Cancer of the Pancreas, Small Bowel and Hepatobiliary Tract from the 2009-2016 Gastrointestinal Cancer Symposium were studied. Ongoing or completed clinical trials based on the question formulation were found through the condition: "Pancreatic Neoplasms". Identified duplicates of the same study, when searched in the different databases, were removed. One reviewer (Johansson H) conducted the study selection and data extraction. A second reviewer (Ansari D) independently checked data for omissions or inaccuracies. Extracted information for each study included study characteristics, intervention, comparison, outcome and adverse events. The data was tabulated and narratively synthesized.

database, three articles were selected and one was further retrieved by manually cross-checking bibliographies of relevant articles. In total, four articles were included. An additional 1837 records were identified through United States National Institutes of Health Clinical Trials, resulting in another 25 references. Furthermore, five out of 10 abstracts from "Multidisciplinary Treatment, Translational Research of Cancer of the Pancreas, Small Bowel and Hepatobiliary Tract" from the 2009-2016 Gastrointestinal Cancer Symposiums met the criteria for this study and were therefore selected. Results from clinical trials are presented in Tables 1-3. Results from the meeting abstracts and articles are presented in Tables 4 and 5, respectively. An overview of the checkpoint molecules, their potential expression and interaction and their targeting drugs are presented in Figure 2.

## RESULTS

Out of 1250 identified search records in the PubMed

## CTLA-4

CTLA-4 is a molecule expressed and upregulated on

Table 1 Clinical trials on cytotoxic T lymphocyte antigen 4

Target molecule	Drug name	Study phase	Study design	Status	Condition	Intervention	Cohort	Estimated enrollment	Dose	End point	Study Arm	Adverse Response event	Survival	Ref.
CTLA-4	Ipilimumab (BMS-734016/MDX-010)	1	NA	Active	PC	Palliative	Locally advanced	28	DE	Safety (MTD, DLT)	Ipilimumab, Gemcitabine	NA	NA	NCT01473940
		2	Randomized	Not recruiting	PC	Palliative	Metastatic	92	3 mg/kg	Efficacy (RR, PFS, OS) Safety (AE)	Ipilimumab, GVAX (Arm A) FOLFIRINOX (Arm B)	NA	NA	NCT01896869
	Tremelimumab (CP-675/CP-675,206)	2	NA	Recruiting	PC, BC, BC	Palliative	Metastatic	77	NA	Efficacy (OS, PFS, ORR, DoR) Safety	Tremelimumab (Arm A) Durvalumab (Arm B)	NA	NA	NCT02527434
		2	Randomized	Recruiting	PC	Palliative	Metastatic	130	NA	Safety Efficacy (ORR, DoR, DCR, PFS)	Tremelimumab, Durvalumab (Arm C) Durvalumab (Arm A) Durvalimab, Tremelimumab (Arm B)	NA	NA	NCT02558894
		1	Randomized	Recruiting	PC, NSCLC, HNSCC	Palliative	Locally advanced	108	NA	Safety (AE, DLT) Antidrug Antibody Presence	Durvalumab, Mogamulizumab (Arm A)	NA	NA	NCT02301130
		1	Non-randomized	Recruiting	PC, NSCLC, BC, MM	Palliative	Metastatic	30	1 mg/kg	Safety (AE) Efficacy	Mogamulizumab (Arm B) Durvalumab, Tremelimumab, Radiotherapy	NA	NA	NCT02639026

ORR: Overall response rate; DLT: Dose limiting toxicity; AE: Adverse event; MTD: Maximum tolerance dose; NA: Not available.

activated CD4<sup>+</sup>, CD8<sup>+</sup> T cells and T-regulatory FOXP3<sup>+</sup>, CD4<sup>+</sup>, CD25<sup>+</sup> cells (Tregs)<sup>[9,10]</sup>. It is a member of the costimulatory B7 family of receptor signals (homolog to the CD28 receptor) critical in regulation and development of T-cells in the adaptive immune response<sup>[11]</sup>. Similar to CD-28, CTLA-4 binds to APCs ligands B7.1 (CD80) and B7.2 (CD86) after MHC-TCR binding in APC-T-cell-interaction<sup>[12,13]</sup>. CTLA-4 binds to these ligands competitively with a higher affinity than CD28<sup>[13]</sup>.

Triggering of CTLA-4 result in downregulation of immune response and maintaining of the peripheral resistance by inhibiting co-stimulation and dephosphorization of the MHC-TCR-interaction. CTLA-4 does this through activation of protein phosphatases, PP2A and SHP-2 and removal of CD80 and CD86 ligands on APC surface<sup>[14-16]</sup>. This is in contrast to its homologous Ig-member, CD-28, which potentiates immune response by T cell proliferation, activation, differentiation, cell migration and preventing T cell apoptosis<sup>[16-18]</sup>.

The main function of the molecule is to suppress immune responses by Tregs and to downregulate effector T cells<sup>[19]</sup>. This immunosuppressive function of CTLA-4 is especially prominent in the tumor microenvironment of PDA<sup>[20]</sup>.

Blockade of CTLA-4 has two contradictory effects. It can result in immune response progression with effector T cell enhancement, Treg depletion and consequent

Table 2 Clinical trials on programmed cell death 1

Target molecule	Drug name	Study phase	Study design	Status	Condition	Intervention	Cohort	Estimated enrollment	Dose	End point	Study Arm	Adverse event	Response	Survival	Ref.
PD-1	Nivolumab (BMS-936558/MDX-1106/ONO-4538)	1 2	Randomized	Recruiting	PC	Neoadjuvant Adjuvant	Resectable	50	3 mg/kg	Safety (IRAEs, OS, DFS) Median-[IL17A] in Vaccine-induced Lymphoid Aggregates	Cy/GVAX (Arm A) Cy/GVAX, Nivolumab (Arm B)	NA	NA	NA	NCT02451982
						Palliative	Metastatic	49	NA	Safety	Nivolumab, Temsirolimus (Arm A) Nivolumab, Irinotecan (Arm B)	NA	NA	NA	NA
		1	Non-randomized	Recruiting	PC, NSCLC, CrC, CrC, EC, UC	Palliative	Locally advanced	270	3 mg/kg	Safety (AE) Efficacy (OS; mOS, oYOS; DOR, PFS, ORR; CR, PR) Tolerability	Phase 1a: FPA008 (Arm A) FPA008, Nivolumab (Arm B) Phase 1b: MTD/RD FPA008, Nivolumab	NA	NA	NA	NCT02526017
		1	Non-randomized	Recruiting	PC, OC, BC, CrC, RCC, MM, PrC, NSCLC	Palliative	Locally advanced Metastatic	300	3 mg/kg	Immunogenicity PDA biomarkers Safety Tolerability PK	DE AM0010 (Arm A) DE AM0010, Paclitaxel/Docetaxel, Carboplatin/Cisplatin (Arm B) DE AM0010, FOLFOX, (Arm C) DE AM0010, gemcitabine/nab-paclitaxel (Arm D) DE AM0010, Capecitabine (Arm E) DE AM0010, Paclitaxel (Arm F) DE AM0010, Pazopanib (Arm G) DE AM0010, Pembrolizumab (Arm H) DE AM0010, Nivolumab (Arm I) DE AM0010, Gemcitabine/carboplatin (Arm J)	NA	NA	NA	NCT02009449

Pembrolizumab (MK-3475/SCH 900475)	2	NA	Not recruiting	PC	Palliative	Locally advanced	54	200 mg	Safety (IRAEs) Efficacy (DMFS, OS, LPFS)	Cy/GVAX, Pembrolizumab, SBRT	NA	NA	NA	NCT02648282
	2	Randomized	Active	PC	Palliative	Locally advanced Metastatic	76	NA	Safety (TEAE)	ACP-196 (Arm A)	NA	NA	NA	NCT02362048
	1   2	Randomized	Not recruiting	PC	Neoadjuvant	Resectable Borderline resectable	56	200 mg	Efficacy Safety (DLT) Efficacy (DFS, OS, RR)	ACP-196, Pembrolizumab (Arm B) Pembrolizumab, Capecitabine, Radiotherapy	NA	NA	NA	NCT02305186
	1   2	Non-randomized	Recruiting	PC, SCLC, OC, BC, Sarcoma	Palliative	Metastatic	90	2 mg/kg	[TILs] Safety (AE) Efficacy (ORR, OS, PFS) RD	Pembrolizumab, Gemcitabine (Arm A) Pembrolizumab, Gemcitabine, Docetaxel (Arm B) Pembrolizumab, Gemcitabine, Nab-paclitaxel (Arm C) Pembrolizumab, Gemcitabine, Vinorelbine (Arm D) Pembrolizumab, Irinotecan (Arm E)	NA	NA	NA	NCT02331251
	1	Non-randomized	Recruiting	PC	Palliative	Locally advanced Metastatic	50	200 mg	Safety	Pembrolizumab, Liposomal, Doxorubicin (Arm F) Pembrolizumab, Defactinib, Gemcitabine	NA	NA	NA	NCT02546531
	1	NA	Active Not recruiting	PC, RC, NSCLC, BIC, ASN, RCC, CC, HCC, BC, MM, HNSCC, Sarcoma	Palliative	Metastatic	12	NA	Efficacy Safety Efficacy (Clinical Response) Tolerability	Pembrolizumab, p53MVA	NA	NA	NA	NCT02432963
	2	Non-randomized	Suspended	PC, ChC, GeC, CrC, HCC	Palliative	Metastatic	290	2 mg/kg	Safety Efficacy	Pebrolizumab, Young TIL, Aldesleukin, Cyclophosphamide, Fludarabine	NA	NA	NA	NCT01174121
	1	NA	Recruiting	PC	Palliative	Locally advanced Metastatic	9	2 mg/kg	Safety (DLT)	Pembrolizumab, Reolysin, Gemcitabine/ Irinotecan/ Leucovorin with 5-FU	NA	NA	NA	NCT02620423
	1   2	NA	Recruiting	PC, ChC, GeC, CrC	Palliative	Locally advanced Metastatic	128	DE	Efficacy (ORR, PFS, OS) Safety Efficacy (RR, PFS, OS)	Pembrolizumab, mFOLFOX6, Celecoxib	NA	NA	NA	NCT02268825



1	Non-randomized	Recruiting	PC, OC, BC, CrC, RCC, MM, PrC, NSCLC	Palliative	Locally advanced Metastatic	300	3 mg/kg	Safety	DE AM0010 (Arm A)	NA	NA	NA	NCT02009449
								Tolerability	DE AM0010, Paclitaxel/Docetaxel, Carboplatin/Cisplatin (Arm B)				
								PK	DE AM0010, FOLFOX, (Arm C)				
									DE AM0010, gemcitabine/nab-paclitaxel (Arm D)				
									DE AM0010, Capecitabine (Arm E)				
									DE AM0010, Paclitaxel (Arm F)				
									DE AM0010, Pazopanib (Arm G)				
									DE AM0010, Pembrolizumab (Arm H)				
									DE AM0010, Nivolumab (Arm I)				
2	NA	Suspended	PC	Adjuvant	Resectable	29	3 mg/kg	Safety	DE AM0010, Gemcitabine/carboplatin (Arm J)	NA	NA	NA	NCT01313416
Pdilizumab (CT-011)								Efficacy (Median DFS)	Pdilizumab, Gemcitabine				
1	Non-randomized	Withdrawn	PC, OC, BC, CC, Sarcoma	Palliative	NA	0	DE	Safety	Pdilizumab, p53 Vaccine	NA	NA	NA	NCT01386502
								Efficacy					

AE: Adverse event; NA: Not available.

tumor reduction. However, there is also a risk of autoimmunity development<sup>[19-21]</sup>.

Currently, two human anti-CTLA-4-antibodies, Ipilimumab and Tremelimumab have been approved for use in cancer treatment.

**Ipilimumab**

Ipilimumab (BMS-734016, MDX-010) is a humanized monoclonal IgG1 immunoglobulin antibody, developed against CTLA-4-molecules on T cells. It binds to CTLA-4 and prevents T cell suppression by the inhibitory immune checkpoints, resulting in a cytotoxic T-lymphocyte antitumor-mediated immune response<sup>[22]</sup>.

In PDA, a phase II trial, of Ipilimumab was conducted by Royal *et al*<sup>[23]</sup> for 27 patients with locally advanced or metastatic disease. A 3 mg/kg single dosage was administered each third week with 4 doses per course. It did not demonstrate any objective response according to the standard response evaluation criteria in solid tumors (RECIST). However, delayed progression in one patient was noted with radiographic response in both the primary tumor and the metastatic tumors. Three patients reported episodes of colitis, encephalitis, hypophysitis, grade 3-4 immune-related adverse events (irAEs), with one resulting in treatment-related death.

Preclinical data suggest synergetic effects of Ipilimumab when combined with "GVAX"; an immune response stimulating, granulocyte macrophage colony stimulating factor (GM-CSF) gene transfected tumor-cell vaccine. In a two-armed, randomized, phase I study of 30 patients with advanced PDA, Le *et al*<sup>[24]</sup> reported greater overall survival in patients treated with Ipilimumab and GVAX compared to patients treated with Ipilimumab alone. A 10 mg/kg single dosage of Ipilimumab was administered

Table 3 Clinical trials on programmed cell death ligand 1

Target molecule	Drug name	Study phase	Study design	Status	Condition	Intervention	Cohort	Estimated enrollment	Dose	End point	Study Arm	Adverse event	Response	Survival	Ref.
PD-L1	Durvalumab (MED)4736	2	Non-randomized	Not recruiting	PC, GeC, OC, NSCLC, BC, RCC, CrC	Palliative	Metastatic	136	750 mg > 30 kg, 10 mg/kg < 30 kg	Safety Efficacy (ORR, PFS, OS)	Durvalumab	NA	NA	NA	NCT02669914
		2	NA	Recruiting	PC, BIC, BC	Palliative	Metastatic	77	NA	Safety	Tremelimumab (Arm A)	NA	NA	NA	NCT02527434
		1	Non-randomized	Recruiting	PC, HNSCC, MM, CrC, BC, CEC	Palliative	Locally advanced Metastatic	40	NA	Safety (AE)	Tremelimumab, Durvalumab (Arm C)	NA	NA	NA	NCT02586987
		2	Randomized	Recruiting	PC	Palliative	Metastatic	130	NA	Efficacy (ORR, BoR, DoR) Tolerability PK	Selumetinib	NA	NA	NA	NCT02558894
		1 2	Non-randomized	Recruiting	PC	Palliative	Metastatic	26	NA	Safety (DLT)	Durvalumab, nab-Paclitaxel	NA	NA	NA	NCT02583477
		1	Randomized	Recruiting	PC, NSCLC, HNSCC	Palliative	Locally advanced Metastatic	108	NA	Efficacy (ORR, DoR, DCR, PFS) PK	Gencitabine (Arm A) Durvalumab, AZD5069 (Arm B)	NA	NA	NA	NCT02301130
		1	Non-randomized	Recruiting	PC, NSCLC, BC, MM	Palliative	Metastatic	30	1 mg/kg	Safety (AE) Efficacy	Durvalumab, Mogamulizumab (Arm A) Tremelimumab, Mogamulizumab (Arm B)	NA	NA	NA	NCT02639026

NA: Not available.



Table 4 Immune checkpoint inhibitors based on American Society of Clinical Oncology's meeting abstracts

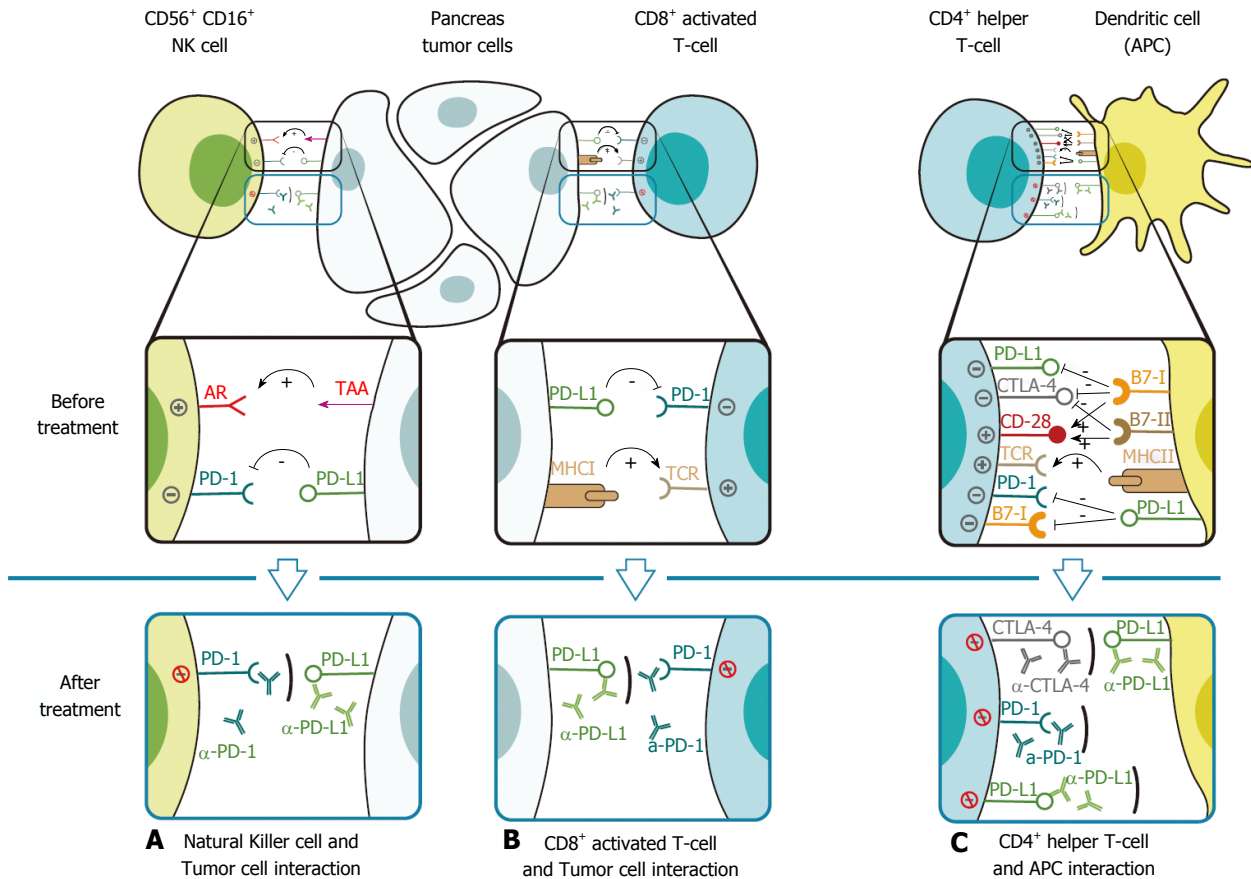
Target molecule	Drug name	Study phase	Study design	Status	Condition	Intervention	Cohort	Estimated enrollment	Dose	End point	Study Arm	Adverse event	Response	Survival	Ref.
CTLA-4	Tremelimumab (CP-675/CP-675,206)	1	Non-randomized	Recruiting	PC	Palliative	Metastatic	60	NA	Safety (ORR, PFS) Tolerability	Durvalumab, SBRT (Arm A) Tremelimumab, SBRT (Arm B) Durvalumab, Tremelimumab, SBRT (Arm C)	NA	NA	NA	Duffy <i>et al</i> <sup>[28]</sup>
PD-1	Nivolumab (BMS-936558/MDX-1106/ONO-4538)	2	Randomized	Recruiting	PC	Palliative	Metastatic	94	3 mg/kg	PK Safety (IRI)	Cy/GVAX/Nivolumab, CRS-207/Nivolumab (Arm A) Cy/GVAX, CRS-207 (Arm B)	NA	NA	NA	Le <i>et al</i> <sup>[25]</sup>
PD-L1	Pembrolizumab (MK-3475/SCH 900475)	1   2	Non-randomized	Recruiting	PC, NSCLC, BC	Palliative	Locally advanced Metastatic	138	3 mg/kg	Safety (DLT, TEAEs) Efficacy (PFS, OS, DCR, ORR, DR)	Nivolumab, nab-Paclitaxel (Arm A) Nivolumab, nab-Paclitaxel, Gemcitabine (Arm B) Part A: Pembrolizumab, DE Pexidartinib (PLX3397) Part B: RD Pexidartinib (PLX3397), Pembrolizumab	NA	NA	NA	Wainberg <i>et al</i> <sup>[50]</sup>
PD-L1	Durvalumab (MEDI4736)	1	Non-randomized	Recruiting	PC	Palliative	Metastatic	60	NA	Safety (ORR, PFS) Tolerability	Durvalumab, SBRT (Arm A) Tremelimumab, SBRT (Arm B) Durvalumab, Tremelimumab, SBRT (Arm C)	NA	NA	NA	Duffy <i>et al</i> <sup>[51]</sup>
PD-L1	Ibrutinib	1   2	NA	Recruiting	PC, NSCLC, mBC	Palliative	Metastatic	160	NA	PK Safety (AE, DLT) Efficacy Tolerability	Part 1: Durvalumab, DLT Ibrutinib Part 2: Durvalumab, RD Ibrutinib	NA	NA	NA	Borazanci <i>et al</i> <sup>[74]</sup>

ORR: Overall response rate; DLT: Dose limiting toxicity; NA: Not available.

Table 5 Immune checkpoint inhibitors based on articles

Target molecule	Drug	Study phase	Study design	Condition	Intervention	Cohort	Enrollment	Dose	End point	Study Arm	Adverse event	Response	Survival	Ref.
CTLA-4	Ipilimumab (BMS-734016/ MDX-010)	2	Non-randomized	PC	Palliative	Locally advanced Metastatic	27	3 mg/kg	Efficacy (ORR, CR, PR)	Ipilimumab	11% Grade 3-4 irAEs (Colitis: 1, Encephalitis: 1, Hypophysitis: 1)	No RECIST-response SD: delayed progression, RECIST-progressive disease (n:1) SD: growth < 20% growth cut-off, 7w (n:1), 22w (n:1) (Arm A) regression 17w (n:1), stabilization 59w (n:1), 71w (n:1) (Arm B)	NA	Royal <i>et al</i> <sup>[23]</sup>
		1	Randomized	PC	Palliative	Locally advanced	30	10 mg/kg	Safety (AE)	Ipilimumab (Arm A)	73%, 80% irAEs (Arm A, B)	mOS (95%CI): IPI vs IPI, GVAX: 3,6 (2,5-9,2), 5,7 (4,3-14,7); HR = 0,51, 95%CI: 0,23-1,08, P = 0,072.	Le <i>et al</i> <sup>[24]</sup>	
						Metastatic			Efficacy (OS, ORR)	Ipilimumab, GVAX (Arm B)	20% Grade 3-4 irAEs (Colitis: 1, GBS: 1, Nephritis: 1) (Arm A), (Rash: 1, Colitis: 1, Pneumonitis: 1) (Arm B) irAEs (p: 0.037)			
	Tremelimumab (CP-675/ CP-675,206)	1	Non-randomized	PC	Palliative	Metastatic	34	15 mg/kg	Safety (AE, DLT, MTD) Efficacy (OS, OR, PFS)	Tremelimumab DE (C6, C10, C15), Gencitabine	Grade 3-4 irAEs (Asthenia: 1, Nausea: 1, Diarrhea: 1, Anemi: 1, Pruritus: 1, Hypertransaminasemia: 1) (C 10), (Asthenia: 3, Nausea: 2, Diarrhea: 1, Anemi: 1, Neutropenia: 2, Hypertransaminasemia: 1, Thrombocytopenia: 2) (C 15) SAE: 11 (Dehydration-diarrhea: 1, ACS: 1, PE: 1, Hyperbilirubinemia: 1, Hematemesis: 1) (C10) (AKI: 1, GIB: 1, Hyperbilirubinemia: 2) (C15)	PR: 8w (n:2) (10,5%: 2/19) SD: > 10w [n:7 (n:2 completed study)]	mOS (95%CI): C6 (6 mg/kg Tremelimumab), C10 (10 mg/kg Tremelimumab), C15 (15 mg/kg Tremelimumab): 5,3 (1,2-14,6), 8,0 (2,3-16,9), 7,5 (6,0-9,5)	Aglietta <i>et al</i> <sup>[25]</sup>
PD-L1	BMS-936559	1	Non-randomized	PC, NSCLC, MM, CrC, OC, GeC, RRC, BC	Palliative	Locally advanced	207 (14 PDA)	DE	Safety (AE, MTD, DLT)	BMS-936559	NA	No objective PDA-response	NA	Brahmer <i>et al</i> <sup>[26]</sup>
						Metastatic			Efficacy (ORR) PK					

PDA: Pancreatic ductal adenocarcinoma; MTD: Maximum tolerance dose; ORR: Overall response rate; DLT: Dose limiting toxicity; NA: Not available; CTLA-4: Cytotoxic T lymphocyte antigen 4; PD-L1: Programmed cell death ligand 1.



**Figure 2 Check-point inhibition.** A: Natural killer cell (NK cell) interacts with a PDA cell and gets activated through the binding of its activating receptor (AR) with PDA's tumor associated antigen (TTA). Tolerance occurs when the programmed-death-receptor (PD-1) molecule on the NK cell interacts with its ligand (PD-L1) on the PDA cell. Treatment with monoclonal antibody to bind these inhibitory proteins such as either  $\alpha$ -PD-1 (Nivolumab, Pembrolizumab, Pidilizumab) or  $\alpha$ -PD-L1 (Druvalumab, BMS-936559) prevents this interaction; B: CD8<sup>+</sup> T cell interacts with a PDA cell and gets activated through the binding of its T cell receptor (TCR) with PDA's major histocompatibility complex (MHC). Tolerance occurs when the PD-1 molecule on the NK cell interacts with PD-L1 on the PDA cell. Treatment with monoclonal antibody to bind these inhibitory proteins such as either  $\alpha$ -PD-1 (Nivolumab, Pembrolizumab, Pidilizumab) or  $\alpha$ -PD-L1 (Druvalumab, BMS-936559) prevents this interaction, resulting in suppression of T cell tumor attack; C: CD4<sup>+</sup> helper T cell interacts with an antigen presenting cell (APC) through the binding of its TCR with APC'sMHC that present TAA. The co-stimulatory APC-signal-binding is induced by the CD28-B7-I/II interaction. The inhibitory checkpoint molecules: PD-L1, PD-1 and CTLA-4 are either presented before CD4<sup>+</sup> activation or upregulated after activation and might result in inhibition and anergy of the helper T cell via PD-L1-PD-1, PD-L1-B7-1 and/or CTLA-4-B7-1/II interactions. Treatment with monoclonal antibody to bind these inhibitory proteins such as either  $\alpha$ -PD-1 (Nivolumab, Pembrolizumab, Pidilizumab),  $\alpha$ -PD-L1 (Druvalumab, BMS-936559) or  $\alpha$ -CTLA-4 (Ipilimumab, Tremelimumab) can prevent this interaction, thus maintain antitumor activity.

in Arm A and a 10 mg/kg dosage of Ipilimumab with GVAX was administered in Arm B. The results met the criteria for stable disease (SD) according to RECIST in four patients (Arm A, B) and one further according to the immune-related response criteria (irRC) (Arm B). In treatment Arm A, two patients with constant disease progression reported SD for seven and 22 wk. In treatment Arm B, three patients reported SD, with 17 wk of regression in one, 59 wk of stabilization in one and till week 71 in another. Median overall survival was reported as 3.6 months (95%CI: 2.5-9.2) and 5.7 mo (95%CI: 4.3-14.7), (HR = 0.51, 95%CI: 0.23-1.08,  $P = 0.072$ ), and one-year overall survival to 7% (1%-45%), and 27% (11%-62%), in Arm A and in Arm B respectively. Furthermore, 73% vs 80% of the patients reported irAEs in Arm A and Arm B. In total 20% grade 3-4 irAEs were reported, including three episodes of colitis, Guillain-Barre syndrome and

nephritis (Arm A) and three episodes of colitis, rash and pneumonitis (Arm B). According to Le *et al.*<sup>[24]</sup> these rates were similar to previous studies testing Ipilimumab 10 mg/kg. The conclusion was that even though insufficient data was conducted to conclude whether Ipilimumab alone, or in combination therapy result in better clinical outcome, combination therapy of Ipilimumab and GVAX had the potential for efficacy improvement in patients with longer life expectancy and that immune checkpoint therapy should start early in the PDA treatment course.

A phase II multicenter study of Ipilimumab and GVAX treatment in metastatic PDA patients is ongoing in patients who have been earlier treated with FOLFIRINOX-chemotherapy (Arm A) or patients continuously treated with FOLFIRINOX (Arm B) (NCT01896869). Survival comparison between continuous FOLFIRINOX-treatment vs FOLFIRINOX

followed by Ipilimumab and GVAX is the primary end point<sup>[25]</sup>.

NCT01473940 is another clinical trial studying safety and efficacy of Ipilimumab, when administered together with the cytostatic Gemcitabine hydrochloride, in locally advanced, or metastatic, unresectable PDA patients. Researchers in this trial are hypothesizing the synergetic effect of this combination<sup>[26]</sup>.

### **Tremelimumab**

Tremelimumab (CP-675, CP-675,206) is a humanized monoclonal IgG2 immunoglobulin antibody also developed against CTLA-4-molecules on T-cells. It binds to CTLA-4 and inhibits immune checkpoint mediated T-cell suppression, resulting in cytotoxic T-lymphocyte antitumor-mediated immune response<sup>[22]</sup>.

In a non-randomized, dose increasing, phase I study of Tremelimumab and Gemcitabine hydrochloride for 34 patients with advanced PDA, a partial response in two patients was demonstrated. The patients received a 15 mg/kg Tremelimumab treatment-cycle, consisting of one dose of Tremelimumab each 84<sup>th</sup>-day along with up to twelve doses of Gemcitabine hydrochloride 1000 mg/m<sup>2</sup> for three weeks followed with one week of rest. Stable disease over ten weeks was reported in seven patients, where two patients completed the study. Median overall survival was 7.4 mo (95%CI: 5.8-9.4), with 7.5 mo (95%CI: 6.0-9.5) in the Tremelimumab 15 mg/kg study-arm. However, Aglietta *et al*<sup>[27]</sup> did not demonstrate any objective response according to RECIST. Also the confidence interval was wide and overlapped due to limited participants in the respective study-arms. Moreover, the most common grade 3-4 adverse events were asthenia (11.8%), and nausea (8.8%).

In metastatic PDA, a three-armed, phase I, non-randomized, clinical trial testing Tremelimumab alone or in combination with Durvalumab (another immune checkpoint inhibitor discussed later) together with stereotactic body radiation therapy (SBRT) is conducted. The primary objectives are to determine safety, tolerability and efficacy of immune checkpoint therapy together with SBRT. SBRT is expected to enhance Durvalumab's and/or Tremelimumab's immune checkpoint inhibitory effects resulting in systemic antitumor effects<sup>[28]</sup>.

Tremelimumab is currently being tested for safety and efficacy in two randomized phase I and II trials for locally advanced and metastatic PDA, together with MORGALIZUMAB [a monoclonal antibody against C-C chemokine receptor 4 (CCR4) (NCT02301130)] and together with Durvalumab (NCT02558894)<sup>[29,30]</sup>.

NCT02639026 is a phase I, non-randomized trials, that studies the safety and efficacy of Tremelimumab and Durvalumab in combination with hypofractionated radiation therapy in patients with metastatic PDA<sup>[31,32]</sup>.

NCT02527434 is a phase II clinical trial studying safety and efficacy of Tremelimumab alone, or

combined with Durvalumab, in patients with metastatic PDA<sup>[33]</sup>.

### **PD-1**

PD-1 is an immune checkpoint molecule expressed on activated T cells, B cells, NK cells, monocytes and DCs<sup>[34]</sup>. Like CTLA-4, PD-1 is a member of the CD28 family of receptor providing costimulatory signals, critical in regulation and development of T cells in the adaptive immune response<sup>[12]</sup>. PD-1 binds to APCs programmed death ligands PD-L1 and PD-L2, and to solid tumor cell ligands PD-L1<sup>[34]</sup>. The strength of this binding depends on the strength of the TCR-signal, where low TCR-MHC interaction results in great PD-1 binding<sup>[35]</sup>. When bound to its ligands, PD-1 induces effector T cell inhibition through downstream, kinase inhibition, TCR decreased signaling and a decrease in INF- $\gamma$ , IL-2 secretion by phosphatase SHP1, SHP2 phosphorylation<sup>[35,36]</sup>.

PD-1 is, similar to CTLA-4, expressed on CD4<sup>+</sup> Tregs, where it increases immunosuppression<sup>[37]</sup>. Its function in the immune response is described as limiting T-cell activity in peripheral tissues, in both the inflammatory infectious response and in potential autoimmunity immune responses<sup>[34,35]</sup>. Within the PDA tumor microenvironment, as well as in the peripheral blood of PDA patients, PD-1 molecules are largely expressed on tumor infiltrating lymphocytes (TILs), such as CD4<sup>+</sup> T-cells, resulting in the tumor exerting a potentiated immune resistance by inducing T-cell apoptosis, and promoting tumor growth<sup>[38,39]</sup>. PD-1 is further described to create T-cell anergy among cognate antigen-specific T cells due to chronic antigen exposure in cancer tumors<sup>[40]</sup>.

Besides this expression, PD-1 is expressed on other activated immune-cells, such as NK cells and B cells, in which it limits its lytic activity<sup>[34,41]</sup>. This results in immune suppression. Also, this is thought to result in enhancement of tumor immune surveillance<sup>[42]</sup>.

Conversely, blockade of PD-1 results in immune response progression, with antitumor immunity<sup>[39,43]</sup>.

Since its discovery in 1992 PD-1 has been another promising immune-checkpoint target molecule in cancer tumor immunotherapy to enhance intratumoral immune response<sup>[34]</sup>.

In PDA, the human PD-1-antibody-drugs Pidilizumab, Nivolumab and Pembrolizumab are now tested in clinical trials.

### **Nivolumab**

Nivolumab (BMS-936558, MDX-1106, ONO-4538) is a humanized monoclonal IgG4 immunoglobulin antibody developed against PD-1-checkpoint-molecule-receptors on T cells, NK cells and B cells. It binds to and inhibits either PD-1-PD-L1 or PD-1-PD-L2 cell interaction, resulting in immune function reinstating by activation of NK cells and cytotoxic T lymphocyte (CTL) antitumor

immune response<sup>[22]</sup>.

In previously chemotherapy-treated patients, with PDA, a 1:1 randomized, two-armed, phase II study, of Cyclophosphamide-GVAX-vaccine, (CY/GVAX) and CRS-207 (a live-attenuated *Listeria monocytogenes*-expressing mesothelin drug) is being conducted both with and without Nivolumab. Patients receiving two doses of CY/GVAX and Nivolumab, together with four doses of CRS-207 and Nivolumab (Arm A) will be compared with patients receiving two doses of CY/GVAX and four doses of CRS-207 (Arm B). Overall survival is the primary objective of the study. CY/GVAX-vaccine together with CRS-207, have shown promising results in PDA-patients by priming tumor antigen-specific T cells, through Cyclophosphamide Treg-inhibitor-function; GVAX induced immune response-function against PDA tumor antigens *via* its GM-CSF-expressing- modified allogeneic pancreatic cancer cells; and CRS-207 enhanced antitumor PDA immune response-function by NK cells and T cells through its tumor-associated mesothelin antigens. Le *et al*<sup>[44]</sup> expect this combination therapy, together with Nivolumab to result in priming tumor antigen-specific T cells and simultaneously blocking immune-checkpoints.

In another ASCO-symposium Firdaus *et al*<sup>[45]</sup> presented their two-armed, two-part, phase I study of Nivolumab and Nab-paclitaxel-cytostatic, with and without Gemcitabine in patients with advanced PDA. Patients administered 125 mg/m<sup>2</sup> Nab-paclitaxel and 3 mg/kg Nivolumab (Arm A), will be compared with patients administered 125 mg/m<sup>2</sup> Nab-paclitaxel, 1000 mg/m<sup>2</sup> Gemcitabine and 3 mg/kg Nivolumab (Arm B). Evaluation of dose limiting toxicity (DLT) (Part I), together with evaluation of the safety of Nab-paclitaxel/Nivolumab combination (Part I and II) are the primary objectives in this still recruiting clinical trial.

Furthermore, Nivolumab is currently being tested for safety and efficacy in two, phase I and II trials for resectable, (NCT02451982) and metastatic (NCT02423954) PDA. In NCT02451982 patients with resectable PDA are randomized to receive 200 mg/m<sup>2</sup> CY/GVAX (Arm A) or 200 mg/m<sup>2</sup> CY/GVAX together with 3 mg/kg Nivolumab (Arm B)<sup>[46]</sup>. NCT02423954 is a non-randomized three-armed study. In Arm A, patients are given 25 mg Temsirolimus (a protein kinase inhibitor) and Nivolumab every 14<sup>th</sup> day. In Arm B, 150 mg/m<sup>2</sup> Irinotecan-cytostatic and Nivolumab every 14<sup>th</sup> day. In Arm C, 175 mg/m<sup>2</sup> Irinotecan is administered on day one then every 14 d. A dosage of 1000 mg Capecitabine-cytostatic is given on days 1-5, on days 6-7, off each 7 d period in combination with Nivolumab<sup>[47]</sup>.

Two phase I, non-randomized trials in locally advanced or metastatic PDA patients are being conducted (NCT02009449, NCT02526017). NCT02009449, a

study of AM0010 (recombinant human IL-10) is being conducted with one cohort treated with 20 µg/kg AM0010 daily subcutaneous injections, together with 3 mg/kg Nivolumab on day one of each 14-d cycle to study dose escalation, where safety and tolerability of AM0010 in patients with advanced solid tumors, dosed daily as a monotherapy or in combination with chemotherapy or immunotherapy, is evaluated<sup>[48]</sup>. NCT02526017 is a study of FPA008 (a monoclonal humanized antibody) with monotherapy every other week. This is compared to combination therapy consisting of FPA008 and 3 mg/kg Nivolumab every second week. Safety of FPA008 with Nivolumab is being evaluated<sup>[49]</sup>.

### **Pembrolizumab**

Pembrolizumab (MK-3475, SCH 900475) is also a humanized monoclonal IgG4 immunoglobulin antibody, developed against the PD-1-checkpoint-molecule-receptor on T cells. It binds to and inhibits either PD-1-PD-L1 or PD-1-PD-L2 cell interaction, resulting in activation of a CTL antitumor immune response<sup>[22]</sup>.

In a two-part immunotherapy clinical trial, testing Pexidartinib [a colony-stimulating factor 1 receptor (CSF1R) inhibitor] and Pembrolizumab in patients with PDA and other solid tumors, safety, efficacy, pharmacokinetics and pharmacodynamics is to be evaluated. In part I, a safety and tolerability combination-dose will be established; 200 mg Pembrolizumab each third week, with daily 200 mg oral doses of Pexidartinib. Thereafter, overall response rate (ORR) and progression free survival (PFS) will, in solid tumor-patients with the given established combination-dose, be evaluated. Pexidartinib is expected to target tumor associated macrophages (TAMs) and Myeloid-derived suppressor cells (MDSCs). TAMs enhance tumor growth and contribute to tumor resistance to radiation and chemotherapy. MDSCs suppress tumor surveillance and cause resistance to PD-1-checkpoint therapy<sup>[50]</sup>.

Three other phase I and II clinical trials are currently running.

NCT02305186 is a randomized, safety and efficacy study. 200 mg of Pembrolizumab is given every third week during concurrent neoadjuvant Capecitabine-cytostatic treatment and radiation therapy in resectable PDA patients<sup>[51]</sup>. NCT02331251 is a non-randomized, safety and efficacy study of 2 mg/kg Pembrolizumab given every third week, together with a different cytostatic in different study arms in metastatic PDA<sup>[52]</sup>. NCT02268825 is a safety and efficacy dose-escalating study were phase I evaluates the maximum tolerance dose (MTD) of Pembrolizumab in combination with mFOLFOX6-cytostatic. Phase II combines an additional dose of Celecoxib (NSAID) to determine the clinical benefit rate, the objective response rate, PFS and overall survival in locally advanced or metastatic PDA<sup>[53]</sup>.



Two phase II clinical trials of Pembrolizumab in patients with locally advanced or metastatic PDA are being conducted. NCT02648282 is a safety and efficacy study of combination therapy using 200 mg Pembrolizumab together with 200 mg/m<sup>2</sup> Cyclophosphamide-cytostatic and GVAX-vaccine (CY/GVAX) and SBRT<sup>[54]</sup>. NCT02362048 is an active, non-recruiting, two-armed, randomized safety and efficacy study where ACP-196 alone (Arm A) and ACP-196 in combination with Pembrolizumab (Arm B) are studied<sup>[55]</sup>.

Four phase- I clinical trials of Pembrolizumab in PDA are being conducted.

NCT02546531 and NCT02009449 are two non-randomized, safety and efficacy trials in locally advanced and metastatic PDA. In NCT02546531, Pembrolizumab is tested together with Gemcitabine and Defactinib in advanced PDA. Defactinib, a selective cancer stem cell inhibitor, directed against focal adhesion kinase is hoped to reduce stromal fibrosis in tumors during immune-checkpoint-therapy<sup>[56]</sup>. NCT02009449 is a dose escalation study of AM0010 (recombinant human IL-10) administered in incremental dosages through daily subcutaneous injections, together with 2 mg/kg Pembrolizumab on day one of each 14-d cycle. Safety and tolerability of AM0010 in patients with advanced solid tumors, either as a monotherapy or in combination with chemotherapy or immunotherapy, is evaluated<sup>[48]</sup>.

NCT02432963 and NCT02620423 are two phase-I Pembrolizumab studies. NCT02432963 is an active, non-recruiting safety trial of Pembrolizumab in combination with p53MVA (a gene-modified virus vaccine) administered to enhance antitumor immune response and reduce tumor growth in patients with metastatic PDA<sup>[57]</sup>. NCT02620423 is a safety and efficacy study of Pembrolizumab 2 mg/kg in combination with Reolysin (a human oncolytic reovirus that lysates cancer cells) and with chemotherapy in locally advanced or metastatic PDA<sup>[58]</sup>.

NCT01174121 is a non-randomized, phase-II trial of Pembrolizumab, 2 mg/kg Pembrolizumab, 60 mg/kg per day Cyclophosphamide, along with 25 mg/m<sup>2</sup> per day Fludarabine-cytostatic, Aldesleukin (a humanised IL-2-molecule) and tumor infiltrating cells (TILs) was conducted to study if tumor reduction could be reached by immunotherapy and TILs in PDA. The study was suspended<sup>[59]</sup>.

### **Pidilizumab**

Pidilizumab (CT-011, MDV9300) is a humanized monoclonal IgG1 immunoglobulin antibody developed against PD-1-molecule-receptors on T cells, NK cells, and B cells. It binds to and inhibits either PD-1-PD-L1 or PD-1-PD-L2 cell interaction, resulting in activation of CTL and an NK-cell antitumor immune response<sup>[22]</sup>. In PDA, two clinical trials, NCT01313416, and NCT01386502 of Pidilizumab were partially conducted, then suspended

and withdrawn<sup>[60,61]</sup>.

## **PD-L1**

PD-L1 is a molecule expressed on solid tumors (such as PDA) and on tumor-infiltrating dendritic cells and macrophages<sup>[62,63]</sup>. PD-L belongs to the B7 family consisting of PD-L1 (CD274/B7-H1) and PD-L2 (CD273/B7-DC)<sup>[63,64]</sup>. PD-L1 is a co-inhibitory ligand that binds to PD-1 and to CD80 (the co-stimulatory molecule to CD28 and co-inhibitory molecule to CTLA4 of T-cells) on T-cells and APCs<sup>[65]</sup>. When bound to its receptors, PD-L1 induces effector T-cell inhibition, apoptosis, anergy and exhaustion *via* PD-1-PD-L1 interaction<sup>[39,40,66]</sup>. Immunosuppression is enhanced through inhibitory receptor-signaling *via* B7-1-molecules in the B7-1-PD-L1 binding. However, this is a finding that not have been observed in tumors<sup>[34]</sup>. Moreover PD-L1 expression was observed to increase Treg infiltration in the PDA tumor microenvironment, also resulting in immune suppression<sup>[67]</sup>.

PD-L2 is primarily expressed on dendritic cells and macrophages<sup>[68]</sup>.

PD-L1 is the major PD-L-molecule expressed on solid tumors<sup>[39]</sup>. The expression of PD-L1 on PDA tumor cells is induced *via* CD8<sup>+</sup> T-cell INF $\gamma$ -secretion<sup>[69,70]</sup>. The expression of PD-L1 molecules in PDA is associated with assessed tumor proliferation, accelerated tumor cell carcinogenesis and drug resistance, defining the tumor as highly malignant<sup>[71]</sup>.

Blockade of PD-L1 is described to result in significant immune response progression, with enhanced T-cell activation<sup>[72]</sup>. In PDA enhanced upregulation and secretion of INF $\gamma$ , cytokines and proteases by infiltrated and activated CD8<sup>+</sup> T-cell in the tumor microenvironment was reported, after PD-L1 blockade<sup>[73]</sup>. Furthermore, as already stated, blockade of the PD-1 to PD-L1-pathway, results in tumor and antitumor immunity reduction by Treg depletion. Likewise, blockade of the CD80 to PD-L1-pathway results in immune response enhancement. The CD80-PD-L1-pathway is reported to be a bidirectional interaction of CD80 and PD-L1, where CD80, as stated above, interacts co-stimulatory with CD28-receptor and co-inhibits CTLA4-receptor on T-cells. PD-L1 also interact with PD-1, indicating a specific and significant T-cell response-inhibition, regulation and tolerance<sup>[34]</sup>.

Thus, PD-L1 is another promising immune checkpoint target molecule to enhance intratumoral immune response in cancer tumor immunotherapy. In PDA, Durvalumab, and BMS-936559, human anti-PD-1-antibody-drugs, are now tested in clinical trials.

### **Durvalumab**

Durvalumab (MEDI4736) is a humanized FC optimized monoclonal IgG1 immunoglobulin antibody, developed against PD-L1-checkpoint-ligand on solid tumors and TILs, such as dendritic cells and macrophages.

When bound, Durvalumab inhibits PD-1 cell interaction, potentially resulting in T cell upregulation and antitumor immune potentiation against PD-L1 expressing tumors by CTL response exertion. To avoid antibody-dependent cytotoxicity or complement-dependent cytotoxicity Durvalumab's FC-region is modified<sup>[22,74]</sup>.

As stated above, Duffy *et al.*<sup>[28]</sup> have, in metastatic PDA, conducted a three-armed, phase I, non-randomized clinical trial testing Durvalumab alone or in combination with Tremelimumab, together with SBRT. The primary objectives were to determine safety, tolerability and efficacy of immune checkpoint therapy together with SBRT. SBRT is expected to enhance Durvalumab's and/or Tremelimumab's immune checkpoint inhibitory effects, resulting in systemic anti-tumor effects.

In a phase I b/II clinical trial, Borazanci *et al.*<sup>[74]</sup> expect to evaluate the safety, tolerability and efficacy of Durvalumab in combination with Ibrutinib (a Burton's tyrosine kinase inhibitor). Preclinical data suggest that Ibrutinib inhibit tumor cell proliferation *via* angiogenesis and collagen deposition in TME by PDA mast cell degranulation<sup>[75]</sup>. DLT in phase I will determine recommended phase II dosage to treat up to 160 patients.

In two non-randomized, phase I clinical trials, Durvalumab is tested in locally advanced and metastatic PDA (NCT02586987) or metastatic PDA (NCT02639026). NCT02586987 is an open label, multicenter study, assessing safety, tolerability and anti-tumor activity<sup>[76]</sup>. NCT02639026 studies safety and efficacy of Tremelimumab and Durvalumab in combination with hypofractionated radiation therapy<sup>[32]</sup>.

NCT02301130 is a randomized, two-armed, phase I trial studying safety and efficacy. In Arm A, Durvalumab is combined with Mogamulizumab (monoclonal antibody against CCR4). In Arm B, Tremelimumab is combined with Mogamulizumab<sup>[29]</sup>. Two phase II randomized clinical trials of Durvalumab in metastatic PDA studying safety and efficacy together with Tremelimumab are conducted<sup>[30,33]</sup>. Two non-randomized, safety and efficacy clinical trials of Durvalumab in metastatic PDA were conducted. NCT02669914 is a phase II non-recruiting study of Durvalumab against refractory or recurrent brain metastases from solid tumors such as in PDA<sup>[77]</sup>. NCT02583477 is a two-armed, phase I b/II recruiting study of Durvalumab evaluated in combination with gemcitabine and nab-paclitaxel-cytostatics or in combination with the C-X-C motif chemokine receptor 2 (CXCR2) reversible antagonist with antineoplastic activity potentiality, AZD5069, in patients with metastatic PDA<sup>[78]</sup>.

### **BMS-936559**

BMS-936559 is a Bristol-Myers Squibb humanized monoclonal IgG4 antibody directed against PD-L1-

checkpoint-ligand on solid tumors and TILs such as dendritic cells and macrophages. BMS-936559 inhibits PD-1 and CD80 cell interaction. In a phase I, non-randomized safety and efficacy study, Brahmer *et al.*<sup>[79]</sup> reported objective response rates of 6% to 17% and prolonged disease stabilization rates of 12% to 41% at 24 wk in advanced cancers patients, when treated with BMS-936559. The study consisted of 207 patients with locally advanced, or metastatic cancer; 75 with NSCLC, 55 with melanoma, 18 with colorectal cancer, 17 with renal-cell cancer, 17 with ovarian cancer, 7 with gastric cancer, four with breast cancer, and 14 with PDA. BMS-936559 was tested with escalating doses ranging from 0.3 mg/kg to 10 mg/kg. In patients with melanomas, objective response was observed in 29% at a dose of 3 mg/kg. In PDA, no objective response was observed.

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## **FURTHER IMMUNE CHECKPOINT MOLECULES**

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Besides the above defined inhibitory lymphocyte receptors (CTLA-4, PD-1) and the ligand (PD-L1), other immune checkpoint molecules have been observed to be expressed on tumors and TILs in the tumor microenvironment. Lymphocyte activation gene 3 (LAG-3), B and T lymphocyte attenuator (BTLA/CD272), T cell membrane protein 3 (TIM-3/HAVCR2), adenosine A2a receptor (A2aR) and the B7-family inhibitory receptors (B7-H3/CD276, B7-H4/B7S1) are immune checkpoint molecules described to exert immune suppression<sup>[80-84]</sup>. B7-H3 is a molecule expressed on tumors, while B7-H4 is expressed on monocytes and macrophages<sup>[84]</sup>. TIM-3 is expressed on T helper cells and co-expressed with PD-1 on CD8<sup>+</sup> cells, preventing immune response<sup>[82]</sup>. A2aR interacts with adenosine, resulting in T-cell suppression<sup>[83]</sup>. LAG-3 is an inhibitory receptor molecule expressed on NK cells, APCs, Tregs, T-cells, B cells<sup>[80]</sup>. In TIL anergic or exhausted T cells, LAG-3 is co-expressed with PD-1<sup>[80]</sup>. It binds to MHC-II molecules on CD4<sup>+</sup> and CD8<sup>+</sup>, resulting in immune suppression<sup>[80]</sup>. Blockade of LAG-3 is reported to slow tumor growth and act in synergy with anti-PD-1 antibodies<sup>[80,85]</sup>.

In PDA one non-randomized, phase-I clinical trial testing IMP321 (an LAG-3 immune checkpoint blocker) was conducted, but was terminated, due to company manufacturing production inability<sup>[86]</sup>.

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## **DISCUSSION**

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In regulating the human immune system, immune checkpoint molecules are considered essential. CTLA-4, PD-1 and PD-L1 are three well-described inhibitory checkpoint molecules expressed on immune cells in the pancreatic ductal adenocarcinoma tumor microenvironment. Together with other molecules



derived by the tumor cells and different tumor-specific antigens, these checkpoint inhibitors create a heterogeneous protein expressing tumor, able to thwart immune surveillance and suppress and evade immune response. Checkpoint inhibition may enhance the T-cell response against the tumor. Hence immune checkpoint therapy is expected to have a place in the treatment arsenal for solid tumors such as pancreatic ductal adenocarcinoma. In patients with melanoma and lung cancer, treatments with immune checkpoint therapy have shown promising results in terms of tumor regression, objective response rate and overall survival progression, whereby FDA approval has been received.

Among patients with pancreatic ductal adenocarcinoma, immune checkpoint therapies also appear to be effective. Royal *et al.*<sup>[23]</sup>, noted delayed progression in one patient when treated with 3 mg/kg Ipilimumab. Le *et al.*<sup>[24]</sup> observed stable disease in five patients (two Arm A, three Arm B), four according to RECIST (two Arm A, two Arm B) and one according to irRC (Arm B), when treated with 10 mg/kg Ipilimumab alone (Arm A) or in combination with GVAX-vaccine (Arm B). Furthermore greater overall survival of 5.7 mo in patients treated with Ipilimumab and GVAX-vaccine, compared to 3.6 mo of Ipilimumab monotherapy was in this study noted. Aglietta *et al.*<sup>[27]</sup> demonstrated partial response in two patients with advanced pancreatic ductal adenocarcinoma, receiving 15 mg/kg Tremelimumab. Moreover a median overall survival of 7.4 mo with Tremelimumab was observed. Brahmer *et al.*<sup>[79]</sup> reported objective response rates of 6% to 17%, and prolonged disease stabilization rates of 12% to 41% at 24 wk in advanced cancers patients of melanoma and NSCLC, but not in PDA, when treated with BMS-936559.

However, these studies are limited, both in numbers and enrolled patients. These results of treatment with immune checkpoint inhibitors in PDA instead indicate that further research is needed. The number of currently running clinical trials described in this analysis serves as evidence that immune checkpoint therapy is a new and novel treatment perspective in the fight against PDA. A couple of trials are combining two of the checkpoints inhibitors, Durvalumab and Tremelimumab<sup>[29-33]</sup>. Other trials are testing checkpoint inhibitors, in combination with cytostatics<sup>[54,60]</sup>; in combination with vaccine<sup>[25,57,87]</sup>; or as monotherapy<sup>[30,49]</sup>. Currently, no results from these trials are presented.

Further knowledge is needed regarding the tumor of pancreatic ductal adenocarcinoma, its tumor microenvironment, its immune response and carcinogenesis to determine the activated immunosuppressors within an individual patient tumor in order to develop a personalized combination treatment. Improved evaluation of key-molecules within the tumor microenvironment of PDA, may as Bailey *et al.*<sup>[8]</sup> reported, enable distinctions of PDA,

where distinct combination therapies can be given in order to increase efficacy and response rate. The repertoire of immune checkpoints is expanding with further research. LAG-3 is a checkpoint molecule found to have been under investigation in a clinical trial by blocking it with IMP321, an immune checkpoint blocker, which was subsequently terminated due to company manufacturing production inability<sup>[86]</sup>. Identifying and blocking this molecule, as well as other novel reported checkpoint molecules, for example TIM-3 and BTLA might result in improved efficacy in treating the cancer, since these molecules are reported being co-expressed with PD-1<sup>[82]</sup>. As stated above, Le *et al.*<sup>[24]</sup> reported that combination therapy of Ipilimumab and GVAX-vaccine improved overall survival compared to Ipilimumab monotherapy. Vaccine therapies are described to potentiate associated antigen specific T-cells in the tumor microenvironment. Likewise, cytostatic and radiation therapies are described to result in tumor cell death with antigen releases, resulting in T-cell activation in the tumor microenvironment. In order to create and above all maintain the tumor microenvironment as an immunogenic environment, checkpoint inhibitors might be essential.

Outside the scope of this paper, but still worth mentioning is the finding of McGranahan *et al.*<sup>[2]</sup>. It describes the potential of combining immune checkpoint therapy in advanced cancers which both show sensitivity to immune checkpoint therapy and also express multiple tumor-antigens such as clonal neoantigens, and CD8<sup>+</sup> specific TILs (that recognize these clonal neoantigens) and identifying and potentiating these cells. Such an approach could make possible a general potentiation of a CD8<sup>+</sup> T-cell immune response against the whole tumor. This could lead to a new generation of personalized cancer treatment that could treat and perhaps even cure a cancer like pancreatic ductal adenocarcinoma.

This particular research is in its infancy. While the results are rather bleak the promise of future success lies in one important fact: that the treatment is leveraging the body's own immune response to a tumor. Great strides have been made in medicine, but we do not yet fully understand the wonders of the immune system and the force that drives the healing process within the human body.

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## CONCLUSION

Pancreatic cancer is one of the most treatment resistant human malignancies. Due to its heterogenic tumor microenvironment it has long been considered as a non-immunogenic cancer. However, in recent years more emphasis has been put on the importance of the immune system and the immune escape mechanisms of pancreatic cancer. Novel immune-based treatment strategies have been developed.

One of the more promising types of immunotherapies includes immune checkpoint inhibition, which is currently being tested in clinical trials in combination with vaccines, radiation or cytotoxic agents. If proven clinically beneficial, immune checkpoint therapy may become a valuable tool in our armamentarium.

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