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Exploring novel systemic therapies for pancreatic cancer: a review of emerging anti-PD-1/PD-L1 combination therapy

Minireview

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Despite attempts to apply single therapy such as surgical treatment, chemotherapy, or radiotherapy, pancreatic cancer (PC) is still one of the most lethal solid tumors. Moreover, immune checkpoint inhibitors against PD-1/PD-L1, which have shown good efficacies against many other solid tumors, have not shown encouraging results in PC treatment. Therefore, some studies are evaluating the efficacies of combination therapies based on anti-PD-1/PD-L1 for PC. In this review, we summarized the emerging anti-PD-1/PD-L1 combination therapies for PC in these years. We realized that anti-PD-1/PD-L1-based combination therapies have the potential to be efficacious in PC treatment, and further relevant studies are needed. Moreover, we elucidated the reasons for the ineffectiveness of anti-PD-1/PD-L1 alone in PC treatment. We concluded that this was mainly because PC has an immunosuppressive tumor microenvironment and develops drug resistance during treatment. Anti-PD-1/PD-L1-based combination therapeutic regimens that alter the immunosuppressive tumor microenvironment and reduce the development of drug resistance in PC are summarized in this review, and we expect that these regimens will achieve good clinical application prospects.

Key words: PD-1, PD-L1, pancreatic cancer, tumor microenvironment, immunotherapy

Pancreatic cancer (PC) is the seventh most common cause of cancer-associated mortalities worldwide [1]. Nowadays, the incidence of PC in China exhibits an increasing trend, and the mortality rate is not trending down [2]. Globally, it is associated with the highest mortality rates among solid tumors [3]. It has been postulated that PC will be the second leading cause of cancer-associated mortalities by 2030 in the United States [4] due to its increasing incidence and remaining high mortality rate. There are multiple pathological types of PC, approximately 95% of PC are exocrine cell tumors [5], and pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of PC. PDAC is mainly composed of glands with duct-like structures with different degrees of differentiation, accompanied by abundant fibrous stroma that makes its tumor-specific molecular information difficult to obtain and leads to unoptimistic therapeutic effects. Most PDAC patients have progressed to unresectable, locally advanced, or metastatic disease when they are diagnosed due to its pathological features [6]. Endocrine pancreatic cancer is generally a more indolent tumor with a relatively good prognosis [7]. As the primary pathological subtype of PC, PDAC is one of the most aggressive cancers associated with high morbidity and mortality. Apparently, new therapeutic regimens for patients with PC are essential now, especially for PDAC.

Previous studies have shown that current therapeutic regimens for PC are not effective. Patients with metastatic ductal adenocarcinoma of the pancreas treated with durvalumab plus tremelimumab had a response of 3.1%, while patients did not respond to durvalumab single [8]. Under the development of neoadjuvant therapy, resectable PC can be treated by surgery, while borderline unresectable PC can be treated with chemotherapy. Targeted therapy and interstitial therapy are also promising regimens. However, PC treatment is facing multiple challenges, including relative failure of single-agent immune checkpoint inhibitors (ICI),

difficulties in identifying high-risk populations for screening and prevention, early PC detection through advanced imaging and new biomarkers, as well as in the selection of better treatment regimens, to overcome drug resistance to current treatment modalities [9].

A key factor for cancer cell survival and tumor formations is that they can inhibit immune responses or assist through other cells in the tumor microenvironment (TME) [9]. Therefore, a feasible therapeutic strategy is immunotherapy against cancer cells or reshaping the TME. The application of immunotherapy in cancer has been evaluated in recent years. The simplest form involves using tumor-specific antigen cancer vaccines [10]. However, this approach is not available for PC [11].

Besides, immune checkpoint receptors are potential targets for cancer therapy, and they can generate long-lasting anti-tumor responses in advanced cancers [12]. Immune checkpoint inhibitors have shown encouraging results in the treatment of many tumors [9], while monotherapeutic regimens have not shown favorable responses in many cancer patients [8, 12]. For example, a PDAC treatment approach against the PD-1/PD-L1/CTLA-4 immune checkpoint was performed in early clinical trials for PC treatment. It was found that this single treatment approach does not have a good efficacy [13]. Nonetheless, successful reports of immune checkpoint inhibitors in the treatment of other solid tumors hold promise for PC treatment [9]. Future studies are needed to evaluate how to best combine immune checkpoint blockade with other agents. Some studies have combined anti-PD-1/PD-L1 agents with vaccines, radiotherapy, and chemotherapy to treat PDAC [13]. Moreover, there are therapeutic regimens that combine genetic aspects or inhibit other antibodies as well as blockers to treat PC.

From this perspective, anti-PD-1/PD-L1 combination therapy may become an effective therapy for PC in the future. In this review, we summarized the emerging anti-PD-1/PD-L1 combination therapies for PC and elucidated the reasons for the ineffectiveness of anti-PD-1/PD-L1 alone in PC treatment.

Current status and challenges of PC immunotherapy

Immunotherapy for PC

Due to the high rate of PC metastasis, low pathologic complete response (pCR) rate for surgical resection, and limitations associated with chemotherapy and radiotherapy, immunotherapy has shown many advantages. Single immunotherapies for PC are focused on various aspects.

Advance the specificity of tumor antigen. Improving antigen specificity is the first step in activating specific cellular immune systems. Currently, the most remarkable research in this field involves therapeutic cancer vaccines, which include four subtypes: cell vaccines (including whole cell vaccines and dendritic cell vaccines), protein vaccines, peptide vaccines, and genetic vaccines [14], all of which can stimulate tumor-associated autoantigens to be presented to the immune system, thereby, activating antigen-specific T cells and producing specific acquired immune responses. The relevant clinical trials involving various vaccines, such as human telomerase (hTERT), GVAX, PancVAX, and PANC02 vaccines, are ongoing [15].

Eliminating the inhibitory effects of the microenvironment. During PC immunotherapy, the low response rates and drug resistance of immune checkpoints are closely associated with the immunosuppressive tumor microenvironment. Infiltrations of pancreatic cancer stromal cells (including myelogenous suppressor cells, macrophages, fibroblasts, and Treg cells, among others) inhibit various functions of T cells, NKs, and DCs, leading to the poor efficacy of immunotherapy and poor prognostic outcomes [16]. Effective myeloid-based immunotherapy and matrix-modulating immunotherapy play essential roles in cancer therapy. The relevant clinical trials involving CCR2 inhibitors, CXCR2 inhibitors, CSF-1R inhibition, and IDO1 inhibition are ongoing. Another factor that cannot be ignored is that the pancreas has a unique microbiome. Some studies have found that the bacteria in the pancreas may potentially affect the development of pancreatic pathophysiological processes [17]. The role of this unique microbiome in the diagnosis and treatment of pancreatic diseases needs to be further explored.

Enhance T cell immune response. The most effective immunotherapy for enhancing T cell immune responses in metastatic pancreatic cancer involves activating innate and specific immune systems. Enhancing T cell immune responses is key to overcoming resistance in tumor cells. In addition to immune checkpoint blockade, other approaches encompassing adoptive cell transfer therapy and agonist immunotherapy have been found to have positive outcomes. Among them, CAR-T (Chimeric Antibody Receptor Engineered T Cell) is a specific adoptive cell transfer therapy, which can activate cellular immune responses in a non-MHC-restricted form by expressing chimeric antigen receptors (CARs) on T cells from transgenic patients [18].

Immunotherapy targeting PD-1/PD-L1 in PC

Programmed death-1 (PD-1) is a member of the CD28 family [19]. It can be expressed on the surface of activated T cells after antigen recognition. Moreover, it is a crucial immune checkpoint molecule involved in the classical type of programmed cell death [20]. PD-L1, one of PD-1's ligands, can be expressed in different human cancers, including PC, breast cancer, urothelial cancer, ovarian cancer, cervical cancer, colorectal cancer, gastric cancer, non-small cell lung cancer, melanoma, and glioblastoma [21, 22]. When PD-1 binds to PD-L1, they can promote T cell apoptosis, thereby

protecting tumor cells. Blocking PD-1/PD-L1 binding can activate T cells and enhance immune responses [22], thereby achieving cancer treatment.

Gao et al. performed IF detection of PD-1/PD-L1 and found that PDAC cells express both PD-1 proteins and PD-1 genes. They also reported that PD-1/PD-L1 selectively binds and activates the MAPK signaling pathway in PDAC, thereby promoting PC cell proliferation [23]. Preclinical studies and clinical trials of mouse models have evaluated the role of anti-PD-1/PD-L1 as an immune checkpoint blocking therapy in overcoming fatal malignant tumors. Anti-PD-1/PD-L1 antibodies have shown good clinical efficacies in many cancer types [20, 24, 25], such as ovarian cancer [26], melanoma [27], renal cell carcinoma [28], bladder cancer [29], Hodgkin lymphoma [30], and non-small cell lung cancer [31]. However, they have not shown good efficacies in PC [20]. Although no significant efficacy has been shown in PC, based on the considerable effect in the treatment of other types of cancer as well as some preclinical studies, anti-PD-1/PD-L1 can potentially be used in PC treatment.

Combination therapy based on anti-PD-1/PD-L1

Combination therapy based on anti-PD-1

Chemotherapy. Chemotherapy has been widely used in the treatment of many cancer types. Some studies have evaluated the efficacy of chemotherapies and their applications in PC treatment. Chemotherapy can significantly improve disease-free survival and overall survival rates for patients after radical resection of PC [32].

Enzyme inhibitor. Many enzyme inhibitors can be used in combination with PD-1 inhibitors. Kim et al. reported that the HDAC inhibitor, CG-745, can induce or prolong expressions of IL-2 and IFN-γ, promote the proliferation of cytotoxic T and NKs, and inhibit regulatory T cell proliferation. Alterations in TME induced by CG-745 can promote the anticancer effects of PD-1 antibodies [33]. Therefore, a combination of CG-745 and pembrolizumab can achieve good results in PC treatment. PDAC is associated with a lack of specific biomarkers, low responsiveness to chemotherapy, and low response rates to targeted therapies against MEK [34–37]. Gao et al. reported that PD-1/PD-L1 binding could activate the MAPK signaling pathway in PDAC and promote tumor cell growth. The MEK1/2 inhibitor, trametinib, can block the MAPK pathway and enhance cytotoxic effects when used in the combination regimen with the PD-1 inhibitor, pembrolizumab [23]. Therefore, the combination of trametinib and pembrolizumab in PC/PDAC treatment is expected to achieve good results. Yan et al. found that DCLK1, a cancer stem cell marker, regulates PD-L1 expression through the Hippo signaling pathway, and the inhibition of DCLK1 can downregulate PD-L1 expression in human PC through the HIPPO signaling pathway [38]. Accordingly, we postulated that inhibition of DCLK1 combined with anti-PD-1 therapy may achieve better results.

Immunomodulator. Combining various immunomodulators, pixatimod and TGFβ, with anti-PD-1 exerts a specific therapeutic effect in PC. Pixatimod is the international non-proprietary name and was previously described in the literature as PG545 [39]. It inhibits the infiltration of tumor-associated macrophages (TAMs) [40, 41], stimulates dendritic cells (DCs) [42], and activates natural killer cells (NKs). Hammond et al. experimentally verified the safety of pixatimod use and showed that pixatimod could enhance the anti-tumor activities of PD-1 inhibitors [43]. Therefore, studies should investigate the effects of pixatimod in combination with the PD-1 inhibitor, nivolumab, among others, in PC treatment. TGFβ is a vital immunomodulator. It can inhibit the activation of cytotoxic T lymphocytes (CTLs) in early PDAC [44]. Inhibition of the TGFβ signaling pathway can improve lymphocyte activity and enhance the antitumor effect. Principe et al. showed that mice treated with a TGFβ-targeting agent galunisertib+anti-PD-1 had reduced pancreatic weights, consistent with reduced tumor stroma. This combination regimen promoted T cell-mediated clearance of advanced PDAC [44]. The efficacy and safety of this combination approach in the treatment of PC patients should be further investigated. The lack of immunotherapeutic efficacy in human PDAC is associated with a lack of immunogenicity or generation of tumor-specific T cell responses [45, 46]. However, Seo et al. reported that ineffective treatment outcomes could be because clonally expanded tumor-reactive T cells are blocked outside the tumor by the juxtatumoral stroma [47]. They also evaluated the effect of targeted PD-1, combined with CXCR4 blockade in PDAC and found that the combination therapy reactivated intratumoral $CD8^+$ T cell clonal proliferation, promoted $CD8^+$ T cell migration, enhanced cytotoxicity, and then promoted the killing effect on PDAC cells [47, 48]. These findings lay the basis for further PDAC studies. More details are shown in the additional figure and table files (Figure 1, Table 1).

Silicone carrier. Irinotecan (IRIN) is a potent anticancer drug with multiple effects that can trigger chemoimmunotherapy responses in PC models. We can induce ICD response in the orthotopic Kras-induced pancreatic cancer (KPC) model by administering IRIN via a silicone carrier, and this response could be enhanced by anti-PD-1 therapy [49]. This way of administration through a particular carrier provides a new approach for chemotherapy of PC and may have good therapeutic effects in the future.

Combination with a vaccine. The most straightforward approach to immunotherapy involves cancer vaccines using tumor-specific antigens [10]. However, this direct approach has so far been ineffective for PC [11]. Progress has been made in dual or triple therapies combined with anti-PD-1 and tumor vaccines. This has shown great potential for future PC treatment.

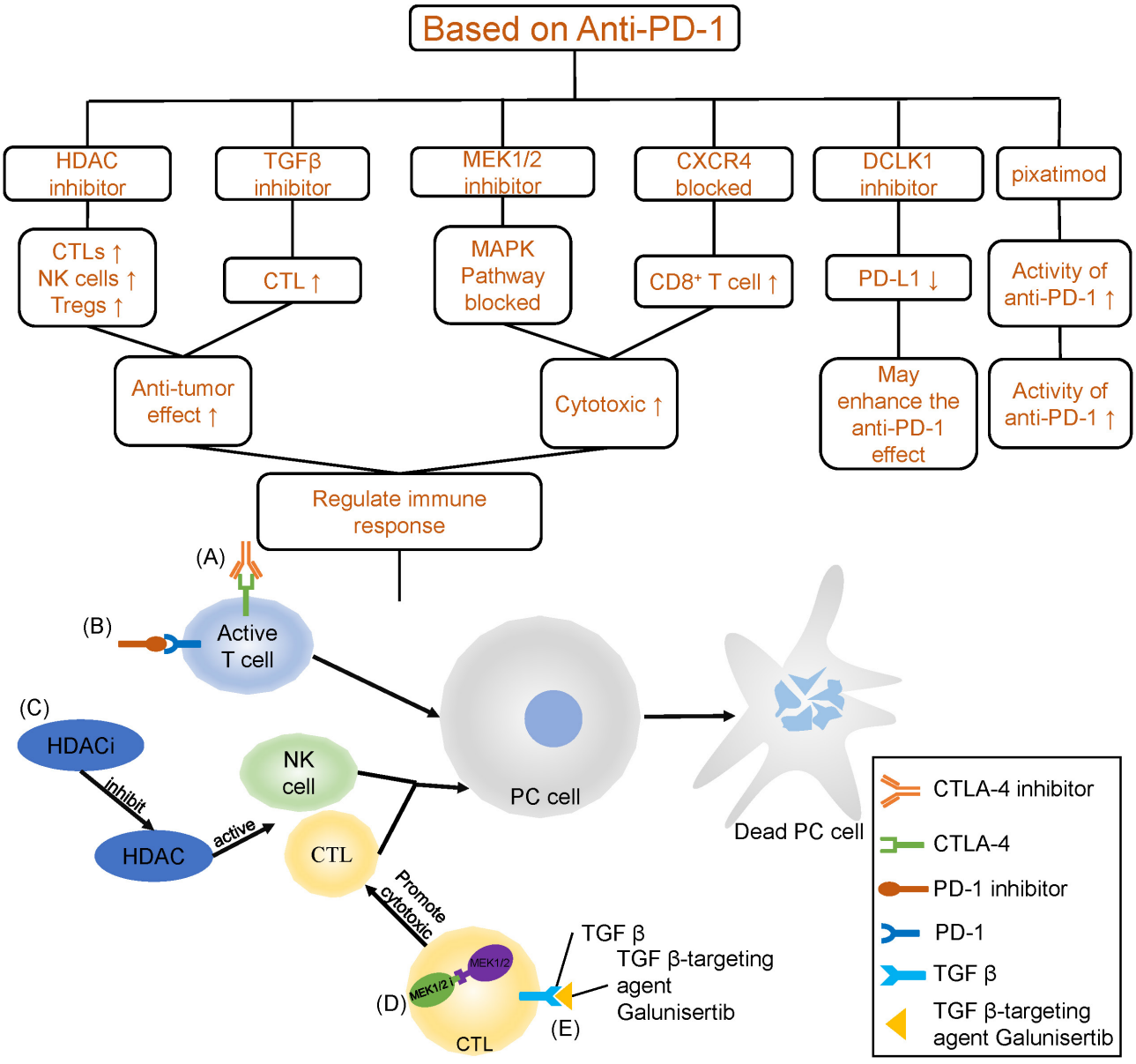

Figure 1. Mechanism of anti-PD-1-based combination regimens. A, B) Anti-CTLA-4 may exhibit synergy when combined with anti-PD-1 in PC treatment. C) HDAC inhibition can promote the anti-cancer effect of anti-PD-1 treatment. D) The inhibition of MEK1/2 can block the MAPK pathway and enhance the cytotoxic effect when combined with anti-PD-1. E) TGFβ blockade can improve CTL activity and enhance anti-tumor responses. Abbreviations: PC-pancreatic cancer; PD-1-programmed cell death 1; NK-natural killer; MEK-methyl ethyl ketone; CTLs-cytotoxic lymphocytes; MAPK-mitogen-activated protein kinase; Tregs-regulatory T cells; HDAC-histone deacetylase; TGFβ-transforming growth factor-β; CTLA-4-cytotoxic T-lymphocyte antigen 4

Dual therapies. In dual therapies, such as the gastrin vaccine PAS (polyclonal antibody stimulator, a vaccine against gastrin), survival outcomes for PC patients can be prolonged. Gastrin inhibits PC growth by acting on PC cells *in vitro* [50]. Osborne et al. investigated the effect of PAS combined with anti-PD-1 for PC treatment. They found that PAS stimulated the production of specific, high-affinity polyclonal anti-gastrin antibodies, and this regimen could activate $CD4^+$, $CD8^+$, TEMRA cells, which play essential roles in T cell-mediated tumor cell death and memory. Mice treated with the PD-1 antibody and PAS100 exhibited less tumor fibrosis, which was significant when using a combination of PAS250 and PD-1Ab [51]. Therefore, this combination is essential as a PC treatment strategy.

Annexin A2 (ANXA2) is a PDAC-related protein that promotes tumor metastasis. Immunotherapies that target

ANXA2 can stimulate immune responses with anti-tumor effects. Kim et al. found that Listeria-based immunotherapy (Lm-ANXA2) induced ANXA2 expression in PDAC mice models and also induced tumor antigen-specific T cell responses in mice TME, thereby prolonging the survival time of mice. Moreover, a sequential combination of Lm-ANXA2 and anti-PD-1 antibody was superior to LM-ANXA2 alone in the treatment of PDAC in mice models, with more effective anti-tumor activities [52]. Studies should compare this combination regimen with anti-PD-1 alone. In conclusion, this protocol has encouraging application potential.

Triple therapy. PancVAX+anti-PD-1+OX40. PancVAX is a novel antigen-targeted vaccine that can activate novel T cell families and then cause tumor regression. Kinkead et al. demonstrated that PancVAX induced vaccine-specific tumor-infiltrating lymphocytes (TIL), decreased T cell

Table 1. Preclinical studies of combination regimens based on PD-1.

Target	Drug/Reagents	Model	Function
HDAC and PD-1 [33]	CG-745 PD-1 Ab Anti-mouse PD-1 (BP0146; Bio X cell, West Lebanon, NH, USA)	Subcutaneous Hepa1-6 mouse model CT26 syngeneic mouse model	Anti-cancer effect ↑
MEK1/2 and PD-1 [23]	Trametinib (anti-MEK1/2 small molecule, Novartis) Pembrolizumab nivolumab (anti-PD-1 monoclonal antibody, Bristol Myers Squibb) Pembrolizumab (anti- PD-1 monoclonal antibody, Merck)	Human PC cell lines MIAPaCa-2 and PANC-1 Human pancreatic duct epithelial cell line (H6c7) Human Jurkat cells Patient-derived organoids (PDOs) Patient-derived tumor xenografts (PDTXs)	Cytotoxic effect ↑
DCLK1 and PD-1 [38]	LRRK2-IN-1(catalog T2246) XMD8-92(catalog S7525) Verteporfin (catalog HY-B0146) PLVX-IRES-Zsgreen vector Rabbit anti-DCLK1 (Abcam, ab31704; 1:1000)	AsPC-1 cells BxPC-3 cells PANC-1 cells	Regulation of PD-L1 ↓
Immune cell and PD-1 [43]	Pixatimod Clone RMP1–14 or isotype control antibody 2A3, Bio-X-Cell, NH, USA	Cells: Immune cells from the collagenase Animals: Female Balb/c mice (6–8 weeks)	Anti-tumor activity of PD-1 inhibitors ↑
TGFβ and PD-1 [44]	Galunisertib (LY2157299) RMP1-14 (anti-PD-1 antibody)	Animals: P48-Cre x LSL-KRASG12D (KC), Tgfbr1+/-, KC/Tgfbr1+/-, and Pdx1-Cre x LSL-KRASG12D x LSL-TP53R172H (KPC) mice	PC tumor stroma ↓ T cell-mediated clearance of advanced PDAC ↑
Gastrin and PD-1 [51]	PAS (100 μg/150 μg/250 μg) PD-1Ab (150 μg)	Cells: The murine pancreatic cancer cell lines mT3-2D (mT3) Animals: Male (6 weeks) C57BL/6 mice	Cytotoxic effect ↑ PC tumor stroma ↓
Lm-ANXA2 and PD-1 [52]	Lm-ANXA2 PD-1Ab	Cells: KPC tumor cells Animals: Six- to eight-weeks old C57Bl6 female mice	Cytotoxic effect ↑
PancVAX and OX40 and PD-1 [53]	PancVAX PD-1Ab OX40 agonists	Cells: Panc02 cell line Animals: Male 6-week-old C57BL/6 mice	T cell-mediated clearance of advanced PDAC ↑
GVAX and CSF-1R and PD-1 [58]	GVAX vaccine Anti-CSF-1R antibody PD-1Ab	Cells: KPC tumor cells Animals: Seven- to eight-weeks old C57Bl6 mice	Anti-cancer effect ↑
T cell generating vaccine and CD40 and PD-1 [60]	PANC02vac CD40 agonist mAb (Dacetuzumab) PD-1Ab	Cells: NT2.5cells, 3T3neuGM(vaccine)cells, T2Dqcells, PANC02mouse pancreatic tumor cells and B78H1-GM cells Animals: Male C57BL/6 mice (age 7-8 weeks) and Female neu-N mice	PC tumor stroma ↓ T cell-mediated clearance of advanced PDAC ↑
Radiotherapy and PD-1 [63]	RT PD-1Ab	Cells: The KPC tumor cell line Animals: Female C57Bl/6 mice.	Anti-cancer effect ↑
ISG15 and PD-1 [64]	ISG15 pathway knockdown PD-1Ab	Cells: Panc02 murine pancreatic cancer cells and Stable, ISG15 and UbcH8 knockdown clonal cells Animals: 6-weeks old female C57BL/6 mice	Regulation of PD-L1 ↓ $CD8^+$ T-cell infiltration ↑
Oncolytic virus and PD-1 [68]	OBP-502 PD-1Ab	Cells: The murine colon cancer cell line CT26 and PAN02 Animals: 6-week-old female BALB/c mice and BALB/c nude mice, 6-week-old female C57BL/6 mice	T-cell infiltration ↑

activation threshold, and reduced TIL depletion. The triple regimen of PancVAX with anti-PD-1 and OX40 agonists can induce a robust anti-tumor immune response in the PC mice model and also achieve durable tumor clearance [53].

GVAX+anti-PD-1+anti-CSF-1R. CSF-1R is a receptor for CSF-1. The binding of CSF-1 to CSF-1R can affect the migration, differentiation, and survival of macrophages and other myeloid cells [54, 55]. GM-CSF cell-based vaccines (GVAX) is a PC vaccine that can secrete GM-CSF (granulocyte-macrophage colony-stimulating factor), which induces lymphocyte accumulation and infiltration into the TME of PDAC [56, 57]. Saung et al. reported that a triple therapy regimen of GVAX vaccine, anti-PD-1, and anti-CSF-1R antibody improved survival rates for PDAC mice models and increased the proportion of PD-1 + OX40 + CD40 + T-cells in tumors, which highly express IFN-γ that can improve anti-tumor activities [58]. This triple regimen is worthy of further studies and is expected to be an effective regimen for the clinical treatment of PDAC.

PANCO2vac+anti-PD-1+CD40 agonist. CD40 is a member of the tumor necrosis factor (TNF) receptor superfamily. It is expressed in various immune cells, including DCs, macrophages, B cells, and NKs [59]. Activation of CD40 can enhance the activity of these immune cells. PANC02 cells treated with PANC02vac (PANC02vaccine) can be mixed with GM-CSF secreting cell lines [60]. Ma et al. showed that CD40 agonists could improve the antitumor effects of anti-PD-1 therapies. Application of PANC02vac plus anti-PD-1 and CD40 agonist triple therapy in mice enhanced T cell infiltration and function in solid tumors, promoted the development of functional T cell memory, converted T cells in the TME from simple T cells and Tregs to activated CTLs, changed the medullary component of the TME, and induced anti-tumor immune responses [60]. This regimen has shown good therapeutic results in mice models; its safety and clinical effects should be further verified.

These findings imply that both dual and triple therapies combined vaccine with anti-PD-1 have encouraging therapeutic effects in PC. These combination therapies have great potential for clinical applications in PC treatment; their safety and efficacies should be further evaluated. An additional table file shows more details (Table 1).

Other emerging methods

Radiotherapy. Radiotherapy (RT) is beneficial in treating many cancer types, while its efficacy in PDAC has not been established [61–63]. RT can locally induce innate immune responses in PDAC and can also induce PD-L1 as well as indoleamine 2,3-dioxygenase 1 (IDO1) production by PDAC tumor epithelial cells [63]. Fujiwara et al. investigated the efficacy of dual or triple combinations of RT and anti-PD-1. They found that the combination could significantly prolong the survival times of PDAC mice models. In comparison, triple therapies with RT, anti-PD-1, and anti-IDO1 tended to reduce the survival rate [63]. In summary, combined RT and anti-PD-1 have positive therapeutic effects on PC, and subsequent safety and more studies should be performed.

Gene knockout. Gene therapy is a challenging but precise treatment. Gene knockout has a role in PC treatment and can enhance the efficacy of anti-PD-1. For example, the interferon-stimulated gene (ISG15) is a 15 kDa protein induced by type I interferons [64]. ISG has a tumor-promoting effect, while free ISG15 has an anti-tumor or a tumor-promoting effect depending on the tumor type in which it is located [65, 66]. Burks et al. showed that knockdown of the ISG15 gene reversed the KRAS-related phenotype of PDAC cells, thereby inhibiting PDAC growth and suppressing PD-L1 expression to enhance infiltration of $CD8^+$ T cells in the tumor [64]. Moreover, the ISG15 gene knockdown enhanced the efficacy of anti-PD-1, as shown by the synergistic anti-tumor effects [64]. This combined strategy is promising to improve the survival rate of clinical PDAC patients and warrants further relevant research efforts.

Virus induction. Pelareorep (oncolytic virus), an intravenously administered oncolytic reovirus, is a proprietary isolate of unmodified human reovirus that has been developed as an immuno-oncovirus agent for systemic administration in the treatment of solid tumors and hematologic malignancies [67]. Treatment with pelareorep allows reovirus replication in tumor tissues, T-cell infiltration, and PD-L1 upregulation [68]. Mahalingam et al. conducted a phase 1b trial of pelareorep combined with ICI and chemotherapy in patients with advanced PDAC. They found that the treatment was well tolerated with no significant toxic effects and considerable efficacy [68]. Further assessment of pelareorep and anti-PD-1 therapy is ongoing. An additional table file shows more details (Table 1).

Combination therapy based on anti-PD-L1

In combination with chemotherapy

PRMT inhibitors. PRMT1 is a type I protein arginine methyltransferase (PRMT). It is involved in post-translational modifications, such as cell cycle control, RNA processing, and DNA replication, among others [69]. PRMT1 promotes cancer cell transformation, proliferation, invasion, and survival by methylation of arginine residues on histone and non-histone substrates. Regulation of PRMT1 has anti-tumor effects. However, the anti-tumor mechanism has not been established [70]. In PC mice models, the combination of the PRMT1 inhibitor and anti-PD-L1mAb up-regulated tumor infiltration of the $CD8^+$ T cells and enhanced tumor cell apoptosis. Moreover, PRMT1 inhibited the expression of PD-L1 on tumor cell surfaces [71] (Figure 2, Table 2).

ATM inhibitors. Another enzyme inhibitor is Ataxia Telangiectasia Mutated (ATM), which is an apical kinase of significance in radiation-induced DNA damage response (DDR) [72]. ATM inhibitors improve the activation of type

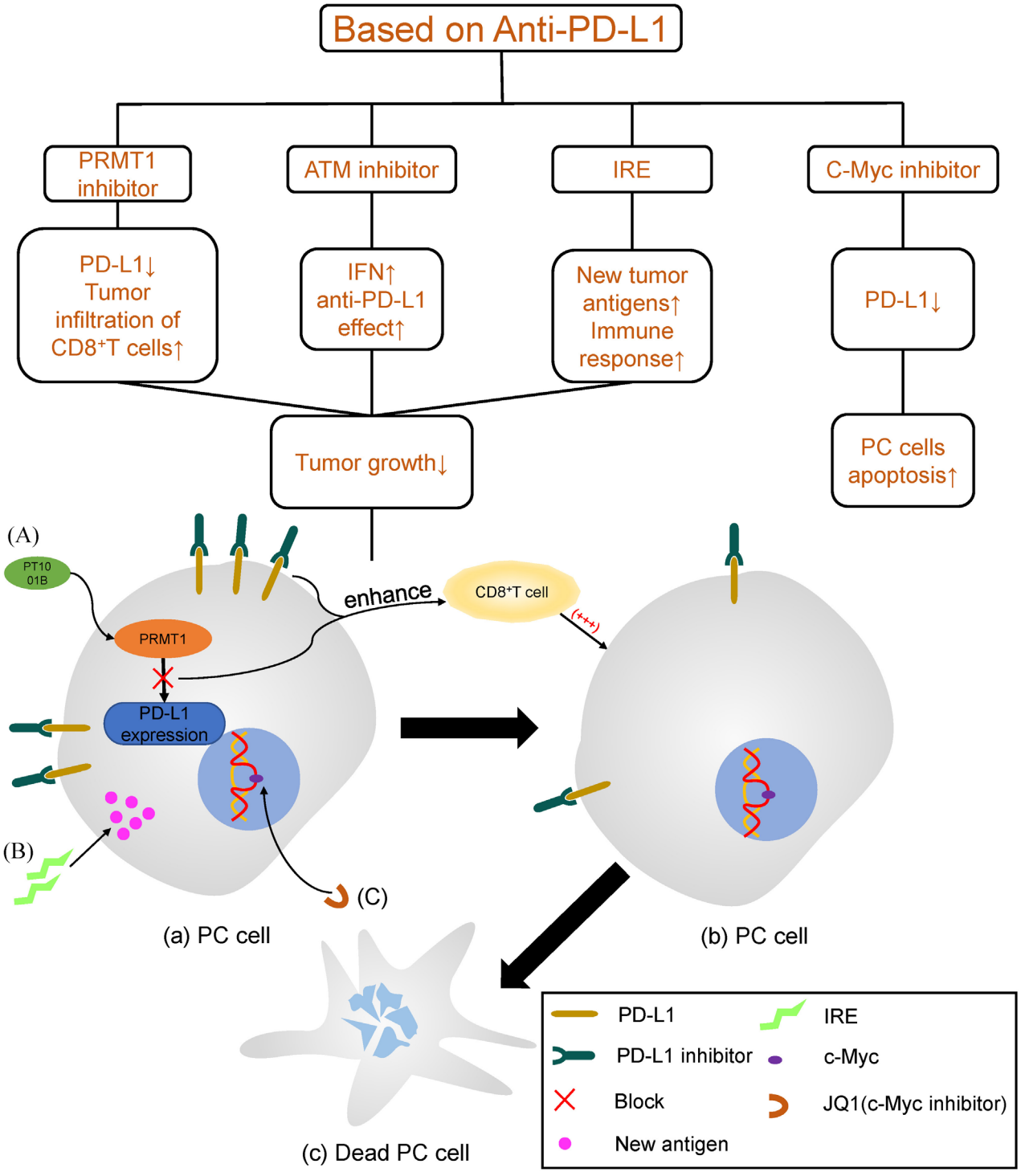

Figure 2. Mechanism of tumor cell growth inhibition by anti-PD-L1-based combination regimens. A) PRMT inhibits the expression of PD-L1 in PC cells, and combined therapy inhibits tumor cell proliferation. B) IRE enhances antigen presentation by releasing new tumor antigens, improves immune response, and inhibits the proliferation of distant metastatic cancer cells. C) JQ1 can reduce PD-L1 expression and induce apoptosis of tumor cells through c-Myc inhibition. b) Reduce the expression of PD-L1 on PC cells. Abbreviations: PD-L1-PD-1 ligand; PRMT-protein arginine methyltransferase; ATM-Ataxia Telangiectasia Mutated; IFN-interferon; IRE-irreversible electroporation

Table 2. Studies of combination regimens based on PD-L1.

Target	Drug	Model	Effect	Function
PRMT1 and PD-L1 [71]	PT1001B combined with PD-L1mAb	Cells: The murine PDAC cell line Panc02 Animals: Female C57BL/6 mice aged 5 weeks	PRMT inhibits the expression of PD-L1 in pancreatic cancer cells and combined therapy inhibits the proliferation of tumor cells	Expression of PD-L1 ↓ Tumor infiltration of CD8+T cells ↑ proliferation of tumor cells ↓
ATM and PD-L1 [73]	ATM inhibitor KU60019 combined with PD-L1mAb	Cells: Human PC cell line Panc1 and Capan1, murine pancreatic cancer cell line mT4 and KPC2 Animals: 10- to 12-week-old NSG mice and wild type C57BL/6 and FVB/NJ mice	Increase the secretion of IFN and significantly inhibit the growth of tumor	Secretion of IFN ↑ Tumor growth ↓
c-Myc and PD-L1 [76]	c-Myc inhibitor JQ1 combined with PD-L1mAb	Cell: The murine PDAC cell line Panc02 and MPC-83, Human PC cell lines including PANC-1, BxPC-3, SW1990, CFPAC-1, and AsPC-1 Animals: C57BL/6 mice and Kunming (KM) mice	Reduce the expression of PD-L1 and induce apoptosis of tumor cells	Expression of PD-L1 ↓ Tumor cell apoptosis ↑
Irreversible electroporation, PD-L1 and TLR7 [78]	Irreversible electroporation combined with PD-L1mAb and TLR7 agonists	Cells: Male KPC4580P cell line Animals: 6-8week old male C57BL/6 or Rag-1 knockout mice	Enhance antigen presentation by releasing new tumor antigens Improve immune response and inhibit the proliferation of distant metastatic cancer cells	Antigen presentation ↑ Proliferation of tumor cells ↓
Irreversible electroporation and PD-L1 [77]	Irreversible electroporation and PD-L1mAb (Nivolumab)	Patients who met the eligibility criteria	Promote the release of IFN- γ and regulate cellular immune function	Release of IFN-γ ↑ Expression of PD-L1 ↑ Immune evasion of tumor cells ↓

I interferon signaling through the TBK1 pathway, enhancing the anti-tumor effect of anti-PD-L1 therapy. An additional table file shows more details (Table 2). PD-L1 antibody, ATM silencing, and radiation combined therapy can significantly inhibit tumor growth due to the synergistic effect of radiotherapy and ATM inhibitors [73].

MET inhibitors. Li et al. reveal the positive correlation between high MET expression and PDAC upregulation in PC and explore the effect of MET inhibitor carbamazepine in a PC mouse model. The results showed that capmatinib could enhance the efficacy of anti-PD-L1, and the combination therapy strategy of the two had potential benefits for the treatment of pancreatic cancer [74]. Further clinical trials are still needed to investigate the effect of dual blockade of MET and PD-L1.

Gene or electrochemical therapy

c-Myc gene. Several studies have explored the anti-tumor effects of targeting c-Myc, which is associated with tumor evasion in PC [75]. c-Myc inhibitors enhance the anti-tumor effects of anti-PD-L1 therapy by inhibiting PD-L1 expression [76].

Irreversible electroporation (IRE). In a clinical phase 1b trial of PC, IRE was found to destroy tumor stroma, promote IFN-γ release, upregulate PD-L1 expression, and relieve tumor cells' immune evasion when combined with nivolumab [77]. In another study of anti-PD-L1 combination therapy, the combination regimen included IRE to increase tumor mutation burden, thereby acting as an *in situ* vaccine, which inhibited PC non-immunogenicity. Combination of the regimen with a toll-like receptor-7 (TLR7) agonist activated the innate immune system in PC, and this regimen did slow down tumor proliferation when compared to monotherapy [78]. More details are shown in the additional figure and table files (Figure 2, Table 2).

Others

Sonodynamic therapy (SDT) is a targeted anticancer therapy that uses ultrasound to activate sensitizers and produce reactive oxygen species (ROS), which can directly lead to cell death through apoptosis and necrosis. In the PC model of Nesbitt et al., SDT combined with anti-PD-L1 therapy can activate the adaptive immune system, and the levels of tumor-infiltrating CD4 and CD8 cells are significantly increased. SDT has a specific supplementary effect on PD-L1 inhibition therapy in the PC mice model, which synergistically inhibits tumor growth. SDT combined with anti-PD-L1 therapy can provide a beneficial treatment regimen for PC, especially for patients with advanced PC [79].

Discussion

The incidence of pancreatic cancer is still growing, and PC is expected to be the second leading cause of cancer-

related deaths by 2030 [4]. Some studies have shown that the prognosis of PC is abysmal, with 5-year overall survival (OS) of less than 5% [80] and 1-year OS of 24% [81] after standard treatment. Although immunotherapy has been efficacious in some other solid tumors (ovarian cancer [26], melanoma [27], renal cell carcinoma [28], bladder cancer [29], and Hodgkin's lymphoma [30] among others), the only potential application population for PC immunotherapy is the patients with microsatellite instability-high (MSI-H) tumors, in which PD-1 inhibitors (pembrolizumab) have shown encouraging results [82, 83]. Additionally, it is time to bring up the slightly improved responses for MSI-H patients, as the relatively low tumor mutational burden associated with PC appears to be a major barrier to ICIs in PC. One phase II trial investigated the therapeutic effect of anti-CTLA-4 monoclonal antibody (ipilimumab) in patients with advanced or metastatic PC [84], and the other phase II trial investigated the efficacy of a dual blocking regimen combining anti-CTLA-4 and anti-PD-L1 drugs [8], but none of the experiments showed satisfactory benefits. A phase Ib/II trial investigated the efficacy of the combination regimen of standard chemotherapy plus PD-1 inhibitor pembrolizumab, and the results showed that the OS was improved to 9.1 months [85], with a significant effect compared with OS at 4.6 months in patients who were not effectively treated. However, a phase II trial investigated the efficacy of dual immune checkpoint blockade combined with first-line chemotherapy regimens, and the results showed that the addition of ICI did not significantly improve patient OS [86].

We concluded that this is because most PCs have an immunosuppressive TME since they lack specific common antigens with immunogenicity and a low positive rate of PD-1 gene detection. The TME of human PC is composed of a complex of immune cells, in which T cells (including effector memory T cells, Tregs) account for a large proportion [87]. However, PC has a dense fibrous TME [6] that suppresses nor immune effects of T cells. The suppressive para-tumor stroma sequesters expanded tumor-reactive T cells [47], making TME devoid of infiltration by $CD8^+$ T cells. Therefore, the TME cannot exert normal anti-tumor immune effects. Extensive proliferation of connective tissues reduces interstitial vascularization, which alters the normal infiltration process of immune cells, further inducing tumor growth and hindering drug activity [88]. Moreover, drug resistance is likely to occur during treatment. The lack of tumor antigens and PD-1 gene results in impaired T cell recognition, rendering anti-PD-1/PD-L1 therapies ineffective in PC. Additionally, the relatively low tumor mutational burden associated with PC appears to be a major barrier to ICIs in PC.

In this review, we summarized the preclinical model and clinical research status of anti-PD-1/PD-L1-based combination therapies for PC. Anti-PD-1 based immune checkpoint inhibitors (ICI), enzyme inhibitors, immunomodulators, vaccines, radiotherapy, gene knockout and viruses, anti-PD-L1 based enzyme inhibitors, gene expression inhibitors, and IRE have been experimentally used in combination therapies. This review elucidates on hyporesponsiveness of immunotherapy alone for PC/PDAC, more importantly, the importance and effectiveness of combination immunotherapy. Despite the new immune combination treatment regimens mentioned in this review, perhaps the choice of treatment timing is the future direction of PC treatment. PC is characterized by early locoregional spread and distant metastasis, and most patients are incurable by surgical resection at the time of diagnosis. If there is no effective treatment, their OS is only 4.6 months, especially for patients with metastatic cancer, and the survival time is 2.8 to 5.7 months [89]. This reflects the importance of monitoring high-risk groups [90] and collecting pancreatic juice [91], which is helpful for early detection and intervention to improve the effectiveness of treatment. There was no significant difference in OS between the combination therapy and chemotherapy alone, while the progression-free survival of the combination therapy was longer than that of chemotherapy alone. Combination therapy can improve treatment efficiency without causing severe adverse effects [92]. Because of the low positive rate of PD-1 gene detection in clinical patients, it is vital to grasp the opportunity of anti-PD-1 therapy. Opening the dense and immune-repulsive TME of PC by targeting Hedgehog, TGFβ, and other targets mentioned in this review, and then grasp the timing of anti-PD-1 therapy, which can improve the therapeutic effect of anti-PD-1. Neoadjuvant immune combination chemotherapy can transform patients with advanced PC into resectable, achieving tumor-free survival of patients. Cancer immunotherapy is a promising strategy for PC treatment. We suggest further exploration of methods for early monitoring and detection of carcinogenesis and the effect of combined chemotherapy, radiotherapy, and other treatment regimens in patients with PC in the future. Especially study the timing, individualization, and precision treatment of immune combination therapy regimens to improve the survival rate of patients further and prolong the survival time.

In conclusion, most PCs have an immunosuppressive TME, and drug resistance is likely to occur during monotherapy. These cause poor treatment efficacies for PC. Compared to immune checkpoint blockade therapy alone, combination therapies were shown to alter the PC tumor microenvironment and improve responsiveness to immunotherapy by blocking or activating signaling pathways, inhibiting targets, regulating immune pathways, and inducing immune activation. Most of the current studies are in the preclinical model research stage, and further studies are needed to investigate the clinical safety, responsiveness, and effectiveness of combination therapies. Additionally, we should pay attention to earlier surveillance and detection of lesions while studying combination immunotherapy in the future, such as improving the methods of high-risk population monitoring and the collection of pancreatic juice. The

correct application timing of immune combination therapy should be further studied to achieve high efficacy, individualization, and precision treatment. In the near future, these combination therapies will have considerable clinical treatment benefits or survival benefits in PC treatment, improving treatment responsiveness as well as patient quality of life while reducing side effects.

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