Chapter 14
Targeting the Immune System in Pancreatic Cancer

D. Kabacaoglu, D. A. Ruess, and Hana Algül

The Immune Response in Pancreatic Cancer and Its Major Players

The immune system can be both harmful and beneficial during carcinogenesis and progression of pancreatic cancer (PC). The ability of both innate and adaptive immune cells to exert either tumor-suppressive or tumor-promoting properties yields a mosaic pattern of immune cell composition in the tumor microenvironment (TME). Therefore, an understanding of the individual components of this mosaic is required to develop efficient therapeutics.

Chronic inflammation is an important characteristic of PC, which is maintained by a complex interplay of immune cells in the TME [1, 2]. The myeloid compartment has many components, undoubtedly the most important one of them being tumor-associated macrophages (TAM). TAMs are found as M1 or M2 macrophages, which are classified according to the cytokine profile and surface markers they express [3]. Both M1 and M2 macrophages derive from monocytes. M1 macrophages, as "good cops," produce pro-inflammatory cytokines like TNF, IL12, IL-1β, and IFN-γ and show tumoricidal activity and induce an antitumor Th1 immune response. On the other hand, M2 macrophages, as the "bad cops," produce anti-inflammatory tumor-promoting cytokines like TGFβ and IL-10 and stimulate a Th2 immune response [3]. Next to TAMs, myeloid-derived suppressor cells (MDSC) are produced from immature myeloid cells and are known to suppress

D. Kabacaoglu · H. Algül (✉)
Department of Internal Medicine II, Klinikum rechts der Isar, Technische Universitat München, Munich, Germany
e-mail: hana.alguel@mri.tum.de

D. A. Ruess
Department of Surgery, Faculty of Medicine, Medical Center, University of Freiburg, Freiburg, Germany

C. W. Michalski et al. (eds.), *Translational Pancreatic Cancer Research*, Molecular and Translational Medicine,
https://doi.org/10.1007/978-3-030-49476-6_14

adaptive immunity with the recruitment of regulatory T cells (T_{reg}) to the TME and by reducing antitumor T cell activation [4, 5]. In line with this, the presence of immunosuppressive cells like M2 macrophages, MDSCs, and T_{reg} cells in the pancreatic TME has been shown to negatively correlate with overall survival [6–11]. Although both pro- and antitumorigenic abilities of neutrophils are reported, the inhibition of neutrophil recruitment to the TME remains a promising option in preclinical studies [12–14].

In the adaptive immune system, antigen-presenting cells (APC) such as dendritic cells (DC) can prime naïve T cells broadly into functional $CD4^+$ helper T cells (Th) or $CD8^+$ cytotoxic T cells (CTL) [15]. Th cells are further mainly characterized as Th_1, Th_2, and T_{reg}, and their coordination is highly deterministic for the type of tumor immune response [15]. Th_1 cells as conductors of an antitumorigenic response promote antigen presentation on APCs and cytolytic activity of $CD8^+$ T cells and boost M1 macrophages [16, 17]. However, Th_2 and T_{reg} cells are pro-tumorigenic since they can oppose the Th_1 immune response and escalate T cell exhaustion. Their presence is correlated with reduced survival in PC patients [18–23]. $CD8^+$ CTLs are the "best cops" in tumors, since they can directly recognize tumor cell-specific antigens and induce cancer cell death [15, 24].

Immunotherapy for PC: Obstacles and Potential Solutions

Boosting the adaptive immune response is one of the most attractive goals in cancer therapeutics: Other than generating a repertoire of T cells recognizing tumor-specific antigens, the ability of the adaptive immune system to form an immunological memory holds promise for long-term disease control [25]. Immunotherapeutic approaches, currently being established as a fourth pillar of cancer therapeutics (next to chemo-/targeted therapy, radiotherapy, and surgery), augment the antitumor adaptive immune response [26]. Immune checkpoint inhibitors are the best studied candidates in immunotherapeutic options so far. While checkpoint inhibitors like anti-CTLA-4 and anti-PD-1 antibodies showed very promising results in clinical studies for many solid tumors and hematologic malignancies, as single agents or in combination, they appear to be ineffective in PC [27–36]. Therefore, precise understanding of the immune cell network in PC is essential to explore ways to exploit immunotherapeutic approaches for treatment of patients with PC.

Immune Checkpoint Inhibition

CTLA-4 and PD-1 were the first immune checkpoint targets discovered and evaluated for cancer immunotherapeutics [37–39]. During APC:MHC molecule engagement with T cell receptor (TCR) on T cells, axes of co-stimulatory and co-inhibitory signals in T cells mediate T cell activity. These co-signaling pathways are essential

for physiological homeostasis since an imbalance can cause either autoimmunity or disability to fight invaders. Tumors may evolve the ability to skew this balance by reducing co-stimulation and inducing co-inhibition to impair antitumor T cell activity [40]. CTLA-4 and PD-1 are such co-inhibitory molecules leading to T cell anergy and exhaustion [41–44]. Antibodies targeting CTLA-4 and PD-1 can impair such signaling pathways in T cells and boost an antitumor cytotoxic immune response in tumors.

The question is though, why checkpoint inhibitors are not effective in PC as opposed to other solid tumor entities. PC owes this to its extreme immune-privileged nature [45]. Immune privilege is the ability to retain the production of antigens, without creating an anti-tumor immune response [46]. Normally, during carcinogenesis, tumor cells produce unique antigens (de novo mutations, re-expression of embryonic stage proteins), which may be recognized by the immune system, potentially leading to tumor cell elimination. During the immunosurveillance process (a hypothesis developed by Paul Ehrlich), the immune system continuously inspects the body for any malignant transformation [47–49]. However, some transformed cells have the ability to escape detection in a process called immunoediting. Immunoediting proposed by Schreiber and colleagues comprises three phases (triple E): elimination, equilibrium, and escape [50]. During the elimination phase, most of the transformed somatic cells die due to immunosurveillance, while the remaining survivors in the equilibrium step no more respond to immune reaction. Through a Darwinian-like selection, these clones proliferate and expand within the escape phase. While many tumors undergo the triple E of immunoediting process, PC holds a unique state [51, 52].

PC carcinogenesis is different in terms of the immunoediting process compared to many other solid tumors. With the use of genetically engineered mouse models (GEMMs), PC was shown to have an immunosuppressive microenvironment and a scarcity of antitumor T cells already during the carcinogenesis process [45]. Due to immunosuppression, the adaptive immune system is not educated toward recognition of any tumor-specific antigens, bypassing the elimination phase of triple E. With this rather immune quiescence-like phenotype, PC limits the entry of antitumor immune cells into the microenvironment maintaining its immune privileged status [51].

Overall, an approach to augment T cell entry and activity in the PC microenvironment may have the ability to render PC cells responsive toward immune checkpoint inhibitors. The factors which will determine such responsiveness are (1st) antigenicity of cancer cells and (2nd) immunogenicity of the tumor in general [53].

Antigenicity is the degree to which tumor cells produce and present neoantigens to generate an antitumor adaptive immune response [53]. These antigens can be divided into tumor-specific antigens (TSA) and tumor-associated antigens (TAA). TSAs are produced upon tumor-specific mutations of genes or reactivation of genes for embryonic development, which are not occurring in healthy somatic cells, while TAAs are wild-type proteins but expressed higher in tumor cells compared to somatic ones [54]. Production and MHC-mediated presentation of such antigens determine the level of antigenicity of tumors [53, 54].

Tumors carrying a high mutational burden generally respond better to checkpoint inhibition since they have a diverse tumor-antigen responsive T cell repertoire [55–57]. PC on the other hand doesn't carry such mutational load, compared to other entities [58, 59]. However, a subgroup of PC patients, representing around 1% of a patient cohort, carry mutations leading to mismatch repair (MMR) deficiency and microsatellite instability (MSI) and may profit from checkpoint inhibitors [60, 61]. As a result, anti PD-1 immunotherapy is approved by FDA for solid tumors including PC with MMR deficiency and MSI [62]. Moreover, one study identified long-term survivors in a PC patient cohort based on their ability to express good quality neoantigens, but not quantity [63]. Most importantly, a decrease in neoantigen quality of metastatic tumors compared to their respective primaries implied the importance of immunosurveillance in cancer metastasis and its implication in therapeutics [63]. Other than antigen production, presentation of these antigens via MHC molecules has been shown to be reduced in PC through the activation of oncogenic drivers like RAS [64–66]. Also, reduced MHC expression in disseminated PC cells appears to be an important driver of metastasis [67]. Since a correlation between antigenic load and immune checkpoint inhibition efficacy is absent in PC, as opposed by other solid tumor entities, in addition, factors determining immunogenicity of PC require exploitation.

Tumors with better ability to induce an adaptive immune response are considered immunogenic. This ability can be modulated both at the tumor cell level and at the level of cross talk of tumor cells with cells of the TME [53]. Transcriptomic analyses revealed an immunogenic subtype of PC, showing higher cytolytic T cell activity, antigen presentation, and CTLA-4 and PD-1 signatures [68]. Signatures as those may help to predetermine the prognostic value of checkpoint inhibitor therapy in the context of "personalized medicine" [69].

Tumor cell-specific immunogenicity can be decreased upon co-inhibitory checkpoint ligand expression in tumor cells, such as PD-L1. In various solid tumors, PD-L1 expression by tumor cells is increased due to oncogenic signaling pathways like PI3K, Hippo, Myc, and JAK-STAT [70–74]. In PC, the myeloid compartment was shown to induce EGFR-dependent MAPK signaling, leading to an increase of PD-L1 production in tumor cells [75]. An imbalance of autophagic modulation in mitochondrial iron homeostasis also may induce PD-L1 expression by pancreatic cancer cells [76].

Reprogramming the Tumor Microenvironment

Even if specific cancer cells are sufficiently antigenic and immunogenic, they may still not respond well to checkpoint inhibition due to an overall impaired immunogenicity mediated by the corresponding tumor tissue. The immunosuppressive TME is the main player in this context. An understanding of the responsible TME compartments, and of their cross talk with antitumor adaptive immune cells, is essential to reveal options for boosting immune checkpoint inhibitor response (Fig. 14.1).

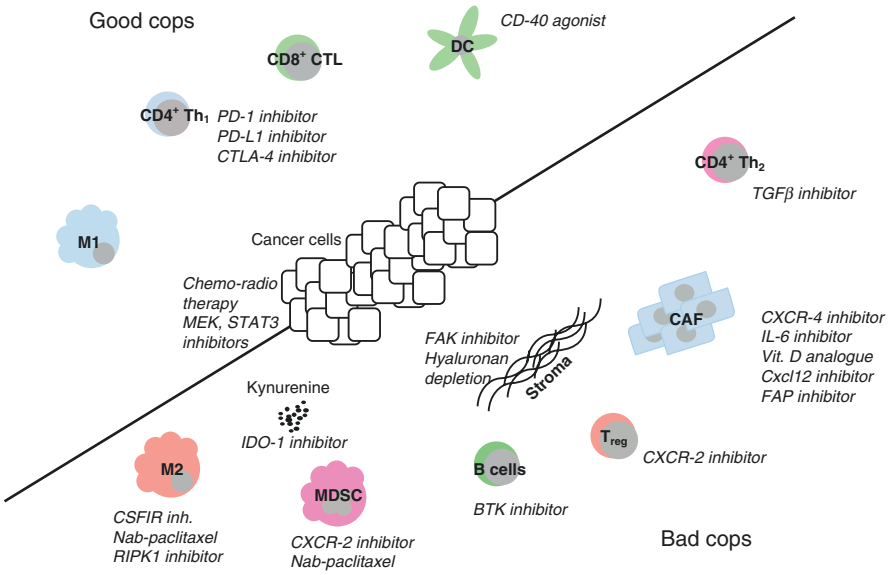

Fig. 14.1 The good and the bad cops of the tumor microenvironment and how to target them to boost a favorable immune response in PC. M1: M1 macrophages, M2: M2 macrophages, MDSC: myeloid-derived suppressor cells, CTL: cytotoxic T lymphocytes, DC: dendritic cells, T_{reg}: regulatory T cells, CAF: cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) are the leading actors regarding the characteristic desmoplastic stroma formation in PC. Various studies revealed a binary action of stromal cells in the immunogenicity of PC. One study revealed a positive correlation between type-I collagen production and CTL infiltration in tumor specimens of PC patients, whereas another showed the inhibition of CTL activity by αSMA$^+$ CAFs [77]. Other studies demonstrated an inhibitory action of CAFs toward CD8$^+$ T cell infiltration [78]. While most of the research so far implies the prognostic value of "stromal remodeling" in PC, an understanding of CAF action heterogeneity in the TME may provide options to improve the efficacy of immune checkpoint inhibitors. For example, with the use of preclinical mouse models, impairment of CXCR4 or IL-6 signaling in CAFs was shown to be synergistic with anti PD-L1 therapy [79, 80]. Stromal remodeling with FAK inhibitors reduced the immunosuppressive milieu in the TME, increasing chemotherapy-checkpoint inhibitor combination therapy efficacy [81]. Previous studies showed the benefit of hyaluronan depletion and vitamin D receptor activation in stromal remodeling [82–85]. Here, a combination therapy with immune checkpoint inhibitors may have therapeutic impact.

The myeloid compartment is a double-edged sword as also mentioned earlier. Years of research dissected the complex roles of individual components in PC. Studies focusing on CD40 agonist treatment of PC actually revealed the quite unique properties of PC. Treatment of preclinical mouse models with a CD40 agonist (acting on APCs increasing their capability to prime CTL) in combination with

gemcitabine created an only mild response by remodeling the stroma and reprogramming immunosuppressive myeloid cells inside the TME [86]. However, this regimen was not enough to create an adaptive immune response in tumors. The subsequent studies identified a subtype of immunosuppressive macrophages ($Ly6C^{low}$ $F4/80^{+}$), accumulating in the tumor periphery. These macrophages were shown to prevent CTL migration into the TME [87]. Finally, a combination therapy of nab-paclitaxel with gemcitabine and CD40 agonist revealed a synergism allowing penetration of active CTLs [88].

Re-education of neutrophils, MDSCs, and TAMs can also be achieved via various inhibitors targeting CSF1R, CXCR2, or RIPK1, which demonstrated synergism with immune checkpoint inhibitors in preclinical studies [14, 89, 90]. Other than directly targeting the myeloid compartment, inhibition of B cell-specific Bruton's tyrosine kinase (BTK) reprogrammed tumor resident macrophages indirectly, increasing the antitumor immunity [91].

Immunosuppressive immune cells impair immunosurveillance not only via cytokine-chemokine release but also through generation of a metabolite-restricted TME. Arginine depletion via arginase-1 produced by TAMs and MDSCs limits T cell activity [92, 93]. Further, the immunosuppressive metabolite kynurenine is produced from tryptophan as a by-product of indoleamine 2,3-dioxygenase (IDO-1) enzymatic activity. IDO-1 expression from cancer cells, TAMs, and MDSCs not only limits tryptophan availability for antitumor T cells but also increases inhibition of T cell activity by kynurenine [94]. Adenosine production by T_{reg} cells and prostaglandin E2 production from TAMs and MDSCs are also responsible for antitumor T cell activity impairment [95, 96].

Immunotherapeutic Properties of "Classical" Treatment Approaches

Other than targeted inhibitors, chemotherapeutic agents and radiotherapy also have the ability to convert nonresponsive, "immunologically cold," tumors to responsive, "immunologically hot," tumors. Chemo- and radiotherapy can boost both, antigenic properties of cancer cells due to their mutagenic effect and also immunogenicity of the tumor due to the induction of immunogenic cell death and subsequently enhanced inflammation [97, 98]. Next to their direct effect on cancer cells, such treatments may also alter the composition of immunosuppressive immune cells in the TME [88, 99]. Strikingly, immune checkpoint inhibition in cancer may not only enhance the response to radiation therapy in primary tumors but also has the potential for an abscopal response in metastatic sites [100, 101]. In conclusion, while chemotherapy and radiotherapy still are the gold standard therapies for cancer treatment, their combination with checkpoint inhibitors may be the next step to both increase the treatment response and T cell memory for long-term disease control, even for PC. Essentially, analysis of respective clinical trials may inform about dosing, sequence of treatment, and specific subgroups profiting most from the expected synergism.

Other Strategies for Boosting the Antitumor Immune Response

Immunotherapeutic approaches are not only limited to immune checkpoint inhibitors.

Oncolytic viruses (OV) can be designed to only target tumor cells, but not healthy somatic ones. This specificity can be achieved at multiple levels [102]. At the physiological level, OVs are not equipped to win a combat against healthy cells. Tumor cells, however, already may have imbalanced interferon signaling and increased cellular metabolism coupled with proliferation making them vulnerable towards viral infection. OVs can also be designed to take advantage of tumor-specific expression of cell entry receptors or transcription factors, limiting their action on healthy cells.

Cancer vaccines aim to boost adaptive immune response in the host against tumors. They can be produced as either whole cell (e.g. GVAX) or antigen-specific vaccines. GVAX is composed of pancreatic cancer cells genetically engineered to secrete GM-CSF with the aim to convert "cold" tumors to "hot" ones, and these cells are irradiated to prevent further proliferation [103]. *Listeria* vaccine is an engineered bacterial strain to secrete TAAs such as human mesothelin, boosting antitumor CTL activity. An approach with total cell followed by antigen-specific vaccine may recapitulate a "prime and boost" scenario [104].

Chimeric antigen receptor T cells (CAR-T) are genetically designed to express a receptor construct comprising an antibody-like ectodomain targeting TSAs and a TCR-like endodomain, bypassing the need for MHC engagement [105]. Upon antigen recognition they exert their cytotoxic properties. CAR-T cell therapy requires adoptive T cell transfer (ATC), in which patient's T cells have to be isolated, expanded, and genetically engineered. Without a genetic manipulation, in vitro induction and expansion of TILs (TIL-ATC) is also a valuable approach to exploit tumor targeting not only by a single antigen but a pool of them [106, 107].

Currently Ongoing Clinical Trials for Immunotherapy of Patients with PC

An overview of clinical trials based on abovementioned preclinical studies is given in Table 14.1. Overall, these studies reveal that PC is actually antigenic enough to create an antitumor adaptive immune response. However, the main barrier to be exceeded is the immunosuppressive microenvironment, which blocks the antitumor T cell priming and infiltration. One important factor is that many of these studies for PC are still in their early stages. Thorough analysis of each of these trials will pave the way to dissect individual rationales for combination therapies.

Table 14.1 Selected clinical trials aiming to induce an antitumor immune response in pancreatic cancer

Combination-arm 1	Combination-arm 2	Status	Patient eligibility criteria	Trial ID
Ipilimumab (αCTLA-4), gemcitabine	–	Phase 1	Stage III–IV or recurrent pancreatic cancer, uneligible to surgery	NCT01473940
Nab-paclitaxel, gemcitabine, nivolumab (αPD-1)	Nab-paclitaxel and nivolumab	Completed/ phase 1	Multiple solid tumors including pancreatic cancer	NCT01473941
Cyclophosphamide, GVAX, pembrolizumab (αPD-1), radiation (SBRT-6.6 Gy)	–	Recruiting/ phase 2	Locally advanced pancreatic ductal adenocarcinoma upon standard chemotherapy	NCT02648282
Durvalumab (αPD-L1), radiation (SBRT-6.6 Gy)	–	Recruiting/ phase 1–2	Borderline resectable and locally advanced pancreatic adenocarcinoma, treated with standard of care (SOC)	NCT03245541
Cyclophosphamide, GVAX, nivolumab (αPD-1), radiation (SBRT-6.6 Gy)	–	Recruiting/ phase 2	Borderline resectable pancreatic cancer	NCT03161379
Durvalumab (αPD-L1), radiation (SBRT-6.6 Gy)	–	Recruiting/ phase 1–2	SOC treated, borderline resectable, and locally advanced pancreatic adenocarcinoma	NCT03245541
Durvalumab (αPD-L1), tremelimumab (αCTLA4), radiation (SBRT-6.6 Gy)	Radiation (SBRT-6.6 Gy) with either durvalumab or tremelimumab	Recruiting/ phase 1	Uunresectable, nonmetastatic, locally advanced adenocarcinoma of pancreas	NCT02868632
Avelumab (αPD-L1), binimetinib (MEK inhibitor), talazoparib (PARP inhibitor)	Avelumab, binimetinib	Recruiting/ phase 2	Locally advanced or metastatic Ras-mutant solid tumors, including pancreatic cancer	NCT03637491
Durvalumab (αPD-L1), AZD9150 (STAT3 antisense)	–	Recruiting/ phase 2	Advanced pancreatic cancer	NCT02983578
Pembrolizumab (αPD-1), paricalcitol (vit D analogue)	Pembrolizumab, placebo	Recruiting/ early phase 2	Stage IV pancreatic cancer	NCT03331562

Table 14.1 (continued)

Combination-arm 1	Combination-arm 2	Status	Patient eligibility criteria	Trial ID
PEGPH20 (hyaluronidase), pembrolizumab (αPD-1)	–	Phase 2	Hyaluronan high (HA-high) metastatic pancreatic ductal adenocarcinoma	NCT03634332
PEGPH20 (hyaluronidase), avelumab (αPD-L1)	–	Recruiting/ early phase 1	Chemotherapy-resistant advanced or locally advanced pancreatic ductal adenocarcinoma	NCT03481920
Galunisertib (TGFβ inhibitor), durvalumab (αPD-L1)	–	Phase 1	Metastatic pancreatic cancer	NCT02734160
Spartalizumab (αPD-1), NIS793 (TGFβ inhibitor)	NIS793 (TGFβ inhibitor)	Recruiting/ phase 1	Advanced malignancies including pancreatic cancer	NCT02947165
Pembrolizumab (αPD-1), defactinib (FAK inhibitor)	–	Recruiting/ phase 1–2	Advanced solid malignancies including pancreatic neoplasms	NCT02758587
Pembrolizumab (αPD-1), defactinib (FAK inhibitor), gemcitabine	–	Recruiting/ phase 1	Advanced solid malignancies including pancreatic cancer	NCT02546531
Pembrolizumab (αPD-1), defactinib (FAK inhibitor)	Pembrolizumab (αPD-1)	Recruiting/ phase 2	SOC treated, neoadjuvant, and adjuvant treatment for resectable pancreatic ductal adenocarcinoma	NCT03727880
Cyclophosphamide, GVAX, pembrolizumab (αPD-1), IMC-CS4 (CSF1R inhibitor)	–	Recruiting/ early phase 1	Borderline resectable pancreatic ductal adenocarcinoma	NCT03153410
Durvalumab (αPD-L1), pexidartinib (CSF1R, FLT3, and KIT inhibitor)	–	Recruiting/ phase 1	Metastatic/ advanced pancreatic or colorectal cancers	NCT02777710
Nivolumab (αPD-1), cabiralizumab (αCSF1R)	Cabiralizumab	Phase 1	Advanced solid tumors including pancreatic cancer	NCT02526017

(continued)

Table 14.1 (continued)

Combination-arm 1	Combination-arm 2	Status	Patient eligibility criteria	Trial ID
Pembrolizumab (αPD-1), AMG820 (CSF1R inhibitor)	–	Phase 1–2	Advanced solid tumors including pancreatic cancer	NCT02713529
Pembrolizumab (αPD-1), BL-8040 (CXCR4 inhibitor)	BL-8040	Phase 2	Metastatic pancreatic adenocarcinoma	NCT02826486
Olaptesed pegol (CXCL12 inhibitor) + Pembrolizumab	Olaptesed pegol	Phase 1–2	Metastatic colorectal and pancreatic cancer	NCT03168139
APX005M (CD40 agonist), gemcitabine, nab-paclitaxel, nivolumab (αPD-1)	APX005M, gemcitabine, nab-paclitaxel	Recruiting/ phase 1–2	Previously untreated metastatic pancreatic adenocarcinoma	NCT03214250
CDX-1140 (CD40 agonist), CDX-301 (CD135 agonist)	CDX-1140	Recruiting/ phase 1	Advanced malignancies including pancreatic adenocarcinoma	NCT03329950
Pembrolizumab (αPD-1), acalabrutinib (BTK inhibitor)	Acalabrutinib	Phase 2	Metastatic pancreatic cancer	NCT02362048
Durvalumab (αPD-L1), ibritunib (BTK inhibitor)	–	Completed/ phase 1–2	Relapsed or refractory solid tumors including pancreatic cancer	NCT02403271
Epacadostat (IDO-1 inhibitor), pembrolizumab (αPD-1)	–	Phase 2/ withdrawn	Advanced pancreatic cancer with chromosomal instability/ homologous recombination repair deficiency (HRRD)	NCT03432676

Table 14.1 (continued)

Combination-arm 1	Combination-arm 2	Status	Patient eligibility criteria	Trial ID
Atezolizumab (αPD-L1), chemotherapy, selicrelumab (CD40 agonist)	Nab-paclitaxel, gemcitabine (chemotherapy)	Recruiting/ phase 1–2	Cohort 1 treatment to be performed on patients with no prior systemic therapy for metastatic pancreatic ductal adenocarcinoma	NCT03193190
Atezolizumab (αPD-L1), chemotherapy, selicrelumab (CD40 agonist), bevacizumab (αVEGF)				
Atezolizumab (αPD-L1) + chemotherapy + bevacizumab (αVEGF)				
Atezolizumab (αPD-L1) + chemotherapy + emactuzumab (αCSF1R)				
Atezolizumab (αPD-L1) + cobimetinib (MEK inhibitor)	Nab-paclitaxel and gemcitabine or mFOLFOX6 (chemotherapy)		Cohort 2 treatment to be performed on patients with disease progression upon control chemotherapy of cohort 1	
Atezolizumab (αPD-L1) + PEGPH20 (hyaluronidase)				
Atezolizumab + BL-8040 (CXCR4 inhibitor)				
Atezolizumab (αPD-L1) + RO6874281 (FAP-IL2 fusion protein)				
Atezolizumab (αPD-L1) + emactuzumab (αCSF1R)				

References

1. Guerra C, Schuhmacher AJ, Cañamero M, Grippo PJ, Verdaguer L, Pérez-Gallego L, Dubus P, Sandgren EP, Barbacid M. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell. 2007;11:291–302.
2. McKay CJ, Glen P, McMillan DC. Chronic inflammation and pancreatic cancer. Best Pract Res Clin Gastroenterol. 2008;22:65–73.
3. Ruffell B, Affara NI, Coussens LM. Differential macrophage programming in the tumor microenvironment. Trends Immunol. 2012;33:119–26.
4. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol. 2009;182:4499–506.
5. Gabrilovich DI. Myeloid-derived suppressor cells. Cancer Immunol Res. 2017;5:3–8.
6. Hiraoka N, Onozato K, Kosuge T, Hirohashi S. Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. Clin Cancer Res. 2006;12:5423–34.
7. Tjomsland V, Niklasson L, Sandström P, Borch K, Druid H, Bratthäll C, Messmer D, Larsson M, Spångeus A. The desmoplastic stroma plays an essential role in the accumulation and modulation of infiltrated immune cells in pancreatic adenocarcinoma. Clin Dev Immunol. 2011;2011:212810.

8. Kurahara H, Shinchi H, Mataki Y, Maemura K, Noma H, Kubo F, Sakoda M, Ueno S, Natsugoe S, Takao S. Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. J Surg Res. 2011;167:e211–9.
9. Greten TF, Manns MP, Korangy F. Myeloid derived suppressor cells in human diseases. Int Immunopharmacol. 2011;11:802–7.
10. Ino Y, Yamazaki-Itoh R, Shimada K, Iwasaki M, Kosuge T, Kanai Y, Hiraoka N. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. Br J Cancer. 2013;108:914–23.
11. Goedegebuure P, Mitchem JB, Porembka MR, Tan MCB, Belt BA, Wang-Gillam A, Gillanders WE, Hawkins WG, Linehan DC. Myeloid-derived suppressor cells: general characteristics and relevance to clinical management of pancreatic cancer. Curr Cancer Drug Targets. 2011;11:734–51.
12. Leliefeld PHC, Koenderman L, Pillay J. How neutrophils shape adaptive immune responses. Front Immunol. 2015;6:471.
13. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell. 2009;16:183–94.
14. Steele CW, Karim SA, Leach JDG, et al. CXCR2 inhibition profoundly suppresses metastases and augments immunotherapy in pancreatic ductal adenocarcinoma. Cancer Cell. 2016;29:832–45.
15. Gao GF, Rao Z, Bell JI. Molecular coordination of alphabeta T-cell receptors and coreceptors CD8 and CD4 in their recognition of peptide-MHC ligands. Trends Immunol. 2002;23:408–13.
16. Steimle V, Siegrist CA, Mottet A, Lisowska-Grospierre B, Mach B. Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA. Science. 1994;265:106–9.
17. Stout RD, Bottomly K. Antigen-specific activation of effector macrophages by IFN-gamma producing (TH1) T cell clones. Failure of IL-4-producing (TH2) T cell clones to activate effector function in macrophages. J Immunol. 1989;142:760–5.
18. Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. Cell Res. 2017;27:109–18.
19. Dobrzanski MJ. Expanding roles for CD4 T cells and their subpopulations in tumor immunity and therapy. Front Oncol. 2013;3:63.
20. Tassi E, Gavazzi F, Albarello L, Senyukov V, Longhi R, Dellabona P, Doglioni C, Braga M, Di Carlo V, Protti MP. Carcinoembryonic antigen-specific but not antiviral CD4+ T cell immunity is impaired in pancreatic carcinoma patients. J Immunol. 2008;181:6595–603.
21. De Monte L, Reni M, Tassi E, Clavenna D, Papa I, Recalde H, Braga M, Di Carlo V, Doglioni C, Protti MP. Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. J Exp Med. 2011;208:469–78.
22. Gabitass RF, Annels NE, Stocken DD, Pandha HA, Middleton GW. Elevated myeloid-derived suppressor cells in pancreatic, esophageal and gastric cancer are an independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13. Cancer Immunol Immunother. 2011;60:1419–30.
23. Formentini A, Prokopchuk O, Sträter J, Kleeff J, Grochola LF, Leder G, Henne-Bruns D, Korc M, Kornmann M. Interleukin-13 exerts autocrine growth-promoting effects on human pancreatic cancer, and its expression correlates with a propensity for lymph node metastases. Int J Color Dis. 2009;24:57–67.
24. Nathan CF, Murray HW, Wiebe ME, Rubin BY. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J Exp Med. 1983;158:670–89.
25. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64.

26. Iwai Y, Hamanishi J, Chamoto K, Honjo T. Cancer immunotherapies targeting the PD-1 signaling pathway. J Biomed Sci. 2017;24:26.
27. Royal RE, Levy C, Turner K, et al. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother. 2010;33:828–33.
28. Brahmer JR, Tykodi SS, Chow LQM, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455–65.
29. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711–23.
30. Weber JS, D'Angelo SP, Minor D, 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;16:375–84.
31. Ribas A, Puzanov I, Dummer R, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16:908–18.
32. Rizvi NA, Mazières J, Planchard D, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. Lancet Oncol. 2015;16:257–65.
33. Motzer RJ, Rini BI, McDermott DF, et al. Nivolumab for metastatic renal cell carcinoma: results of a randomized phase II trial. J Clin Oncol. 2015;33:1430–7.
34. Powles T, O'Donnell PH, Massard C, et al. Efficacy and safety of durvalumab in locally advanced or metastatic urothelial carcinoma: updated results from a phase 1/2 open-label study. JAMA Oncol. 2017;3:e172411.
35. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature. 2014;515:558–62.
36. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med. 2015;372:311–9.
37. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8:793–800.
38. 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;11:3887–95.
39. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271:1734–6.
40. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell. 2015;27:450–61.
41. Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. Curr Opin Immunol. 2013;25:214–21.
42. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, Thompson CB, Bluestone JA. CTLA-4 can function as a negative regulator of T cell activation. Immunity. 1994;1:405–13.
43. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995;182:459–65.
44. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192:1027–34.
45. Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, Vonderheide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. Cancer Res. 2007;67:9518–27.
46. Hong S, Van Kaer L. Immune privilege: keeping an eye on natural killer T cells. J Exp Med. 1999;190:1197–200.
47. Ehrlich P, Himmelweit F, Marquadt M, Dale HH. The collected papers of Paul Ehrlich of Paul Ehrlich: immunology and cancer research. London: Pergamon Press; 1957.
48. Burnet FM. The concept of immunological surveillance. Prog Exp Tumor Res. 1970;13:1–27.
49. Thomas L. On immunosurveillance in human cancer. Yale J Biol Med. 1982;55:329–33.

50. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol. 2004;22:329–60.
51. Evans RA, Diamond MS, Rech AJ, et al. Lack of immunoediting in murine pancreatic cancer reversed with neoantigen. JCI Insight. 2016. https://doi.org/10.1172/jci.insight.88328.
52. Kabacaoglu D, Ciecielski KJ, Ruess DA, Algül H. Immune checkpoint inhibition for pancreatic ductal adenocarcinoma: current limitations and future options. Front Immunol. 2018;9:1878.
53. Beatty GL, Gladney WL. Immune escape mechanisms as a guide for cancer immunotherapy. Clin Cancer Res. 2015;21:687–92.
54. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. N Engl J Med. 2017;377:2500–1.
55. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, Stephens PJ, Daniels GA, Kurzrock R. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther. 2017;16:2598–608.
56. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348:124–8.
57. Yarchoan M, Johnson BA, Lutz ER, Laheru DA, Jaffee EM. Targeting neoantigens to augment antitumour immunity. Nat Rev Cancer. 2017;17:569.
58. Campbell BB, Light N, Fabrizio D, et al. Comprehensive analysis of hypermutation in human cancer. Cell. 2017;171:1042–1056.e10.
59. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. Science. 2013;339:1546–58.
60. Germano G, Lamba S, Rospo G, et al. Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth. Nature. 2017;552:116–20.
61. Humphris JL, Patch A-M, Nones K, et al. Hypermutation in pancreatic cancer. Gastroenterology. 2017;152:68–74.e2.
62. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357:409–13.
63. Balachandran VP, Łuksza M, Zhao JN, et al. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. Nature. 2017;551:512–6.
64. Delp K, Momburg F, Hilmes C, Huber C, Seliger B. Functional deficiencies of components of the MHC class I antigen pathway in human tumors of epithelial origin. Bone Marrow Transplant. 2000;25(Suppl 2):S88–95.
65. Lohmann S, Wollscheid U, Huber C, Seliger B. Multiple levels of MHC class I downregulation by ras oncogenes. Scand J Immunol. 1996;43:537–44.
66. Seliger B, Harders C, Wollscheid U, Staege MS, Reske-Kunz AB, Huber C. Suppression of MHC class I antigens in oncogenic transformants: association with decreased recognition by cytotoxic T lymphocytes. Exp Hematol. 1996;24:1275–9.
67. Pommier A, Anaparthy N, Memos N, et al. Unresolved endoplasmic reticulum stress engenders immune-resistant, latent pancreatic cancer metastases. Science. 2018. https://doi.org/10.1126/science.aao4908.
68. Bailey P, Chang DK, Nones K, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature. 2016;531:47–52.
69. Zhang J, Wolfgang CL, Zheng L. Precision immuno-oncology: prospects of individualized immunotherapy for pancreatic cancer. Cancers (Basel). 2018. https://doi.org/10.3390/cancers10020039.
70. Feng J, Yang H, Zhang Y, Wei H, Zhu Z, Zhu B, Yang M, Cao W, Wang L, Wu Z. Tumor cell-derived lactate induces TAZ-dependent upregulation of PD-L1 through GPR81 in human lung cancer cells. Oncogene. 2017;36:5829–39.
71. Casey SC, Tong L, Li Y, et al. MYC regulates the antitumor immune response through CD47 and PD-L1. Science. 2016;352:227–31.
72. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med. 2007;13:84–8.

73. Lastwika KJ, Wilson W, Li QK, et al. Control of PD-L1 expression by oncogenic activation of the AKT-mTOR pathway in non-small cell lung cancer. Cancer Res. 2016;76:227–38.
74. Song TL, Nairismägi M-L, Laurensia Y, et al. Oncogenic activation of the STAT3 pathway drives PD-L1 expression in natural killer/T-cell lymphoma. Blood. 2018;132:1146–58.
75. Zhang Y, Velez-Delgado A, Mathew E, Li D, Mendez FM, Flannagan K, Rhim AD, Simeone DM, Beatty GL, Pasca di Magliano M. Myeloid cells are required for PD-1/PD-L1 checkpoint activation and the establishment of an immunosuppressive environment in pancreatic cancer. Gut. 2017;66:124–36.
76. Li C, Zhang Y, Cheng X, et al. PINK1 and PARK2 suppress pancreatic tumorigenesis through control of mitochondrial iron-mediated immunometabolism. Dev Cell. 2018;46:441–455.e8.
77. Carstens JL, Correa de Sampaio P, Yang D, Barua S, Wang H, Rao A, Allison JP, LeBleu VS, Kalluri R. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. Nat Commun. 2017;8:15095.
78. Ene-Obong A, Clear AJ, Watt J, et al. Activated pancreatic stellate cells sequester CD8+ T cells to reduce their infiltration of the juxtatumoral compartment of pancreatic ductal adenocarcinoma. Gastroenterology. 2013;145:1121–32.
79. Feig C, Jones JO, Kraman M, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. Proc Natl Acad Sci U S A. 2013;110:20212–7.
80. Mace TA, Shakya R, Pitarresi JR, et al. IL-6 and PD-L1 antibody blockade combination therapy reduces tumour progression in murine models of pancreatic cancer. Gut. 2018;67:320–32.
81. Jiang H, Hegde S, Knolhoff BL, et al. Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy. Nat Med. 2016;22:851–60.
82. Jacobetz MA, Chan DS, Neesse A, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. Gut. 2013;62:112–20.
83. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell. 2012;21:418–29.
84. Thompson CB, Shepard HM, O'Connor PM, et al. Enzymatic depletion of tumor hyaluronan induces antitumor responses in preclinical animal models. Mol Cancer Ther. 2010;9:3052–64.
85. Sherman MH, Yu RT, Engle DD, et al. Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. Cell. 2014;159:80–93.
86. Beatty GL, Chiorean EG, Fishman MP, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011;331:1612–6.
87. Beatty GL, Winograd R, Evans RA, et al. Exclusion of T cells from pancreatic carcinomas in mice is regulated by Ly6C(low) F4/80(+) extratumoral macrophages. Gastroenterology. 2015;149:201–10.
88. Byrne KT, Vonderheide RH. CD40 stimulation obviates innate sensors and drives T cell immunity in cancer. Cell Rep. 2016;15:2719–32.
89. Zhu Y, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, Wang-Gillam A, Goedegebuure SP, Linehan DC, DeNardo DG. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res. 2014;74:5057–69.
90. Wang W, Marinis JM, Beal AM, et al. RIP1 kinase drives macrophage-mediated adaptive immune tolerance in pancreatic cancer. Cancer Cell. 2018;34:757–774.e7.
91. Gunderson AJ, Kaneda MM, Tsujikawa T, et al. Bruton tyrosine kinase-dependent immune cell cross-talk drives pancreas cancer. Cancer Discov. 2016;6:270–85.
92. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, Gilbert J, Ochoa AC. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. J Exp Med. 2005;202:931–9.
93. Rodriguez PC, Ernstoff MS, Hernandez C, Atkins M, Zabaleta J, Sierra R, Ochoa AC. Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. Cancer Res. 2009;69:1553–60.

94. Platten M, Wick W, Van den Eynde BJ. Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. Cancer Res. 2012;72:5435–40.
95. Ohta A. A metabolic immune checkpoint: adenosine in tumor microenvironment. Front Immunol. 2016;7:109.
96. Sreeramkumar V, Fresno M, Cuesta N. Prostaglandin E2 and T cells: friends or foes? Immunol Cell Biol. 2012;90:579–86.
97. Brown JS, Sundar R, Lopez J. Combining DNA damaging therapeutics with immunotherapy: more haste, less speed. Br J Cancer. 2018;118:312–24.
98. Azad A, Yin Lim S, D'Costa Z, et al. PD-L1 blockade enhances response of pancreatic ductal adenocarcinoma to radiotherapy. EMBO Mol Med. 2017;9:167–80.
99. Zheng Y, Dou Y, Duan L, Cong C, Gao A, Lai Q, Sun Y. Using chemo-drugs or irradiation to break immune tolerance and facilitate immunotherapy in solid cancer. Cell Immunol. 2015;294:54–9.
100. Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res. 2013;1:365–72.
101. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst. 2013;105:256–65.
102. Naik S, Russell SJ. Engineering oncolytic viruses to exploit tumor specific defects in innate immune signaling pathways. Expert Opin Biol Ther. 2009;9:1163–76.
103. Lutz ER, Wu AA, Bigelow E, et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. Cancer Immunol Res. 2014;2:616–31.
104. Le DT, Wang-Gillam A, Picozzi V, et al. Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. J Clin Oncol. 2015;33:1325–33.
105. Akce M, Zaidi MY, Waller EK, El-Rayes BF, Lesinski GB. The potential of CAR T cell therapy in pancreatic cancer. Front Immunol. 2018;9:2166.
106. Poschke I, Faryna M, Bergmann F, et al. Identification of a tumor-reactive T-cell repertoire in the immune infiltrate of patients with resectable pancreatic ductal adenocarcinoma. Onco Targets Ther. 2016;5:e1240859.
107. Hall M, Liu H, Malafa M, Centeno B, Hodul PJ, Pimiento J, Pilon-Thomas S, Sarnaik AA. Expansion of tumor-infiltrating lymphocytes (TIL) from human pancreatic tumors. J Immunother Cancer. 2016;4:61.