REVIEW

Immune checkpoints in targeted-immunotherapy of pancreatic cancer: New hope for clinical development

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Abstract

Immunotherapy has been recently considered as a promising alternative for cancer treatment. Indeed, targeting of immune checkpoint (ICP) strategies have shown significant success in human malignancies. However, despite remarkable success of cancer immunotherapy in pancreatic cancer (PCa), many of the developed immunotherapy methods show poor therapeutic outcomes in PCa with no or few effective treatment options thus far. In this process, immunosuppression in the tumor microenvironment (TME) is found to be the main obstacle to the effectiveness of antitumor immune response induced by an immunotherapy method. In this paper, the latest findings on the ICPs, which mediate immunosuppression in the TME have been reviewed. In addition, different approaches for targeting ICPs in the TME

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of PCa have been discussed. This review has also synthesized the cutting-edge advances in the latest studies to clinical applications of ICP-targeted therapy in PCa.

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1. Introduction

In recent years, researchers have highlighted the importance of pancreatic cancer (PCa) in its severity and generalizability. In the past few decades, the concept of PCa has been faced with many arguments in many aspects, however the general view toward the PCa has been kept consistent, and PCa is still one of the most fatal cancers in the world. Accordingly, PCa is defined as the disease in which malignant cancer cells appear in the tissues of the pancreas. Pancreatic tumors are classified into exocrine or neuroendocrine based on the cell from which they originate. This classification is critical as it provides distinct functional characteristics and treatment strategies between these two types. In the United States, PCa is the 9th and 10th most frequently diagnosed cancer in females and males, respectively. Approximately 93% of PCa patients are exocrine tumors. According to the American Cancer Society, PCa patients account for approximately 3% of all adult cancer cases in the United States, with only about 22% of exocrine PCa patients still living one year after surgery. Above 56,000 Americans are expected to be diagnosed with PCa in 2019, with an average of above 150 diagnoses per day. Recently, significant developments have been made in recognizing the molecular biology, diagnosis, staging, and treatment of PCa in patients. As a turning point, cancer immunotherapy has emerged through monoclonal antibodies (mAbs) that obstruct inhibitory receptors on immune-effector cells or their ligands on tumor cells and antigen-presenting cells (APCs) alleged ‘immune checkpoints’ (ICPs). The main ICPs that are expressed on immune cells are programmed death 1 (PD-1), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), and lymphocyte-activation gene 3 (LAG-3). The ligands of PD-1 (PD-L1 and PD-L2), CTLA-4 (CD80 and CD86), and LAG-3 (MHC-II) could bind to ICPs and trigger immunosuppression in the tumor microenvironment (TME). This review is an attempt to focus on recent advances as well as an outlook on targeting ICPs for attenuation or elimination of PCa cells.

ICPs are regulatory molecules that maintain immune homeostasis; however, they are overexpressed to suppress the anti-tumoral immune response in the TME. The immune system plays a critical role in the elimination of tumor cells and fight against cancer. It was demonstrated that the immune system unremittingly checks cells recognize any inappropriate and foreign antigens such as tumor antigens. This process of inspection of the immune system over the cells is called immune surveillance. One of the strategies used by tumors such as pancreatic ductal adenocarcinoma (PDA) is to bypass the immune surveillance by the misusage of ICPs to escape immune recognition. It is in view of this particular phenomenon that immunotherapy against ICPs is expounded as a powerful strategy in the field of anti-cancer therapy, in which mAbs against PD-L1 and CTLA-4 were established to be effective. An immunosuppressive hallmark of elevated expression of the ICP is the reduction of T cells activity. B7-H1 or PD-L1 (CD274), as well as B7-DC and PD-L2 (CD273), are ligands of PD-1 (CD279). PD-L1, PD-L2, and PD-1 are transmembrane glycoprotein type I belong to the immunoglobulin (Ig) superfamily B7 and CD28, respectively. The interaction between PD-1 on T cells and PD-L1 on APCs of the TME and PCa cells promotes the suppression and exhaustion of the T cells. Exhausted T cells have a significant role in leading to a defective T cell reaction which weakens the tumor-specific responses. In PDA, CTLA-4, PD-1, and PD-L1 are three major inhibitory checkpoints which are expressed in the TME. CTLA-4 (CD152) is a member of the Ig superfamily that binds to CD28 on T cells and transmits an inhibitory signal to T cells. CTLA-4 has been first demonstrated as an inhibitory ICP molecule, which can suppress T cells, as well as autoreactive T cells, ordinary in lymph nodes. Despite the mode of action of CTLA-4, PD-1 influences T cells with its inhibitory role at late stages of a T cell activity where PD-1 ligands are expressed. The expression of PD-L1 on tumor cells and also myeloid-derived suppressor cells (MDSC) in the TME could inhibit the activation of T cells. A study showed that PD-1 expression in PDA cells enhanced cancer progression. In addition to the direct suppressive effects of the PD-1/PD-L1 axis on effector T cells, studies have shown the role of this pathway in the induction of regulatory T cells (Treg). In vitro studies have indicated that the presence of PD-L1 induces Treg activity. Furthermore, PD-1 inhibitors could hamper the induction of transforming growth factor-β (TGF-β) and retinoic acid (RA)-induced Treg. It was shown the elevated proportion of Treg for T CD8+ cells led to poor prognosis in cancers, suggesting that the induction of Treg is one of the major approaches used by tumor cells to escape immune surveillance.

In PCa, the TME and the immune system show a vital role in tumor growth. PCa features an extremely immunosuppressive microenvironment, described by a dense desmoplasic stroma that inhibits blood flow to the area, prevents delivery of drug, and stops the antitumor immune reaction. This supports cancer development and metastasis through protecting pancreatic tumors from immune surveillance. Moreover, the hypoxic milieu, acidic extracellular pH, and high interstitial fluid pressure in the TME also have a role in augmenting tumorigenesis. Furthermore, the PDA microenvironment is generally composed of Treg, MDSCs, and MDSCs, which stop the anti-tumoral function of effector CD4+ and CD8+ T cells. Treg has been shown to play critical role in PDA tumor development. Tumor-associated macrophages (TAMs) are also responsible for inflammation, development and metastasis of PCa. Uregulation of negative T cell co-stimulatory molecules is another reason of stimulation of PDA immunosuppression. PD-L1 and PD-L2 are overexpressed in PDA patients and correlate with decreased tumor-infiltrating leukocytes (TILs) and a poor prognosis. Therefore, PD-L1 downregulation prevents cell proliferation in PCa. In addition, an augmented expression of
inhibitory molecules on inactivated T cells has been presented as another strategy to stimulate immunosuppression of PCa34.

High CD8+ TIL correlates with a better prognosis due to their cytotoxic functions in several types of solid tumors. The acquisition of effector T cells (CD4+ and CD8+) in human PCa can improve overall survival (OS). Likewise, CD8+ effector T cells reduce whereas suppressive Treg contain a higher CD4 T cell level, which leads to a low number of TIL and a high number of immunosuppressive cells35. PCa is poorly immune-responsive, which leads to a low number of TIL and a high number of suppressive TME. Therefore, PCa requires combinational therapy to provoke an immune response, for instance by employing vaccines to enhance the accumulation of lymphoid aggregates36. Thus, TME, on account of high levels of immunosuppression and poor immunogenicity, provides a unique challenge in PCa immunotherapy36. The significant milestones for the expression profile of immune cell and ICPs in the TME are pointed out in Fig. 1.

2. Immune checkpoint pathways

2.1. CTLA-4 pathway

Activation of T cells is a complex process and requires more than one activating signal. Binding T cell receptor (TCR) to major histocompatibility complex (MHC) is vital for T cell activation, and other required costimulatory signals. In order to activate the stimulatory signal in T cell, B7-2 (CD86) or B7-1 (CD80) molecules bind to CD28 molecules on the APCs. Adequate concentrations of CD28 for binding to B7 1/2 (CD80/CD86) leads to generation of T cell with improved endurance, differentiation in the enhanced cytokines structure like interleukin-2 (IL-2), adjustment of durability of cell for genes and improved energy metabolism. CTLA-4 delivers a negative regulatory signal to the T cell through binding to B7 1/2 molecules. CTLA-4, a CD28 homologue, shows higher binding affinity to B737,38; however, the binding of CTLA-4 to B7 withholds a stimulating signal through CD28 reverse. The competition binding can block the stimulatory signal generated by CD28–B7 binding39–40. Furthermore, the corresponding quantity of CD28–B7 versus CTLA-4–B7 restricts the activation or energy of a T cell16. In addition, some data suggest that CTLA-4–B7 can truly provide inhibitory signals which prevent CD28–B7 and TCR-MHC binding stimulating signals3,42. CTLA-4 location within the cell is under a controlled mechanism and initially located in the intracellular segment of resting naive T cells40,41,42. Consequently, the stimulatory signals, including both TCR and CD28–B7 binding, trigger upregulation of CTLA-4 through CTLA-4–included vesicles egressing on the cell surface42, which explains how TCR signals extract high translocation of CTLA-4 in a categorized feedback loop to the cell surface. Whereas, inhibition of IL-2 production as well as stimulation of the cell cycle progression has limited CTLA-4–B7 binding to fully activate of T cells45. T regs regulate the effector T cells’ activity and are indispensable to maintain peripheral tolerance46. T regs constitutively express CTLA-4 unlike effector T cell, thus, this is deemed crucial for their suppressive capabilities46. In the animals, the absence of CTLA-4 on Treg impaired their suppressive ability46,48. The downregulation of CD80 and CD86 on APCs is one of Treg’s mechanisms to regulate the function of effector T cells48,49.

2.2. PD-1 pathway

PD-1 as a part of the B7/CD28 costimulatory family regulates T cell activation by binding to their ligands, PD-L1 and PD-L2. Unlike CTLA-4, binding to PD-1 blocks the generation of stimulating cytokines, such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and IL-2, which lead to decrease in the activity of T cells50. Where T cell activation coincides with TCR–PD-1 binding, the phosphorylation of intermediate TCR signals is inhibited by PD-1 generated signals, which with terminating TCR signals result in decrease T cell activation41,51. Expression of PD-1 indicates “exhausted” T cells that practice extreme rates of stimulation or reduced CD4+ T cell support52. Dysfunction of T cell which is happening in the suboptimal administration of tumors and some diseases, identifies this stage of fatigue that occurs when chronic infections and cancer occur. Both CTLA-4 and PD-1 receptors have proven negative effects on T cell activation; however, the timing of downregulation, efficiency of negative signaling pathway and the anatomical regions of inhibition are different17,26. While CTLA-4 utilizes T cell activation in the priming state, PD-1 operates at the point of the effector, predominantly within peripheral tissues50. For CTLA-4, the combination of PD-1 ligands varies too. The B7 ligands for CTLA-4 are expressed by special APCs that usually stay in the lymph nodes or spleen, although PD-1 and PD-L2 are expressed in T cell and, APC and more widely cancer cells, respectively17,29,35,45. PD-L1 is expressed on leukocytes, non-hematopoietic cells, non-lymphoid tissues as well as in parenchymal cells through tumorigenic signals or inflammatory cytokines55. Expression of PD-L1 is differently identified in several tumor types and is incorporated with the increase and decrease of the amount of TILs and prognosis, respectively55,56. The expression of PD-L2 is considerable in DCs and monocytes and can be triggered depending on the microenvironment by a broad range of particular immune and non-immune cells55. Binding affinity of PD-1 for PD-L2 compared to PD-L1 showed unique tendency, which can be efficient for the differential immune response involvement of the ligands55. The interactions of PD-1 with PD-L1 and PD-L2 are developed to make tolerance within infiltrated tissue around due to PD-1 ligands are expressed in peripheral tissues. One type of contradictory roles of PD-L1 and
PD-L2 on natural killer (NK) T cells activation includes improved Th2 activity through restriction of PD-L2 binding points, although CD80 binding to PD-L1 was provided to hinder T cell responses. The multiple biologic effects lead to differences in the toxicity and activity for PD-1-directed antibodies which prevent to bind both ligands as compared to interaction of PD-1 with PD-L1. Despite Treg expression on both PD-1 and CTLA-4, the purpose of PD-1 expression remains dubious. PD-L1 not only provides Treg with the exchange of naïve CD4+ T cells, but also prevents T cell responses by enhancing Treg induction and maintenance. Similar to these results, the blockade of PD-1 is capable of inverting the Treg-mediated defeat of the effector T cells in vivo. The immune response is reduced by PD-1 binding to its ligands on T cells, which are busied on the effector T cell responses. This equal restriction of T cell activation, which is linked to CTLA-4 blockade, may describe immune related adverse events through the potentially inferior occurrence of PD-1, equivalent to a CTLA-4 blockade. Comparisons between CTLA-4 and PD-1 demonstrate that they are both B7 receptor family components, expressed by activated T cells, expression affected by the strength and/or continuity of TCR signaling, decreased T cell proliferation, glucose metabolism, cytokine manufacturing, durability and also arranging an extended T cell proliferation.

However, there are such differences include CTLA-4 expressed through T cells, whereas PD-1 expressed through T cells and other immune cells and further CTLA-4 first restricts T cell responses, originally on lymphoid tissues, whereas PD-1 limits T cell response anywhere usually. In peripheral tissues, PD-1 action clashes with more T cell signaling mechanisms than CTLA-4 ligands expressed by professional antigen-presenting neurons. PD-L1/2 expressed by APCs and other immune cells mostly, although the ligands can be expressed on non-immune cells such tumor cells. On the other hand, CTLA-4 inducing Treg functioning, thus, the function of PD-1 on Treg is uncertain.

3. Potential predictive biomarkers

3.1. CTLA-4

The structural similarity of CTLA-4 to CD28, let this inhibitory checkpoint the ability to interact with CD80 and CD86 but in a stronger affinity than CD28. CTLA-4 can mediate the suppression of T cells through several mechanisms. First, CTLA-4 plays an efficient role to reduce CD28 co-stimulation, and its interaction with CD80/86 of APCs can decrease APCs stimulatory role in the T cells activation. Second, expression of CTLA-4 on Treg which gives them the ability to inhibit T cells function. Third, inhibition of TCR genes and CD28-induced genes, decreased T cell proliferation, glucose metabolism, cytokine manufacturing, durability and also arranging an extended T cell proliferation.

In the case of PDA, it was shown that anti-CTLA-4 approach did not provide an optimal clinical benefit and anti-tumor function neither in human nor in cancer models. However, a study stated that there was an induction of the infiltration of CD4+ T cells in the TME, as a result of anti-CTLA-4 approach, which has no remarkable changes in the migration of CD8+ T cells. This is in total contrast with the approach of anti-CTLA-4 therapy for melanoma patients, which showed a significant increase in infiltration of CD8+ T cells. Thus, it can be suggested that CTLA-4 implicates the recruitment of CD4+ T cell in PDA patients. Like these findings, Bengsch et al. exhibited that anti-CTLA-4 therapy promotes T cell infiltration into the TME as well as T cell activation. However, this approach was not clinically useful in PDA patients. The interesting observation was the influence of CD80 inhibition in the induction of T cell recruitment into the TME. Consequently, the interaction of CTLA-4 and costimulatory molecules (CD80/86) can regulate the migration of T cells to the TME. Basso et al. demonstrated that despite expression of PD-L1 and CTLA-4 in splenic DCs, no PDA patients showed increased levels of both PD-L1 and CTLA-4 expressions. They observed that, for the first time, the S100A8/A9, also known as calprotectin, could downregulate CTLA-4. Intriguingly, CTLA-4 negative DCs diminished T cell proliferation. DCs were also shown to augment the inhibitory role of Treg in the inhibition of allogeneic T cell responses through their CD80. This is while CD86 showed opposing function on the Treg activity.

3.2. PD-1

Activated monocytes, DC, NK, T and B cells express another important ICP called PD-1. Like to CTLA-4, the responsibility of PD-1 is to regulate T cell responses. Domain-containing phosphatase-1 and 2 (SHP-1&2) affiliated with immunoreceptor tyrosine-based switch motif (ITSM) of PD-1 for stimulation tyrosine-based switch motif (ITSM) of PD-1. This is while ligation of PD-1 inhibit T cell activation. It was shown that PD-1 acts as an inhibitory molecule in the primary phases of activation of T cells while the interaction of PD-1 and its ligands mainly happens on activated effector T cells in the periphery. In the TME of PDA, the PD-1 expression has predominantly happened in TILs, which leads to immune escape by tumor cells. However, recent research presented PD-1 expression in PDA tumor cells. It was demonstrated that the amount of PD-1, which was expressed on CD8+ T cells elevated in tumor tissue where this expression was significantly correlated with the clinical stage of PDA patients. Indeed, higher expression of PD-1 in PDA tumor tissue was considered as a poor prognostic factor. However, another investigation disclosed elevated stromal PD-1+TILs as a decisive prognostic factor. In fact, they suggested a correlation between intraepithelial and peripheral compartment of PD-1+TILs infiltration with better progression-free survival (PFS) and distant metastases-free survival (DMFS). Nevertheless, investigations on a diversity of cancers such as renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), and melanoma demonstrated an enhanced PD-1 expression, which was affiliated with disease with poor prognosis. Thus, there is a controversy about the role of PD-1 as a satisfactory target for the targeted ICP therapy. It was demonstrated that PDA patients with elevated PD-1 expression and dense stroma were associated with better OS. Interestingly, the stroma of PDA, is observed to have a role in the upsurge of immune cell migration and accumulation, which led to a boosted inflammatory response. The expression of PD-1 in peripheral blood stays stable even after surgical treatment and this stable PD-1 level of peripheral CD8+ T cells was shown to be associated with higher PDA recurrence.

3.3. PD-L1

As conferred, PD-L1 is one of the inhibitory molecules expressed in solid tumors, DCs and MQs of the TME. It was shown that PD-L1 from T γδ cells of PDA could inhibit tumor-specific cytotoxic T lymphocytes and Th1 cells. Interesting
data revealed that not all but some the PD-L1 producing cells have the ability to suppress T cells via binding to PD-1. In general, the B7 family consists of PD-L1 (B7-H1) and PD-L2 (B7-H2). Although there is still a need for further studies to understand the differences between the practical function of PD-L1 and PD-L2, it was shown that PD-L1 is the primary molecule that is presented in solid tumors. PD-L1 can bind to PD-1, CD80 of T cells, and APCs. This binding leads to apoptosis, energy exhaustion, and finally inhibition of effector T cells. In the case of PDA, PD-L1 was demonstrated to have the ability to upregulate Treg infiltration and to induce immune suppression. The expression of PD-L1 in PDA was shown to be associated with tumor growth, drug resistance, and finally to high tumor invasion. Another study in human PDA showed that PD-L1 and PD-L2 were expressed in cancerous pancreatic tissue. There was also a negative correlation between PD-L1 expression and CD8+ T cells. Furthermore, 20 out of 51 PDA patients who were PD-L1 positive showed poor prognosis. The inhibition of blocking colony-stimulating factor 1 (CSF-1) and its receptor resulted in an increase in CD4+CD8+ T cells infiltration and the expression of PD-L1. Similarly, high expression of HLA class I with PD-L1-PDA was indicated to be associated with high CD8+ T cells infiltration and good prognosis. It was suggested that the presence of HLA-I probability possess the potential to promote immunostimulatory condition, whereas PD-L1 could induce the immunosuppressive one. Thus, the immunological response of PDA pertains to the balance of the TME between the two discussed conditions. Intriguingly, there was an association between the expression of PD-L1, low tumor differentiation, and diminished advanced tumor stage. However, PD-L1 expression correlates with the existence of immunosuppressive cells.

It was shown that the remarkable function of PD-L1 happened in the early stages of the disease, while it was previously shown that both PD-L1 and PD-L2 had their strong effect in the advanced stages of esophageal cancer. This is while a correlation exists between the expression of PD-L1 at high levels in PCa and the metastasis of lymph node. In another study, researchers reported that there might be an association between miss match repair (MMR) system deficient-cancer and the anti-PD-L1 treatment. This is a result of the fact that this deficiency promotes the production of multiple neo-antigens, which are further targeted by the augmented immune system.

### 4. Targeting immune checkpoints

Tumor cells can grow fast and spread in part by targeting the immune system of the host. During the past decade, immunotherapy has emerged as an effective and standard method of treating different cancers. Even though, different treatment strategies, such as surgery and conventional chemotherapies, have prolonged patient survival and they do not inhibit restorative. Consequently, new treatment strategies are indispensable. Over the past decade, cancer immunotherapy has paved the path from a promising preclinical use to a clinical reality. Manipulation of ICPs is one of the most current promising strategies for cancer therapy, therefore clinical studies confirm that inhibition of ICPs disarranges adverse immune regulations and induces immune system as well as anti-tumor activities. For this purpose, mAbs target inhibitory ICPs and show significant results in different cancers. Since they are accepted by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), ICP blockade drugs have been developed for application to treat different cancers. Accordingly, it could also be a highly effective therapy against PCa. In this part, the latest advances and future challenges of ICP inhibitors application in PCa are being summarized. Pivotal ICPs in PCa and their interplay with their specific ligands have been shown in Fig. 2.

#### 4.1. CTLA-4 immune checkpoint blockade

Recently, CTLA-4, the first clinically targeted ICP receptor, has been studied extensively in cancer immunotherapy. According to studies conducted in the late 90s, the opposing effect of CD28 and CTLA-4 has demonstrated on T cell activation in vitro, whereas blocking CD28 and CTLA-4 have declined and intensified anti-tumor responses, respectively. Indeed, monotherapy-based mice treated with anti-CTLA-4 antibodies led to the complete tumor elimination and durable immunity. Subsequently, mechanistic studies showed that this anti-tumor function was related to the augmented ratio of both CD4 and CD8 effector cells to FoxP3+ (forkhead box P3). Treg, Iplimunumab (Ipi) and tremelimumab (Tre), two human anti-CTLA-4-antibodies, have been applied clinically in PCa.

![Figure 2 Pivotal ICPs in PCa and their interaction with specific ligand](image-url)
4.1.1. Ipilimumab

Ipi, a fully humanized IgG1 kappa mAb, was the first drug developed against CTLA-4 \textsuperscript{[118]}. In 2011, Ipi was approved by the FDA to treat melanoma and is now under clinical evaluation in various tumors \textsuperscript{[119]}. Royal et al. \textsuperscript{[120]} investigated the effect of Ipi in the regression of advanced pancreatic adenocarcinoma (APA) during the phase II study. Twenty-seven people with APA received 3.0 mg intravenous Ipi, following which response evaluation criteria in solid tumors (RECIST) and toxicity were measured. This study found that the above mentioned dose-scheme of Ipi is not effective in PCa and suggested the use of higher dosages of Ipi at earlier stages of the disease, possibly with combinatorial agents \textsuperscript{[120]}. Granulocyte-macrophage colony-stimulating factor (GM-CSF) of cell-based vaccines have been discovered to provide a synergistic activity to anti-CTLA-4 antibodies. Therefore, in a phase Ib of a study, Ipi has effectively been combined with GM-CSF cell-based vaccines GVAX (a cancer vaccine containing whole tumor cells which modified to secrete the immune stimulatory agents). In this study, thirty patients with PCa were listed and Ipi (arm 1) and Ipi + GVAX (arm 2) were examined \textsuperscript{[121]}. Following the administration, fifth patients indicated stable disease, and seven patients indicated CA19-9 reduction. An enhancement in OS was detected that was related to the clinical activity in the combination arm. In conclusion, this study showed that checkpoint blockade plus GVAX is a potential candidate for checkpoint immunotherapy, and requires assessment in larger studies \textsuperscript{[121]}. Kamath et al. \textsuperscript{[122]} tested a phase Ib clinical trial of CTLA-4 inhibition with Ipi in combination with gemcitabine (Gem) in patients with advanced PCa. This study established a maximum tolerated dose for Gem and Ipi with a good overall safety profile. Although the observed response rate was similar to Gem alone, the durability of responses suggests a component of immune activation that may warrant further investigation \textsuperscript{[122]}. Moreover, Parikh et al. \textsuperscript{[123]} conducted a phase II study of Ipi and Niv with radiation in metastatic PCa. Dual blockade of CTLA-4 and PD-1 with radiation is feasible and reveals hopeful action in these patients.

4.1.2. Tremelimumab

Tre is an anti-CTLA-4 mAb, which is assessed in melanoma, colon cancer, gastric cancer, and mesothelioma in clinical trials. Tre possesses a greater affinity toward CTLA-4 than CD28. \textit{Ex vivo} studies of patient samples with expanded and metastatic melanoma disclosed that application of Tre decreases tumor development via considerable stimulation of cytotoxic T cell activity \textsuperscript{[125]}. A phase I study in PCa patients, analyzed the tolerability, safety, and maximum tolerated dose of Tre plus Gem. This combination indicated a manageable safety and tolerability profile and prolonged OS \textsuperscript{[125]}. A recent study investigated durvalumab (Dur) with or without Tre for patients with metastatic PDA. Treatment was well tolerated, and the efficiency of Dur plus Tre therapy and Dur monotherapy presented a population of patients with mPDA who had poor prognoses and quickly developing disease \textsuperscript{[127]}. Moreover, a phase II study is testing Dur alone or Dur plus Tre in metastatic PCa \textsuperscript{[128]}. Furthermore, a study of hypofractionated radiotherapy in combination with Dur and Tre in metastatic PCa patients is underway \textsuperscript{[129]}. The second ICP receptor is PD-1, which appeared as a marker for successful cancer immunotherapy \textsuperscript{[39]}. It interacts with the PD-L1 and PD-L2 ligands and stops T cell induction \textsuperscript{[130]}. PD-1/PD-L1 axis enables tumor evasion from the immune responses, and this interaction blockade with anti-PD-1 as well as anti-PD-L1 can augment the tumor immunity. Moreover, the proliferation of CD8$^+$ T cell and production of cytokine can be induced by this blockade \textsuperscript{[131]}. Numerous PCa-related anti-PD-1 and anti-PD-L1 antibodies have been assessed in clinical trials. Nivolumab (Niv), pembrolizumab (Pem), and pidilizumab (Pid) are the antibodies that block PD-1. On the other hand, Dur and BMS-936559 are PD-L1 targeted antibodies \textsuperscript{[132]}. 4.2. PD-I/PD-L1 immune checkpoint blockade

The second ICP receptor is PD-1, which appeared as a marker for successful cancer immunotherapy \textsuperscript{[39]}. It interacts with the PD-L1 and PD-L2 ligands and stops T cell induction \textsuperscript{[130]}. PD-1/PD-L1 axis enables tumor evasion from the immune responses, and this interaction blockade with anti-PD-1 as well as anti-PD-L1 can augment the tumor immunity. Moreover, the proliferation of CD8$^+$ T cell and production of cytokine can be induced by this blockade \textsuperscript{[131]}. Numerous PCa-related anti-PD-1 and anti-PD-L1 antibodies have been assessed in clinical trials. Nivolumab (Niv), pembrolizumab (Pem), and pidilizumab (Pid) are the antibodies that block PD-1. On the other hand, Dur and BMS-936559 are PD-L1 targeted antibodies \textsuperscript{[132]}.
4.2.3. **Pdiluzumab**
In recent years, Pd as another anti-PD-1 ICP inhibitor has received consideration. It is a humanized IgG4κ mAb. In PDA, two clinical trials, were administered imperfectly, and stopped henceforth.

4.2.4. **Durvalumab**
Dur is also another mAb of the IgG4κ isotype which targets PD-L1, stops the binding of PD-L1 to the PD-1 and CD80 molecules. In a recent study, Duffy et al. evaluated the impact of combination therapy of radiation together with Tre and/or Dur in PCa patients. This study showed that radiation could intensify the impact of ICP stopping in these patients. Dur with or without Tre in metastatic PCa patients was performed in phase II study. Additionally, a phase Ib/II study assesses the safety and efficacy of Dur plus ibrutinib (BTK inhibitor) in PCa patients. Currently, a phase Ib study examined the combination of galunisertib (Gal) and Dur in recurrent or refractory metastatic PCa. The combination of Gal plus Dur had a suitable tolerability and safety profile. The effect of this combination in second and third line PCa patients warrants additional attention. The study of Dur and stereotactic radiotherapy in locally advanced PCa was safe, well tolerated and appears to be clinically active with high rates of margin-negative resection. Cassier et al. performed a phase I dose escalation research to assess the safety and clinical function of a combined treatment relating an anti-CSF1R (pexidartinib) with Dur in patients with advanced/metastatic PCa. Toxicity was consistent with the expected profiles of the individual drugs and no unpredicted findings were observed with the combination.

4.2.5. **Status of dMMR/MSI and PD1/PD-L1 and CTLA-4 antibodies**
The mismatch repair (MMR) system plays an important role in repair of DNA sequence during replication. Deficiency in the MMR system (dMMR) or lack of performance of one of the MMR proteins, including MLH1, MSH2, MSH6 and PMS2, lead to an accumulation of somatic mutations, resulting in microsatellite instability (MSI) and a higher neoantigen load that enhances proinflammatory cytokines and activation of T cells. Given the recent tissue-agnostic approval of Pem, MSI testing is now recommended by the National Comprehensive Cancer Network (NCCN) for locally advanced or metastatic PDA. A recent study of MSI status in PDA using next generation sequencing (NGS) revealed that dMMR occurs at a low frequency of 0.8% in PDA, and all these cases also have Lynch syndrome. Furthermore, Yamamoto et al. and Macherla et al. examined the prognostic impact of MSI in PCa cases and found that MSI positives patients had longer survival time than negative ones.

PD-L1 overexpression and dMMR/MSI status could indeed be useful predictive biomarkers for the response to immunotherapy. For this purpose, Kim et al. analyzed both PD-L1 and MLH1/MSH2 expressions and showed a remarkable association between PD-L1 expression and MLH1/MSH2 loss. Moreover, Salem et al. studied the correlation between tumor mutational load (TML), dMMR, and PD-L1 and found a lower frequency of TML-high in PDA, and a positive PD-L1 expression in MSI-H and MSS PDA cases at about 11.1% and 9%, respectively. The majority of pancreatic adenocarcinoma patients with either MSI-H or MSI-L showed low TML level. Future studies are needed to indicate the suitability of PD-L1, MSI, and TML as appropriate predictive markers of response to immunotherapy in PDAC.

Pem was recently evaluated in clinical trials in heavily pretreated patients with MSI-H tumors. Interestingly, these studies demonstrated the benefit ICB in patients with MSI-H tumors regardless of their PD-L1 status. Furthermore, Niv, as a PD-1 inhibitor, which is approved for progressed MSI-H/dMMR metastatic colorectal cancer (CRC) following first-line treatment, indicated that dMMR plays a robust predictive of response to ICB in comparison to PD-L1. Furthermore, the activated V-domain immunoglobulin suppressor of T cell activation (VISTA) which is expressed on the MQs pathway, decreases T cell responses in the tumor at a greater rate compared to PD-L1 blockade. Therefore, PD-1/PD-L1 blockade might breakdown PDA treatment due to suppression of the immune response through an untreated VISTA pathway. Enhancement of T cell infiltration, using anti-CTLA-4 mAb with anti-VISTA antibody to target MQs as combination therapy, holds a promising new strategy for PDA treatment.

4.2.6. **BMS-936559**
BMS-936559, a humanized IgG4 and PD-L1-specific mAb, can prevent the interaction of PD-L1 with both PD-1 and CD80. In NSCLC, RCC and melanoma patients, durable tumor regression and long-term disease stabilization were observed by PD-L1 blockade; however, in PCa, no objective response was detected.

4.3. **LAG-3 immune checkpoint blockade**
LAG-3 or CD223, a homolog of CD4, was cloned over 25 years ago. The negative regulatory role for LAG3–MHC-II interaction is the most prominent characteristic of LAG-3, and this fact represents LAG-3 as a potential treatment target. In 2006, targeted immunotherapy through LAG with a soluble LAG-3Ig fusion protein (IMP321) was introduced. While, IMP321 was tested in other novel clinical trials, in RCC, metastatic breast carcinoma, and melanoma previously with average success. It is still being tested in other novel clinical trials, where it may reveal additional therapeutic advantages. LAG-3 was shown to synergize with PD-1 in downregulating T cell activities and stimulating immune evasion by cancer cells. Even though, targeting of LAG-3 alone showed little effect, and blockade of LAG-3 has been revealed to synergize with PD-L1 and augment its anti-tumor effect. These trials are investigating the application of combination anti-LAG-3 and anti-PD-1 versus anti-LAG-3 alone in diverse solid tumors. These results have received certain interest in perspective, hence LAG-3 will be considered as the promising targeted immunotherapy and predictive biomarker. Table summarizes the clinical trials of targeted ICPs in PCa.

5. **In vitro studies of immune checkpoint blockade**
The expression of PD-L1 protein is typically limited to MQ lineage in human and it has the capacity to be induced in B cells, tumor cells such as PCa, as well as other hematological cells, and non-lymphatic tissues. Furthermore, PD-L2 expression is inducible for MQs and DCs. A study was conducted in Panc-02 cells, where they were directly injected into the pancreas. It was shown that blocking antibodies against B7–H1 could suppress tumor growth. Studies in the MiaPaCa-2 and Su86.80 cell lines showed that the anti-inflammatory cytokines...
such as IL-10 resulted in a modest decrease in mRNA level of PD-L1 in PCa cells. In contrast, treatment with IFN-γ upregulated mRNA level of PD-L1.120 Following semi-spleen implantation of tumor Panc-02 cells, which are treated with cyclophosphamide (Cy) and GVAX or αPD-1/αPD-L1 therapy, it showed elevated amounts of IFN-γ secreted from CD8+ T cells and tumor-specific CD8+ T cells in the TME. CD8+ T cells isolated from spleen and TILs with tumor Panc-02 cells as antigenic targets were treated with anti-PD-1, Cy + GVAX + IgG, and Cy + GVAX + anti-PD-1, showing enhanced amounts of IFN-γ secreted from CD8+ T cells in incremental order compared to the untreated cells.180 A syngeneic orthotopic tumor Panc-02 cells poorly responded for blockage of PD-L1 and CTLA-4 as well as Gem, while CD40 agonist antibody (aCD40) treatment significantly delayed tumor growth and increased survival. It also drove maturation of myeloid cells and expansion of memory T cell in spleen and upregulated the PD-L1 mRNA expression in Pan-02 tumors. The combination of aCD40 with aPD-L1 resulted in a significant upsurge of OS rate compared to either agent of the alone treatment trials.181 Many of the cancer studies have observed tumor cells via types I and type II IFN signaling pathways upregulated PD-L1 expression in human and murine PCa cell lines. In many studies, nivolumab on PANC-1 cell lines, ruxolitinib (JAK–STAT pathway inhibitor) on PANC-02-H7 cell and 5-fluorouracil, Gem or paclitaxel (upregulate cell surface PD-L1 expression) on AsPC-1, Mia PaCa-2 and Pan-02 cells have a significant influence in downregulation of PD-1/PD-L1 protein levels on the surface of tumor cells. The combined effect of these drugs and IFNs indicated that PD-L1 is activated through the JAK–STAT1 pathway by both type I and II IFNs. It also uncovered that JAK–STAT pathway inhibition increases the effectiveness of anti-PD-1 immunotherapy to suppress the growth of PCa. Both type I and II interferon cytokines engage JAK–STAT signaling pathways. The expression of PD-L1 is a primary limiting factor for CTL activities in gastric cancers and is significantly upregulated by IFN-γ exposure.184,186,187 Taken together, these findings suggest that PD-1/PD-L1 might be a critical target for controlling the growth of PCa.

Table 1  Clinical trials of targeted ICP in Pca.

| Target    | Drug                                         | Phase | Status            | Clinical Trials identifier   | Ref. |
|-----------|----------------------------------------------|-------|-------------------|------------------------------|------|
| CTLA-4    | Ipilimumab                                    | II    | Completed         | NCT0012580                   | 120  |
|           | Ipilimumab + GVAX                            | I     | Completed         | NCT00836407                  | 121  |
|           | Ipilimumab + Gemcitabine                     | II    | Recruiting        | NCT01896869                  | 175  |
|           | Ipilimumab + nivolumab with radiation        | I     | Recruiting        | NCT03104439                  | 123  |
|           | Tremelimumab (CP-675,206) + gemcitabine      | I     | Recruiting        | NCT00556023                  | 128  |
|           | Tremelimumab + durvalumab                    | II    | Recruiting        | NCT02598894                  | 127  |
| PD-L1     | Pembrolizumab + REOLYSIN + chemotherapy      | II    | Recruiting        | NCT02620042                  | 137  |
|           | Pembrolizumab + ACP-196                      | I/II  | Active but not recruiting | NCT02362048              | 136  |
|           | Pembrolizumab (MK3475)                       | I     | Recruiting        | NCT02305186                  | 135  |
|           | Pembrolizumab + PLX3397                      | I     | Recruiting        | NCT02452424                  | 134  |
|           | Pembrolizumab + oncolytic virus pelareorep   | I     | Recruiting        | NCT02620423                  | 138  |
|           | Pembrolizumab + PEGPH20                      | II    | Recruiting        | NCT03634332                  | 139  |
|           | Pembrolizumab + NOX-A12                      | II    | Recruiting        | NCT03168139                  | 176  |
|           | Nivolumab + GVAX + cyclophosphamide          | I/II  | Active but not recruiting | NCT02451982              | 144  |
|           | Nivolumab + Nab-paclitaxel + gemcitabine     | I     | Recruiting        | NCT02309177                  | 143  |
|           | Nivolumab + GVAX + CRS-207                   | II    | Recruiting        | NCT02243371                  | 142  |
|           | Nivolumab + cabiralinum + chemotherapy        | II    | Active but not recruiting | NCT03336216            | 145  |
|           | Nivolumab + SD-101                          | I     | Recruiting        | NCT04050085                  | 146  |
| PD-L1     | BMS-93659                                     | I     | Completed         | NCT00729664                  | 9,166|
|           | Durvalumab + ibrutinib mesylate               | Lb/I  | Recruiting        | NCT02403271                  | 150  |
|           | Durvalumab + galunisertib                    | I     | Completed         | NCT02734160                  | 151  |
|           | Durvalumab + stereotactic radiotherapy       | I/II  | Recruiting        | NCT03245541                  | 152  |
|           | Durvalumab + pexidartinib                    | I     | Completed         | NCT02777710                  | 153  |
| PD-L1, CTLA-4 | Tremelimumab + MEDI4736                     | I     | Recruiting        | NCT02311361                  | 149  |
|           | Durvalumab + tremelimumab                    | II    | Recruiting        | NCT02558894                  | 129  |

LA-G3 which belongs to immunoglobulin (Ig) superfamily is expressed on activated T cells, NK cells, B cells, and plasmacytoid DCs. Although LA-G3 acts as target of different drug development programs, it has diverse biological effects on T cell functions.167,190–193 The binding of LA-G3 to MHC class II (its ligand) has a higher affinity than CD4 (immune cell surface glycoprotein).194 LA-G3 helps to maintain the tolerogenicity of CD8+ T cells and CD8 exhaustion due to chronic infection, contributing to the maturation as well as activation of DCs, and like CTLA-4 and PD-1, it negatively regulates proliferation, activation, and homeostasis of T cells,191,196,197 therefore, plays a vital role in the suppression of Treg function.198 Co-inhibitory molecules, including IL-12 and TGF-β, which play the role of induction and suppression of LA-G3 and PD-1, respectively, block ICPs to reverse pathogenic Treg. In addition, the effects of co-inhibitors on NK cells indicate different expression in response to cytokine stimulations of IL-15 at least, demonstrating the regulatory role of the co-inhibitors on human NK cells. Furthermore, IL-15 can promote NK cell-mediated killing in pancreatic stellate cells (PSC).199,200

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6. siRNA as immune checkpoints inhibitors

siRNAs, a group of regulatory therapeutic factors, were rabidly designed to combat various diseases, including cancer, neurodegenerative disorders, and infectious diseases. The application of siRNA in PCa to target immune checkpoints was studied in some investigations. A recent study used a PD-L1-specific siRNA conjugated to a magnetic nanocarrier (MN-siPDL1) in combination with gemcitabine as a therapeutic method in murine PCa models. The strategy reduced the tumor growth and elevated the 90% tumor volume reduction was attained. This is while 100% of the control group animals were observed with a tumor by 6 weeks after starting the treatment.

Although the failure of anticancer vaccines in PCa patients, a multilateral effort to develop new vaccines demonstrates an efficient efficacy in case survival. Several strategies have been developed to improve the efficacy and safety of tumor vaccines. These include single immunotherapy approaches such as breaking immunosuppression within the tumor microenvironment and overcoming tolerance to TAA, as well as combinatorial immunotherapy approaches such as the combination of anticancer vaccines with ICP inhibitors and chemotherapy, radiation therapy or even surgery. Recent findings suggest a multipronged approach for therapeutic efficacy, including various types of agents such as vaccines or oncolytic viruses and additional agents to prime the immune microenvironment, followed by ICP inhibitors. Despite the lack of an effective tumor vaccine, a dozen clinical trials are ongoing. For instance, use of heterologous prime-boost followed by a low dose of Cy/GM-CSF gene transfected tumor cell (GVAX) and live-attenuated Listeria monocytogenes-expressing mesothelin (CRS-207) has extended patient survival with minimal toxicity through enhancement of innate and adaptive immunity.

7. Conclusions and perspectives

The adverse effects caused by immunotherapy in the order of their importance include neutropenia, nausea and vomiting, alopecia, fatigue, and thrombocytopenia. Despite of the adverse effects, high levels of immunosuppression in the TME and poor immunogenicity provide significant challenges to prosperous immunotherapy of human PCa. On the other hand, ICPs have been discovered to perform an axial role in the establishment of tumor-induced immunosuppression in the TME. Therefore, targeting of ICPs is suggested to be a hopeful strategy for improving the therapeutic efficacy of PCa immunotherapy. CTLA-4 and PD1/PD-L1 are the most well-known ICPs that have been targeted for overcoming immunosuppression in the TME and induction of effective anticancer immune responses in PCa. The results of clinical studies in patients showed the safety and superior therapeutic efficacy of combination therapy with anti-CTLA-4 and anti-PD1 or anti-PD-L1 mAbs in PCa. Despite the encouraging results, several clinical studies showed poor response to checkpoint blockade with anti-CTLA-4 and anti-PD-1 or anti-PD-L1 immunotherapies in PCa patients due to resistance to CTLA-4 and PD-1/PD-L1-targeted therapies. The lack of significant clinical response to PD-1 or CTLA-4-targeted therapy in PCa is possibly due to the presence of unknown and new emerging ICPs that may be involved in tumor-induced immunosuppression and our incomplete understanding of the function of immune cells and molecules in the TME. Our continued progress toward understanding the immunobiology and targeting of ICPs in this type of human malignancy, as discussed in the present article, promises a new hope in the treatment of PCa in the future.

Author contributions

Seyed Hossein Kiae and Behzad Baradaran designed the work. Seyed Hossein Kiae, Mohammad Javad Sanaei, Masoud Heshmati, Zahra Asadzadeh, and Saleh Hadidi collected data and wrote the manuscript. Seyed Hossein Kiae and Mohammad Javad Sanaei designed and regenerated the conceptual pictures. Seyed Hossein Kiae, Iman Azimi and Reza Jafari checked and revised the article. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Khorana AA, Mangu PB, Berlin J, Engerbretson A, Hong TS, Maitra A, et al. Potentially curable pancreatic cancer: American society of clinical oncology clinical practice guideline update. J Clin Oncol 2017;35:2324–8.
2. Kleeff J, Korc M, Apte M, La Vecchia C, Johnson CD, Biankin AV, et al. Pancreatic cancer. Nat Rev Dis Primers 2016;2:16022.
3. De La Cruz MSD, Young AP, Ruffin MT. Diagnosis and management of pancreatic cancer. Am Fam Physician 2014;89:626–32.
4. Hidalgo M. Pancreatic cancer. N Engl J Med 2010;362:1605–17.
5. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol 2012;24:207–12.
6. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016;351:1463–9.
7. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436–44.
8. Rychsch E, Nötzel T, Hinz U, Autschbach F, Ferguson J, Simon I, et al. Control of T-cell-mediated immune response by HLA class I in human pancreatic carcinoma. Clin Cancer Res 2005;11:498–504.
9. Brahmer JR, Tykodi SS, Chow LQM, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455–65.
10. Kerkar SP, Restifo NP. Cellular constituents of immune escape within the tumor microenvironment. Cancer Res 2012;72:3125–30.
11. Hodi FS, O’Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711–23.
12. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol 2009;10:29–37.
13. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002;8:793–800.
14. Dyck L, Mills KHG. Immune checkpoints and their inhibition in cancer and infectious diseases. Eur J Immunol 2017;47:765–79.
15. Johansson H, Andersson R, Bauden M, Hammes S, Holdenrieder S, Ansari D. Immune checkpoint therapy for pancreatic cancer. World J Gastroenterol 2016;22:9457–76.
16. Alegre ML, Frauwirth KA, Thompson CB. T-cell regulation by CD28 and CTLA-4. Nat Rev Immunol 2001;1:220–8.
17. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunol Rev 2008;224:166–82.
18. Zhao Y, Yang W, Huang Y, Cui R, Li X, Li B. Evolving roles for targeting CTLA-4 in cancer immunotherapy. Cell Physiol Biochem 2018;47:721–34.
19. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated anti-tumor immunity. Nat Med 2003;9:562–7.
20. Loos M, Giese NA, Kleeff J, Giese T, Gaida MM, Bergmann F, et al. Clinical significance and regulation of the costimulatory molecule B7-H1 in pancreatic cancer. Cancer Lett 2008;268:98–109.
21. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med 2009;206:3015–29.
22. Wang L, Pino-Lagos K, de Vries VC, Guleria I, Sayegh MH, Noelle RJ. Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3+CD4+ regulatory T cells. Proc Natl Acad Sci U S A 2008;105:9331–6.
23. Dyck L, Wilk MM, Raverdeau M, Misiak A, Boon L, Mills KHG. Anti-PD-1 inhibits Foxp3+ Treg cell conversion and unleashes intratumoral effector T cells thereby enhancing the efficacy of a cancer vaccine in a mouse model. Cancer Immunol Immunother 2016;65:1491–8.
24. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intratumoral effector T cells thereby enhancing the efficacy of a cancer vaccine in a mouse model. Cancer Immunol Immunother 2016;65:1491–8.
25. Gao Q, Qiu SJ, Fan J, Zhou I, Wang XY, Xiao YS, et al. Intratumoral balance of regulatory and cytotoxic T cells is associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A 2005;102:18538–43.
26. Angelova M, Charoentong P, Hackl H, Fischer ML, Snajder R, Krogsdam AM, et al. Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumor escape mechanisms and novel targets for immunotherapy. Genome Biol 2015;16:64.
27. Feig C, Gopinathan A, Neese A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. Clin Cancer Res 2012;18:4266–76.
28. Waghary M, Yalamanchili M, di Magliano MP, Simeone DM. Deciphering the role of stroma in pancreatic cancer. Curr Opin Gastroenterol 2013;29:537–43.
29. Ino Y, Yamazaki-Itoh R, Shimada K, Iwasaki M, Kosuge T, Kanai Y, et al. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. Br J Cancer 2013;108:914–23.
30. Mitchem JB, Brennan DJ, Knollhoff BL, Belt BA, Zhu Y, Sanford DE, et al. Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. Cancer Res 2013;73:1128–41.
31. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol 2013;13:227–42.
32. Song X, Liu J, Lu Y, Jin H, Huang D. Overexpression of B7-H1 correlates with malignant cell proliferation in pancreatic cancer. Oncol Rep 2014;31:1191–8.
33. Razzaque S, Ashraf N, Chavez JC, Malafa MP, Coppola D, Springer GM, et al. Expression of programmed death ligand 1 (PD-L1) in malignant and nonmalignant pancreatic tissue. J Clin Oncol 2013;31:215.
34. Looi CK, Chung FF, Leong CO, Wong SF, Rosli R, Mai CW. Therapeutic challenges and current immunomodulatory strategies in targeting the immunosuppressive pancreatic tumor microenvironment. J Exp Clin Cancer Res 2019;38:162.
35. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer 2016;16:275–87.
36. Johnson BA 3rd, Yarchoum M, Lee V, Laheru DA, Jaffee EM. Strategies for increasing pancreatic tumor immunogenicity. Clin Cancer Res 2017;23:1656–69.
37. Collins AV, Brodie DW, Gilbert RJc, Iaboni A, Manso-Sancho R, Walse B, et al. The interaction properties of costimulatory molecules revisited. Immunology 2002;107:201–10.
38. Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol 2001;19:565–94.
39. Parry RV, Chemnitz JM, Fauworth KA, Lanfranco AR, Braunstein I, Kobayashi SV, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol 2005;25:9543–53.
40. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. Nat Immunol 2002;3:611–8.
41. Schönfeld D, Matschiner G, Chatwell L, Trentmann S, Gille H, Hülsmeyer M, et al. An engineered lipocalin specific for CTLA-4 reveals a combining site with structural and conformational features similar to antibodies. Proc Natl Acad Sci U S A 2009;106:8198–203.
42. Haspot F, Villemain F, Lafamme G, Coulon F, Olive D, Tiollier J, et al. Differential effect of CD28 versus B7 blockade on direct pathway of allore cognition and self-restricted responses. Blood 2002;99:2228–34.
43. Lu Y, Schneider H, Rudd CE. Murine regulatory T cells differ from conventional T cells in resisting the CTLA-4 reversal of TCR stop-signal. Blood 2012;120:4560–70.
44. Valk E, Rudd CE, Schneider H. CTLA-4 trafficking and surface expression. Trends Immunol 2008;29:272–9.
45. Brunner-Weinzierl MC, Hoff H, Burmeister GR. Multiple functions for CD28 and cytotoxic T lymphocyte antigen-4 during different phases of T cell responses: implications for arthritis and autoimmune diseases. Arthritis Res Ther 2004;6:45–54.
46. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, et al. Immunologic self-tolerance maintained by CD25+CD4+ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 2000;192:303–10.
47. Piccirillo CA, Shevach EM. Naturally-occurring CD4+CD25+ immunoregulatory T cells: central players in the arena of peripheral tolerance. Semin Immunol 2004;16:81–8.
48. Noh MY, Lee WM, Lee SJ, Kim HY, Kim SH, Kim YS. Regulatory T cells increase after treatment with poly (ADP-ribose) polymerase-1 inhibitor in ischemic stroke patients. Int Immunopharm 2018;60:104–10.
49. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science 2011;332:600–3.
50. Brunner-Weinzierl MC, Rudd CE. CTLA-4 and PD-1 control of T-cell motility and migration: implications for tumor immunotherapy. Front Immunol 2018;9:2737.
51. Hsu H, Boudova S, Mvula B, Divala TH, Mungwira RG, Harman C, et al. Prolonged PD1 expression on neonatal Vδ2 lymphocytes dampens proinflammatory responses: role of epigenetic regulation. J Immunol 2016;197:1884–92.
52. Khan N, Vidyarthi A, Amir M, Mushtaq K, Agrewala JN. T-cell exhaustion in tuberculosis: pitfalls and prospects. Crit Rev Microbiol 2017;43:133–41.
53. Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation immunotherapies-inhibiting programmed death-1 and programmed death-1. Clin Cancer Res 2012;18:6580–7.
54. Umezdu N, Okada N, Sakoda Y, Adachi K, Ojima T, Yamaue H, et al. Inhibitory functions of PD-L1 and PD-L2 in the regulation of anti-tumor immunity in murine tumor microenvironment. Cancer Immunol Immunother 2019;68:201–11.
55. Podojil JR, Miller SD. Targeting the B7 family of co-stimulatory molecules: successes and challenges. BioDrugs 2013;27:1–13.
56. Zhao Q, Hu F, Xiao Z, Li M, Wu X, Zhao Y, et al. Comprehensive molecular profiling of the B7 family in gastrointestinal cancer. *Cell Prolif* 2018;51:e12468.

57. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clin Cancer Res* 2014;20:5064–74.

58. Drakes ML, Mehrotra S, Aldulescu M, Potkul RK, Liu Y, Grisoli A, et al. Stratification of ovarian tumor pathology by expression of programmed cell death-1 (PD-1) and PD-ligand-1 (PD-L1) in ovarian cancer. *J Ovarian Res* 2018;11:43.

59. Rozali EN, Hato SV, Robinson BW, Lake RA, Lesterhuis WJ. Programmed death ligand 2 in cancer-induced immune suppression. *Clin Dev Immunol* 2012;2012:656340.

60. Ritprajak P, Hashiguchi M, Akiba H, Yagita H, Okumura K, Zhao Q, Hu F, Xiao Z, Li M, Wu X, Zhao Y, et al. Comprehensive characterization of PD-1/PD-L1 interactions in melanoma patients. *Clin Cancer Res* 2013;19:5300–9.

61. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity* 2007;27:111–22.

62. Stewart R, Morrow M, Hammond SA, Mulgrew K, Marcus D, Zhao Q, Hu F, Xiao Z, Li M, Wu X, Zhao Y, et al. Identification and characterization of MEDI4736, an engineered protein that enhances anti-tumor immunity in cancer patients. *Br J Cancer* 2017;116:1524–9.

63. Comings E, Ouyang Q, Rajagopalan H, Gao M, Lin M, Moffitt RA, Salazar MA, et al. Prognostic value, localization and correlation of PD-1/PD-L1, CD89, and FOXP3 with the desmoplastic stroma in pancreatic ductal adenocarcinoma. *J Immunother Cancer* 2018;6.

64. Huang RR, Jalil J, Economou JS, Chmielowski B, Koya RC, Mok S, et al. CTLA4 blockade induces frequent tumor infiltration by activated lymphocytes regardless of clinical responses in patients. *Clin Cancer Res* 2011;17:4101–9.

65. Basso D, Fogar P, Falconi M, Fadi E, Sperri C, Frasson C, et al. Pancreatic tumors and immature immunosuppressive myeloid cells in blood and spleen: role of inhibitory co-stimulatory molecules PD-L1 and CTLA4. *Am in vivo et in vitro study*. *PLoS One* 2013;8:54824.

66. Zhou H, Wang F, Chen Y, Zhao Y, et al. Prognostic significance of PD-L1 expression in pancreatic adenocarcinoma patients. *Cancer Res* 2011;71:3134–42.

67. Wang Y, Lin J, Cui J, Han T, Jiao F, Meng Z, et al. Prognostic value of programmed cell death protein 1 expression on CD8+ T lymphocytes in pancreatic cancer. *World J Gastroenterol* 2017;23:4954–59.

68. Anderson AC, Joller N, Kuchroo VK, Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity* 2016;44:989–1004.

69. Wang Y, Lin J, Cui J, Han T, Jiao F, Meng Z, et al. Prognostic value of programmed cell death protein 1 expression on CD8+ T lymphocytes in pancreatic cancer. *World J Gastroenterol* 2017;23:4954–59.

70. Wang Y, Lin J, Cui J, Han T, Jiao F, Meng Z, et al. Prognostic value of programmed cell death protein 1 expression on CD8+ T lymphocytes in pancreatic cancer. *Cancer Res* 2017;71:78484.

71. Diana A, Wang LM, D’Costa Z, Allen P, Azad A, Silva MA, et al. Prognostic value, localization and correlation of PD-1/PD-L1, CD8 and FOXP3 with the desmoplastic stroma in pancreatic ductal adenocarcinoma. *Oncotarget* 2016;7:60992–1004.

72. Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. *Clin Cancer Res* 2007;13:1757–61.

73. Waki K, Yamada T, Yoshiyama K, Terazaki Y, Sakamoto S, Matsueda S, et al. PD-1 expression on peripheral blood T-cell subsets correlates with prognosis in non-small cell lung cancer. *Cancer Sci* 2014;105:1229–35.

74. Krönig H, Julia Falchner K, Odendahl M, Brackertz B, Conrad H, Muck D, et al. PD-1 expression on melan-A-reactive T cells increases during progression to metastatic disease. *Int J Cancer* 2012;130:2327–36.

75. Wang Y, Lin J, Cui J, Han T, Jiao F, Meng Z, et al. Prognostic value and clinicopathological features of PD-1/PD-L1 expression with mismatch repair status and desmoplastic stroma in Chinese patients with pancreatic cancer. *Oncotarget* 2017;8:95354–65.

76. Zheng Y, Manzotti CN, Liu M, Burke F, Mead KI, Sansom DM, et al. Pancreatic tumors and immature immunosuppressive myeloid cells in blood and spleen: role of inhibitory co-stimulatory molecules PD-L1 and CTLA4. *Am in vivo et in vitro study*. *PLoS One* 2013;8:54824.
95. Daley D, Zambrinip CS, Seifert L, Akkad N, Mohan N, Werba G, et al. γδ T cells support pancreatic oncogenesis by restraining αβ T cell activation. Cell 2016;166:1845–99.

96. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682–7.

97. Imai D, Yoshizumi T, Okano S, Uchiyama H, Ikegami T, Jelinek T, Mihalyova J, Kascak M, Duras J, Hajek R. PD-1/PD-L1.

98. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682–7.

99. Nomri T, Sho M, Akahori T, Hamada K, Kudo A, Kanehiro H, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res 2007;13:2151–7.

100. Zhu Y, Knothoff BL, Meyer MA, Nywening TM, West BL, Luo J, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res 2014;74:5057–69.

101. Imai D, Yoshizumi T, Okano S, Uchiyama H, Ikegami T, Harimoto N, et al. The prognostic impact of programmed cell death receptor 1 (PD-1) expression on overall survival in patients with pancreatic cancer. Clin Cancer Res 2005;11:2947–53.

102. Marinc-Acevedo JA, Suyano AE, Dohlaria B, Knutson KL, Lou Y, Solinas C, Gombos A, Latifyan S, Piccart-Gebhart M, Kok M, et al. Phase II study of the anti-cytotoxic T-lymphocyte-associated antigen 4 monoclonal antibody, tremelimumab, in patients with refractory metastatic colorectal cancer. J Clin Oncol 2010;28:3485–90.

103. Ribas A, Hansen DC, Noe DA, Millham R, Guyot DJ, Bernstein SH, et al. Tremelimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunother Appl 2013;6:382–9.

104. Ryan DP, Hong TS, Bardeesy N. Pancreatic adenocarcinoma.

105. Brunet LR, Hagemann T, Andrew G, Mudan S, Marabelle A. Have lessons from past failures brought us closer to the success of immunotherapy in metastatic pancreatic cancer?. Oncoimmunology 2015;4:1112942.

106. Ryan DP, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. N Engl J Med 2014;371:1639–49.

107. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature 2011;480:480–9.

108. Solinas C, Gombos A, Latifyan S, Piccart-Gebhart M, Kok M, Buisseter L. Targeting immune checkpoints in breast cancer: an update of early results. ESMO Open 2017:2:000255.

109. Jelinek T, Mihalyova J, Kascak M, Durvalumab with or without tremelimumab for patients with metastatic pancreatic ductal adenocarcinoma: a phase 2 randomized clinical trial. JAMA Oncol 2019;5:1431–8.

110. O'Reilly EM, Oh DY, Dhan N, Renouf DJ, Lee MA, Sun W, et al. Durvalumab with or without tremelimumab for patients with metastatic pancreatic ductal adenocarcinoma: a phase 2 randomized clinical trial. JAMA Oncol 2019;5:1431–8.

111. Bolognese AJ, Riddell SR. Allogeneic hematopoietic cell transplantation and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest 2014;124:1240–7.

112. Hodi FS, Mihm MC, Soffier RJ, Haluska FG, Butler M, Seiden MV, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A 2003;100:4712–7.

113. Quezada SA, Peggs KS, Curran MA, Allison JP. CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest 2006;116:1935–45.

114. Morse MA. Technology evaluation: ipilimumab, medarex/bristol- myers squibb. Curr Opin Mol Therapeut 2007;9:588–97.

115. Le Mercier I, Lines JL, Noelle RJ. Beyond CTLA-4 and PD-1, the generation of negative checkpoint regulators. Front Immunol 2015;6:418.

116. Chen CK, Rohde J, Fereday D, Heideman D, Schadendorf D, et al. Phase II trial of single agent ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunotherapeut 2010;33:828–33.

117. Kamath SD, Kalyan A, Kircher S, Nimeiri H, Fauth AJ, Benso III A, et al. Ipilimumab and gemcitabine for advanced pancreatic cancer: a phase Ib study. Oncology 2019;25:e808–15.

118. Murphy JE, Wo YJ, Ryan DP, Clark JW, Jiang W, Yeap BY, et al. Total neoadjuvant therapy with folinrix in combination with los- arian followed by chemoradiotherapy for locally advanced pancreatic cancer: a phase 2 clinical trial. JAMA Oncol 2019;5:1020–7.

119. Chung KY, Gore I, Fong L, Venook A, Omers B, Solt S, et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunother Appl 2013;6:382–9.

120. Kamath SD, Kalyan A, Kircher S, Nimeiri H, Fauth AJ, Benso III A, et al. Ipilimumab and gemcitabine for advanced pancreatic cancer: a phase I clinical trial. JAMA Oncol 2019;5:1020–7.

121. Le DT, Lutz E, Uram JN, Sugar EA, Omers B, Solt S, et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunotherapeut 2013;6:382–9.

122. Kamath SD, Kalyan A, Kircher S, Nimeiri H, Fauth AJ, Benso III A, et al. Ipilimumab and gemcitabine for advanced pancreatic cancer: a phase I trial with amp-224 (B7-DC Fc) that binds to the PD-1 negative checkpoint regulators. Front Immunol 2015;6:418.
Immune checkpoints in targeted-immunotherapy of pancreatic cancer

133. Scheipsi G, Farolfi A, Conteduca V, Martignano F, De Lisi D, Ravagli G, et al. Immunotherapy for prostate cancer: where we are headed. J Int Med Res 2017;35:2627.

134. Sachdev JC, Hu-Lieskovak S, Patnaik A, Eisenberg PD, Weise A, Hutchinson MA, et al. Phase 1/2a study of double immune suppression blockade by combining a CSF1R inhibitor (pexitinib/PXL2309) with an anti-PD-1 antibody (pembrozilumab) to treat advanced melanoma and other solid tumors. Gynecol Oncol 2016;141:147–8.

135. Katz MH, Bauer TW, Varadhachary GR, Petroni GR, Bullock T, Slingluff CL, et al. A randomized multicenter phase Ib/II study to assess the safety and the immunological effect of chemoradiation therapy (CRT) in combination with pembrozilumab (anti-PD1) to CRT alone in patients with resectable or borderline resectable pancreatic cancer. J Clin Oncol 2015;33:P167.

136. Overman MJ, Lopez CD, Benson AB, Neelapu SS, Neelapu SS, Mett N, Ko AH, et al. A randomized phase 2 study of the Bruton tyrosine kinase (Btk) inhibitor acalabrutinib alone or with pembrozilumab for metastatic pancreatic cancer (mPC). J Clin Oncol 2016;34:4130.

137. Mahalingam D, Fontzilas C, Moseley JL, Noronha N, Cheetham K, Dzugalo A, et al. A study of REOLYSIN in combination with pembrozilumab and chemotherapy in patients (pts) with relapsed metastatic adenocarcinoma of the pancreas (MAP). J Clin Oncol 2017;35:e15753.

138. Mahalingam D, Wilkinson GA, Eng KH, Fields P, Raber P, Moseley JL, et al. Pembrozilumab in combination with the oncolytic virus pelareorep and chemotherapy in patients with advanced pancreatic adenocarcinoma: a phase Ib study. Clin Cancer Res 2020;26:71–81.

139. Chiorean EG, Ritch PS, Zhen DB, Poplin E, George B, Hendifar AE, et al. PCRT16-001: phase II study of PEGPH20 plus pembrozilumab for patients (pts) with hyaluronan (HA)-high refractory metastatic pancreatic ductal adenocarcinoma (mPDA). J Clin Oncol 2020;38:TPS785.

140. Halama N, Pruefer U, Frohming A, Beyer D, Eulberg D, Mahalingam D, Fountzilas C, Moseley JL, Noronha N, Cheetham K, Firdaus I, Waterhouse DM, Gutierrez M, Wainberg ZA, George B, Sachdev JC, Hu-Lieskovan S, Patnaik A, Eisenberg PD, Weise A, et al. Pembrolizumab in combination with PD-1 checkpoint inhibitor pembrolizumab and radiotherapy for treatment of chemotherapy-refractory metastatic pancreatic adenocarcinoma. J Clin Oncol 2020;38:TPS782.

141. CT-011 and p53 genetic vaccine for advanced solid tumors. Available from: https://clinicaltrials.gov/ct2/show/NCT01386502.

142. Khleif SN. Gemcitabine and CT-011 for resected pancreatic cancer. Available from: https://ClinicalTrials.gov/show/NCT01313416.

143. Duff Y, Makrity-Fraud G, Khleif SN, Moseley JL, et al. A pilot study of immune checkpoint inhibition (tremelimumab and/or MEDI4736) in patients (pts) with relapsed or refractory (R/R) pancreatic adenocarcinoma (PAC), a phase Ib/II multicenter study. J Clin Oncol 2016;34:TPS484.

144. Wang-Gillam A, O'Reilly EM, Bendell JC, Wainberg ZA, Veeder MH, et al. Bruttini bi + durvalumab (MEDI4736) in patients (pts) with relapsed or refractory (R/R) pancreatic adenocarcinoma (PAC). J Clin Oncol 2019;37:e124.

145. Tuli R, Nissen N, Lo S, Tighiouart M, Placencio V, Hendifar A. Abstract B58: a phase I/II study of durvalumab and stereotactic radiotherapy in locally advanced pancreatic cancer. Boston, MA, USA. In: AACR special conference on pancreatic cancer: advances in science and clinical care. 2019 September 6–9, Available from: https://cancerres.aacrjournals.org/content/79/24_Supplement/B58.

146. Cassier PA, Garin G, Eberst L, Delord J-P, Chabaud S, Terrer C, et al. MEDIPLEXX: a phase I study of durvalumab (D) combined with pexidartinib (P) in patients (pts) with advanced pancreatic ductal adenocarcinoma (PDAC) and colorectal cancer (CRC). J Clin Oncol 2019;37:2579.

147. Fraune C, Burandt E, Simmon R, Hueb-Magg C, Makrity-Fraud G, Kluth M, et al. MMR deficiency is homogeneous in pancreatic carcinomas and associated with high density of CD8-positive lymphocytes. Ann Surg Oncol 2020;27:1–10.

148. Hu Z, Shua J, Studler ZK, Varghese AM, Capuanu M, Salo-Mullen E, et al. Evaluating mismatch repair defects in pancreatic adenocarcinoma: challenges and recommendations. Clin Cancer Res 2018;24:1326–36.

149. Yamanoto H, Itoh F, Nakamura H, Fukushima H, Sasaki S, Peruchic M, et al. Genetic and clinical features of human pancreatic ductal adenocarcinomas with widespread microsatellite instability. Cancer Res 2001;61:3139–44.

150. Macherla S, Laks S, Naqsh AR, Bulumulle A, Zervos E, Muzaffar M. Emerging role of immune checkpoint blockade in pancreatic cancer. Int J Mol Sci 2018;19:3505.

151. Kim ST, Klemmner SJ, Park SH, Park JO, Park YS, Lim HY, et al. Correlating programmed death ligand 1 (PD-L1) expression, mismatch repair deficiency, and outcomes across tumor types: implications for immunotherapy. Oncotarget 2017;8:77415–23.

152. Salem ME, Puccini A, Grothe A, Raghavan D, Goldberg RM, Xiu J, et al. Landscape of tumor mutation load, mismatch repair deficiency, and PD-L1 expression in a large patient cohort of gastrointestinal cancers. Mol Cancer Res 2018;16:805–12.

153. Oliveira AF, Bretes L, Furtado I. Review of PD-1/PD-L1 inhibitors in metastatic dMMR/MSI-H colorectal cancer. Front Oncol 2019;9:396.

154. Andreada S, Pilas F, Burdin N, Triebel F. Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD273). J Immunol 2002;168:3874–80.

155. Grosso JF, Kelleher CC, Harris TK, Maris CH, Hinkkkis EL, De Marzo A, et al. LAG-3 regulates CD8 T cell accumulation and
effect of treatment in murine self- and tumor-tolerance systems. J Clin Invest 2007;117:3383–92.

163. Le DT, Uram NJ, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–20.

164. Sohal DPS, Kennedy EB, Khorana A, Copur MS, Crane CH, Garrido-Laguna I, et al. Metastatic pancreatic cancer: ASCO clinical practice guideline update. J Clin Oncol 2018;36:2545–56.

165. Eso Y, Shimizu T, Takeda H, Takai A, Marusawa H. Microsatellite instability and immune checkpoint inhibitors: toward precision medicine against gastrointestinal and hepatobiliary cancers. J Gastroenterol 2020;55:15–26.

166. Lee HT, Lee SH, Heo YS. Molecular interactions of antibody drugs targeting PD-1, PD-L1, and CTLA-4 in immune-oncology. Molecules 2019;24:1190.

167. Goldberg MV, Drake CG. LAG-3 in cancer immunotherapy. Curr Top Microbiol Immunol 2011;344:269–78.

168. Huang CT, Workman CJ, Flies D, Pan X, Marson AL, Zhou G, et al. Role of LAG-3 in regulatory T cells. Immunity 2004;21:503–13.

169. Brignone C, Escudier B, Grygar C, Marcu M, Triebel F. A phase I pharmacokinetic and biological correlative study of IMP321, a novel MHC class II agonist, in patients with advanced renal cell carcinoma. Clin Cancer Res 2009;15:6225–31.

170. Brignone C, Gutierrez M, Mefti F, Brain E, Jarcu R, Civitkov F, et al. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3 Ig) enhances immune responses and antitumor activity. J Transl Med 2010;8:71.

171. Romano E, Michielin O, Voelter V, Laurent J, Bichat H, Stravodimov A, et al. MART-1 peptide vaccination plus IMP321 (LAG-3 Ig fusion protein) in patients receiving autologous PBMCs after lymphodepletion: results of a phase I trial. J Transl Med 2014;12:97.

172. Woo S-R, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res 2012;72:917–27.

173. Nguyen LT, Ohashi PS. Clinical blockade of PD1 and LAG3—potential mechanisms of action. Nat Rev Immunol 2015;15:45–56.

174. Ascierio PA, McArthur GA. Checkpoint inhibitors in melanoma and early phase development in solid tumors: what’s the future?. J Transl Med 2017;15:173.

175. Patel RK, Ko AH, Ommers B, Uram NJ, Parkinson R, Sugar E, et al. A phase 2, multicenter study of FOLFIRINOX followed by ipilimumab in early phase development in solid tumors: what’s the future?. J Transl Med 2017;15:173.

176. Okudaira K, Hori K, Tsuzuki Y, Okada Y, Komoto S, Watanabe C, et al. Blockade of B7-1/H1 or B7-DC induces an anti-tumor effect in a mouse pancreatic cancer model. Int J Oncol 2009;35:741–9.

177. Soares KC, Rucki AA, Wu AA, Olino K, Xiao Q, Chai Y, et al. PD-1/PD-L1 blockade together with vaccine therapy facilitates effector T-cell infiltration into pancreatic tumors. J Immunother Appl 2015;38:1–11.

178. Luhshei NM, Coates-Ulrichsen J, Harper J, Mullins S, Sulikowski MG, Martin P, et al. Transformation of the tumour microenvironment by a CD40 agonist antibody correlates with improved responses to PD-L1 blockade in a mouse orthotopic pancreatic tumour model. Oncotarget 2016;7:18508–20.

179. Ding G, Shen T, Yan C, Zhang M, Wu Z, Cao L. IFN-γ down-regulates the PD-1 expression and assist nivolumab in PD-1 blockade effect on CD8+ T-lymphocytes in pancreatic cancer. BMC Cancer 2019;19:1053.

180. Lu C, Tulukder A, Savage NM, Singh N, Liu K. JAK-STAT-mediated chronic inflammatory impairs cytotoxic T lymphocyte activation to decrease anti-PD-1 immunotherapy efficacy in pancreatic cancer. Oncoimmunology 2017;6:1291106.

181. Doi T, Ishikawa T, Okayama T, Oka K, Mizushima K, Yasuda T, et al. The JAK/STAT pathway is involved in the upregulation of PD-L1 expression in pancreatic cancer cell lines. Oncol Rep 2017;38:1545–54.

182. Jiang J, Zhou H, Ni C, Hu X, Mou Y, Huang D, et al. Immunotherapy in pancreatic cancer: new hope or mission impossible?. Cancer Lett 2019;445:57–64.

183. Garcia-Diaz A, Shin DS, Moreno BH, Saco J, Escuin-Ordinas H, Rodriguez GA, et al. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression. Cell Rep 2017;19:1189–201.

184. Mimura K, Teh JL, Okayama H, Shiraiishi K, Kua LF, Koh V, et al. PD-L1 expression is mainly regulated by interferon gamma associated with JAK-STAT pathway in gastric cancer. Cancer Sci 2018;109:43–53.

185. Syn NL, Teng MWL, Mok TSK, Soo RA. De-novo and acquired resistance to immune checkpoint targeting. Lancet Oncol 2017;18:731–41.

186. Engelhardt JJ, Sullivan TJ, Allison JP. CTLA-4 overexpression inhibits T cell responses through a CD28-B7-dependent mechanism. J Immunol 2006;177:1052–61.

187. Kisielow M, Kisielow J, Capoferri-Sollami G, Karjalainen K. Expression of lymphocyte activation gene 3 (LAG-3) on B cells is induced by T cells. Eur J Immunol 2005;35:2081–8.

188. Mason D, Andrè P, Bensussan A, Buckley C, Civeiri C, Clark E, et al. CD antigens 2001. Immunol Rev 2001;170:401–6.

189. Workman CJ, Wang Y, El Kasm KC, Pardoll DM, Murray PJ, Drake CG, et al. LAG-3 regulates plasmacytoid dendritic cell homeostasis. J Immunol 2009;182:1885–91.

190. Workman CJ, Vignali DA. The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells. Eur J Immunol 2003;33:970–9.

191. Sierro S, Romero P, Speiser DE. The CD4-like molecule LAG-3, biology and therapeutic applications. Expert Opin Ther Targets 2011;15:91–101.

192. Workman CJ, Cauley LS, Kim JJ, Blackman MA, Woodland DL, Vignali DAA. Lymphocyte activation gene-3 (CD223) regulates the size of the expanding T cell population following antigen activation in vivo. J Immunol 2004;172:5450–5.

193. Van Audenaerde JRM, De Waele J, Marcq E, Van Loenhout J, Lion E, Van den Bergh MJI, et al. Interleukin-15 stimulates natural killer cell-mediated killing of both human pancreatic cancer and stellate cells. Oncotarget 2017;8:56968–79.

194. Sun H, Sun C, Xiao W. Expression regulation of co-inhibitory molecules on human natural killer cells in response to cytokine stimulations. Cytokine 2014;65:33–41.

195. Davis ME, Zuckerman JE, Choi CHJ, Seligson D, Tölcher A, Alabi CA, et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature 2010;464:1067–70.
199. Hannon GJ, Rossi JJ. Unlocking the potential of the human genome with RNA interference. *Nature* 2004;431:371–8.

200. Yoo B, Jordan VC, Sheedy P, Billig AM, Ross A, Pantazopoulos P, et al. RNAi-mediated PD-L1 inhibition for pancreatic cancer immunotherapy. *Sci Rep* 2019;9:4712.

201. Salman B, Zhou D, Jaffee EM, Edil BH, Zheng L. Vaccine therapy for pancreatic cancer. *Oncoimmunology* 2013;2:26662.

202. Soares KC, Zheng L, Edil B, Jaffee EM. Vaccines for pancreatic cancer. *Cancer J* 2012;18:642–52.

203. Le DT, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G, 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.

204. Sun D, Ma J, Wang J, Zhang F, Wang L, Zhang S, et al. Clinical observation of immune checkpoint inhibitors in the treatment of advanced pancreatic cancer: a real-world study in Chinese cohort. *Therapeut Clin Risk Manag* 2018;14:1691–700.