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**Immune therapies in pancreatic ductal adenocarcinoma: Where are we now?**

Hilmi M *et al*. Immunotherapy for PDAC

Marc Hilmi, Laurent Bartholin, Cindy Neuzillet

**Marc Hilmi, Cindy Neuzillet**, Service d’Oncologie Médicale**,** Hôpital Henri Mondor, Assistance Publique-Hôpitaux de Paris, Université Paris Est Créteil, Créteil 94010, France

**Laurent Bartholin**, Université de Lyon, Université Claude Bernard Lyon 1, Inserm U1052, CNRS 5286, Centre Léon Bérard, Centre de Recherche en Cancérologie de Lyon, Lyon 69008, France

**ORCID number**: Marc Hilmi (0000-0003-4762-6740); Laurent Bartholin (0000-0002-5637-3223); Cindy Neuzillet (0000-0001-7037-7477).

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**Correspondence to**: **Dr. Cindy Neuzillet, MD, MSc,** Service d’Oncologie Médicale**,** Hôpital Henri Mondor, Assistance Publique-Hôpitaux de Paris, Université Paris Est Créteil, 51 Avenue du Maréchal de Lattre de Tassigny, Créteil 94010, France. cindy.neuzillet@gmail.com

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**Abstract**

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers, mostly due to its resistance to treatment. Of these, checkpoint inhibitors (CPI) are inefficient when used as monotherapy, except in the case of a rare subset of tumors harboring microsatellite instability (< 2%). This inefficacy mainly resides in the low immunogenicity and non-inflamed phenotype of PDAC. The abundant stroma generates a hypoxic microenvironment and drives the recruitment of immunosuppressive cells through cancer-associated-fibroblast activation and transforming growth factor β secretion. Several strategies have recently been developed to overcome this immunosuppressive microenvironment. Combination therapies involving CPI aim at increasing tumor immunogenicity and promoting the recruitment and activation of effector T cells. Ongoing studies are therefore exploring the association of CPI with vaccines, oncolytic viruses, MEK inhibitors, cytokine inhibitors, and hypoxia- and stroma-targeting agents. Adoptive T-cell transfer is also under investigation. Moreover, translational studies on tumor tissue and blood, prior to and during treatment may lead to the identification of biomarkers with predictive value for both clinical outcome and response to immunotherapy.

**Key words:** Checkpoint inhibitor; Drug therapy combination; Hypoxia; Immunology; Inflammation; Pancreatic cancer; transforming growth factor β; Tumor-infiltrating lymphocyte; Tumor microenvironment

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**Core tip:** Checkpoint inhibitors (CPI) and other immune therapies remain inefficient when used as single agents in pancreatic ductal adenocarcinoma (PDAC). Here, we present an overview of the biological mechanisms underlying these failures and the lessons learned, giving a rationale for innovative combination therapies. In particular, the latest ongoing studies are attempting to overcome the immunosuppressive microenvironment, the basis of resistance to CPI in PDAC.

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**INTRODUCTION**

Immunotherapy has paved the way for new therapeutic opportunities in cancer. Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death-1 (PD-1) are receptors expressed on the surface of T-cells that regulate the duration and the amplitude of immune responses in physiological conditions[1]. CTLA-4 is involved in the priming phase (lymph node) while PD-1 and its ligand PDL-1 are implicated in the effector phase (tumor) (Figure 1). The hijacking of these immunological "checkpoints" by cancer cells is a major mechanism of immune evasion, a better understanding of which led to the clinical development of anti–CTLA-4 and anti–PD-1/PD-L1 mAb with striking efficacy in several malignancies, including chemoresistant tumors. For example, objective responses associated with prolonged survival were observed in 30%-45% of melanomas[2], 15%-20% of lung cancers[3,4], 13% of pre-treated head and neck carcinomas[5], 22%-25% of pre-treated kidney cancers[6], and more than 60% of Hodgkin lymphomas[7] following anti–PD-1/PD-L1 monotherapies, leading to their clinical approval in these indications. However, immunotherapy failed to improve the outcome of patients in some tumor types[8], notably pancreatic ductal adenocarcinoma (PDAC).

Recent epidemiological projections have predicted that PDAC will become the second leading cause of cancer-associated death in the USA and Europe by 2030[9]. PDAC is the gastrointestinal tumor with the poorest prognosis, with 80% of patients having advanced disease at diagnosis and a 5-year survival rate that does not exceed 7%[10]. PDAC is characterized by its resistance to conventional therapies (chemotherapy, targeted therapy and radiotherapy)[11]; thus innovative therapeutic options are crucially needed. Despite hopes raised by the results of immune therapies in other cancers, these strategies have so far been disappointing in PDAC. Nonetheless, an improved understanding of the biology of its microenvironment has recently provided a rationale for innovative therapeutic combinations to unlock PDAC resistance to immune therapy.

The objectives of this review are (1) to present an overview of the immune therapies that have so far been tested in PDAC; (2) to describe the main mechanisms involved in resistance to these therapies, and (3) to introduce the current strategies to overcome this resistance.

**FAILURE OF IMMUNE MONOTHERAPIES IN PDAC**

Patients with PDAC were treated with anti–PD-1/PD-L1 (pembrolizumab, atezolizumab) and anti–CTLA-4 (ipilimumab) monotherapies in three phase I[12–14] and one phase II trials[14], respectively. Overall, these studies showed no activity of checkpoint inhibitor (CPI) monotherapies in unselected patients with advanced, pre-treated, progressive PDAC (Table 1).

Nevertheless, PD-1 blockade appears to be efficient in a subset of patients with PDAC harboring a mismatch repair (MMR) deficiency. The MMR machinery is encoded by four key genes (MLH1, MSH2, MSH6, PMS2), which behave as genome safeguards by correcting base mispairs occurring during DNA replication. Loss of MMR results in drastically increased rates of somatic mutations[15,16], potentially translated into neoantigens that can be recognized by the immune system[17,18] rendering them responsive to CPI. MMR deficiency can be caused by inherited germline defect in the case of Lynch syndrome, predisposing to a spectrum of tumors [mainly, colorectal (CRC) and endometrial cancers], or emerge from somatic mutations or promoter methylation (*e.g.,* in *BRAF*-mutated CRC)[19]. Microsatellite instability-high (MSI-H) is the phenotypic evidence of MMR deficiency. Recently, the use of pembrolizumab was approved for MSI-H or MMR-deficient tumors based on five clinical trials[20], which including 149 patients with tumors from 15 primary origins, mostly CRC (91/149). The objective response rate was 39.6%, including complete responses in 7.4%, and 78% of responses lasted more than 6 mo. MSI-H is thus recognized as a predictive biomarker of response to PD-1 blockade[21,22].

Six patients with PDAC were included in a multitumor expansion study of pembrolizumab (12 cancer types) with evidence of clinical benefit (one stable disease, three partial responses, and two complete responses). However, MSI-H is a rare event in PDAC[23] as illustrated by a genetic study on 385 PDAC that reported that hypermutated profiles (all related to MMR deficiency) were found in less than 2% of cases (4 out of 385)[24]. Therefore, the subset of PDAC patients eligible for CPI monotherapy is small.

Beside CPI, other immune therapy strategies (vaccines, oncolytic viruses, TGFβ inhibitors) have been tested and also remained inefficient in PDAC patients when used as monotherapies or in combination with gemcitabine chemotherapy (Table 1). Overall, except for MSI-H tumors, PDAC are considered to be resistant to single-agent immune therapy.

***Reasons why checkpoint inhibitor monotherapies failed to show any activity in pancreatic ductal adenocarcinoma***

The “cancer-immunity cycle” theory defines three conditions that are required to obtain an effective anti-tumoral immune response[25]: tumor immunogenicity, T cell recruitment and activation.

**Tumor immunogenicity:** Immunogenicity is related to the degree of epitope structural difference between tumor and normal cells. The more different the epitope, the more likely to be recognized by T cells[26]. Hence, tumor-associated antigens (TAA) loosely fall into two classes based on their tumoral specificity and immunogenicity: (1) low (differentiation antigens, overexpressed self-antigens) and (2) high (viral antigens, cancer-germline genes, and neoantigens) tumoral specificity. Neoantigens are peptides generated from non-silent coding mutations in the cancer cell genome and are highly immunogenic. Several studies have shown that tumor mutation load is linked to neoantigen burden and positively correlated with response to immunotherapy[27,28]. Pancreatic cancer has a low mutation load compared to other solid tumors, with an average mutation rate of 1 mutation per megabase (Mb) (compared to 11 mutations per Mb for melanoma), only occasionally yielding neoantigens[29]. Nevertheless, PDAC has an immunogenic capacity as reflected by the presence of T-cell infiltrates and tertiary lymphoid structures in resected PDAC samples[30-32]. Some studies suggest that although the rate of mutations is low, it is sufficient to create highly immunogenic neoantigens, notably through *KRAS* codon 12 mutations[33,34].

Importantly, DNA mutations do not necessarily translate into immunogenicity because both antigen presentation by major histocompatibility complex (MHC) and recognition by the T cell receptor (TCR) with a high affinity are required to induce T cell response, leading to the concept of neoantigen quality. It has been shown that the *fitness* of a neoantigen, *i.e.*, its distance from the wild type sequence coupled with its binding affinity to the TCR, is correlated with the activation of T cells[35]. High-quality neoantigens (mutation-associated or microbial-like sequences) have been associated with longer survival in PDAC, highlighting the fact that the neoantigen quality outweighs the neoantigen quantity in clinical significance[36].

Determining MHC-antigenic structures (*e.g.,* using mass spectrometry) is useful to (1) predict which neoantigen will be recognized by T cells and (2) identify actionable targets to trigger the immune response (*e.g.,* for vaccine strategies)[37-39]. Nonetheless, such approaches are currently limited by the poor performance of neoepitope predictive algorithms. Indeed, less than 5% of predicted neoepitopes actually give rise to a biological response[34]. The Tumor Neoantigen Selection Alliance initiative is a global bioinformatics collaborative effort aiming to develop a software that can best predict immunogenic mutation-associated cancer antigens from patients’ tumor DNA[40].

**T cells recruitment and activity:** The release of tumor neoantigens following cell death[41] allows antigen-presenting cells (APC), such as dendritic cells to uptake and present them to T cells leading to the activation of the latter[42-44]. Secondly, T cells must be recruited into the tumor after trafficking in blood vessels[45] and passing through the endothelial wall[46]. Finally, tumor-infiltrating lymphocytes (TIL) recognize and kill tumor cells[43].

Depending on the histological pattern of TIL, tumors are classified into T-cell inflamed (also known as “hot” tumors) *vs* non-inflamed (“cold”) tumors, in which T cells are excluded or absent[47]. Preclinical and clinical evidence suggest that only patients who have T-cell inflamed tumors respond to CPI monotherapy[47]. Most PDAC are thought to belong to the non-inflamed tumor group, displaying low levels of TIL along with low PD-L1 expression, which can account for the poor efficacy of single-agent immune therapies[48-50].

PDAC display an abundant desmoplastic stroma, the extent of which is often greater than the epithelial component of the tumor[51,52]. The stroma is a complex structure composed of extracellular matrix proteins and various cell types including cancer associated fibroblasts (CAF), endothelial cells, and immune cells[52]. This fibrotic barrier was believed to physically impede T cell infiltration[53]. However, recent work using multiplex imaging for spatial analysis of desmoplastic elements in PDAC revealed that collagen I deposits are inversely correlated with TIL numbers[54]. This observation has led to the hypothesis that the stroma may be a chemical rather than a physical barrier[55] (Figure 2). Indeed, PDAC is characterized by a high density of immunosuppressive cells including T regulatory cells (TREG) and myeloid cells [*e.g.* dendritic cells, myeloid derived suppressive cells (MDSC) and M2 macrophages], which are negative prognostic factors[56]. Myeloid cells release TGFβ[57], nitric oxide synthase and arginase, preventing TIL recruitment and activity[56,58]. Tumor hypoxia is a predominant driver in the recruitment of these immune cells through CAF activation[59-61]. Activated CAF then secrete immunosuppressive cytokines[62,63], such as CXCL12 and IL-6, which promote MDSC recruitment and inhibit effector T cell recruitment.

In addition, although T cell infiltration seems to be necessary for the response to immune therapy, the presence of TIL is not sufficient to induce an effective anti-tumor response[64]. Indeed, TIL activation is required. However, in PDAC, even in the presence of tumor-specific neoepitopes, T cells display a reduced activation signature[34] and most of them are PD-1–positive[65], suggesting that T cell activation is actively suppressed. Notably, not only MDSC but also TREG and CD8-positive γδT cells restrain activation of αβT cells that are directed against the tumor[66]. These deleterious TIL represent approximately 40% of CD8-positive TIL populations in PDAC and may mislead the interpretation of the biological significance of TIL in PDAC. This may enlighten some negative results showing no prognostic impact of T cell infiltration in PDAC[56,64].

Overall, given its low mutational load, low lymphocyte count, the presence of inflammatory cytokines and hypoxia, PDAC displays a unique microenvironment that is unfavorable to immune therapy according to the cancer immunogram and requires combination strategies[67].

***Research challenges***

**Rational combinations:** Following the failure of CPI monotherapies in PDAC, efforts have been made to develop rational combinations to overcome PDAC resistance to immune therapy. Based on the cancer immunity cycle[25], most of them combine a CPI with another agent aiming to (1) increase tumor immunogenicity; (2) increase TIL number and activity; and/or (3) attenuate immunosuppression in the tumor microenvironment. Combination therapy can employ immune therapy, conventional chemo/radiotherapy, targeted therapy, or vaccine/adoptive T-cell therapy[50,68].

**Increasing tumor immunogenicity:** Chemotherapeutic agents and radiotherapy may play a dual role by directly killing cancer cells, thus reducing the overall tumor burden and indirectly by releasing pro-inflammatory molecules and tumor-associated antigens (TAA) (*e.g.,* calreticulin, ATP) which, when presented in an immunogenic fashion, may function as *in situ* vaccines to attract and activate T cells (so called “immunogenic death”). Among chemotherapeutic agents used in the PDAC therapeutic armamentarium, platinum-based agents and taxanes are preferential combination partners for immunotherapy because they can induce immunogenic cell death, sensitize tumor cells to immune-mediated destruction and enhance T cell activation[69-71]. Although some investigators have shown that FOLFIRI [folinic acid, 5-fluorouracil (5FU) and irinotecan combination] can be given with vaccines to CRC patients without abrogation of the immune response[72], 5FU and irinotecan have been reported to be more immunosuppressive[73]. Therefore, combining them with an immune therapy may impair the immune-mediated anti-tumor response, and a sequential design for immune therapy after induction chemotherapy using these agents may be more effective.

Tumor vaccines and oncolytic viruses both aim at increasing tumor antigen recognition by the immune system through presentation by dendritic cells[74,75]. Although relatively inefficient as monotherapies, vaccine strategies are currently explored in combination with CPI. GVAX is a granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting allogeneic PDAC vaccine. It was first evaluated in combination with anti–CTLA-4 therapy[76]. Thirty pre-treated PDAC patients were randomized to receive ipilimumab alone or combined with GVAX. The latter experienced a longer median overall survival (OS) (3.6 *vs* 5.7 mo, *P* = 0.07) with no additional toxicity. Furthermore, the observation that neoadjuvant GVAX was able to induce intra-tumoral tertiary lymphoid structures and upregulate PD-L1 membranous expression in resected tumor samples[30] provided a rationale for its combination with anti–PD-1. This was also supported by preclinical data in mouse models[77] showing an improved survival rate with the combination of GVAX and PD-1 blockade compared to each agent taken individually. In clinical practice, GVAX is associated to cancer vaccine CRS-207 (an attenuated form of *Listeria monocytogenes*) and/or cyclophosphamide (aiming at downregulating TREG) in clinical trials in the adjuvant setting[78]. GVAX/cyclophosphamide therapy is also currently being tested in PDAC in combination with nivolumab (anti–PD-1) alone (NCT02243271, NCT02451982, NCT03161379) or combined to ipilimumab (anti-CTLA-4) (NCT03190265), or with pembrolizumab (anti–PD-1) alone (NCT02648282) or combined to the indoleamine-2,3 dioxygenase (IDO, an enzyme that inhibits T cells proliferation by catalyzing the degradation of tryptophan[79]) inhibitor epacadostat (NCT03006302). Restoring the proliferation and activation of various immune cells, including T cells[80], may potentiate the response to vaccine therapy. Of note, there is also a rationale for combining GVAX with TGFβ inhibitors in preclinical models[77,81]. However, this combination has not reached clinical trials. GVAX, like peptidic “one-size-fits-all” vaccines, has to face the challenges of (1) the unique tumor antigen landscape specific to each patient and (2) the emergence of immune evasion, both of which can compromise patient response to vaccine therapy[82]. Personalized vaccine approaches are expected to partially overcome these issues but their development remains limited by their logistic complexity and high costs[82-84]. Alternatively, oncolytic viruses combine antigen presentation with the induction of a type I interferon-γ (IFN-γ) response that potentiates effector T-cell activation[74,75]. Similar to the vaccine approach, the oncolytic virus reolysin was tested in metastatic PDAC in combination with carboplatin and paclitaxel but failed to improve progression-free survival (PFS)[85]. However, a phase II study[86] explored the combination of reolysin, pembrolizumab (anti–PD-1) and chemotherapy in 11 patients with pre-treated PDAC and showed antitumor activity with a manageable safety profile. Among the 5 evaluable patients, two had stable diseases (126 and 221 d) and one had partial response lasting more than 6 mo. A phase Ib trial in combination with pembrolizumab and gemcitabine, irinotecan or leucovorin/5-fluorouracil (5-FU) is ongoing (NCT02620423).

**Increase TIL recruitment and activity:**Most anti–PD-1/PD-L1-based combination trials focus on converting the PDAC non-inflamed (immune-excluded or desert) microenvironment into an inflamed pattern by increasing T cells recruitment and activity.

CPI combination : The association of CTLA-4 and PD-1 antibodies resulted in an improved OS in patients with advanced melanoma compared with each agent used as monotherapy, albeit at the price of increased toxicity with 59% of patients experiencing grade 3 or 4 adverse events (*vs* 21%-28% with monotherapy)[87]. The PA.7 randomized phase II trial (NCT02879318)explores the combination of tremelimumab (anti–CTLA-4 mAb) and durvalumab (anti–PD-L1 mAb) with gemcitabine plus *nab*-paclitaxel chemotherapy *vs* chemotherapy alone as a first-line treatment for metastatic PDAC. Co-targeting of other immunomodulatory pathways such as IDO, OX40, CD40, the lymphocyte activation gene 3 protein (LAG3) or T cell immunoglobulin and mucin 3 (TIM3), among numerous candidates, might be as efficient and less toxic than PD-1/CTLA-4 combination[88] but remain to be explored in PDAC patients.

Combination with anti-M2/-MDSC: The CCL2-CCR2 chemokine axis induces the recruitment of immunosuppressive tumor-associated-macrophages (TAM)[89]. A CCR2 inhibitor (PF-04136309) has been tested in combination with FOLFIRINOX chemotherapy in a phase Ib study in patients with borderline resectable/locally advanced PDAC[89]. The objective response rate was 49% and disease control rate reached 97% with a manageable safety profile. Interestingly, ancillary studies showed (1) a decrease in TAM infiltration together with (2) a decrease in circulating monocytes and (3) an increase in bone marrow monocytes in patients treated with the combination, supporting the mechanistic hypothesis of a reduction in intra-tumor monocyte recruitment from the bone marrow[90].

Other inflammatory pathways have been targeted using small molecules or mAb and are currently being explored in clinical trials in combination with CPI based on promising results in mouse models. These include colony stimulating factor 1 receptor (CSF1R)[91] (NCT02777710), IL-6[92], TGFβ (NCT02734160), CCR4 (NCT02301130), CXCR2 (NCT02583477) and CXCR4/CXCL12 (NCT03168139). Nonetheless, similarly to the results obtained following pathway inhibition using tyrosine kinase inhibitors, secondary resistance due to cytokine axes compensation has emerged, leading to disease progression and pleading for combination strategies[93].

Combination with MEK inhibitors: MEK inhibition (MEK-i) was primarily developed in PDAC as a *KRAS* signaling inhibition strategy, given the high frequency of activating *KRAS* mutations in these tumors (> 90%)[94]. MEK-i failed to improve the survival rate of PDAC patients when used as monotherapy or in combination with gemcitabine[94]. However, novel perspectives are opening up for MEK-i as a combination partner with immune therapy. Indeed, MEK-i exerts multifaceted immunostimulatory effects by (1) increasing MHC-I expression and decreasing PD-L1 expression on tumor cells, (2) increasing TIL activity and survival and (3) decreasing macrophage and MDSC infiltrates[95].

A phase Ib study (NCT01988896) has investigated the combination of cobimetinib (MEK-i) with atezolizumab (anti–PD-L1) in pre-treated metastatic CRC; durable objective responses were observed in patients with microsatellite stable (MSS)/MSI-low tumors, mostly *KRAS*-mutated, prompting the evaluation of this combination in PDAC in a clinical trial (NCT03193190).

Targeting tumor hypoxia: Likewise, hypoxia-targeting strategies have been tested with disappointing results in combination with gemcitabine[96]. Evofosfamide (TH-302) is a cytotoxic prodrug that is activated under hypoxic conditions, targeting hypoxic tumor areas. It is now being explored as a combination partner for immunotherapy since it can improve tumor tissue oxygenation and subsequently decrease MDSC recruitment and increase effector T cell activity[59,97]. The use of TH-302 with CPI may therefore be effective in restoring a favorable immune environment. A phase I trial is underway to study the combination of TH-302 with ipilimumab (anti-CTLA-4) in PDAC, melanoma, head and neck cancer and prostate cancer (NCT03098160).

Targeting fibroblasts and the stromal physical barrier: There have been contradictory reports on the roles of the desmoplastic stroma in PDAC (tumor-promoting *vs*  tumor-restrictive effect). CAF elimination using sonic hedgehog inhibitors or genetic strategy for selective depletion of α-smooth muscle actin (α-SMA)-positive cells in transgenic mice resulted in aggressive and undifferentiated tumors with increased vascularization and TREG infiltration, respectively[98,99]. Clinical trials with hedgehog inhibitors in PDAC were negative for any anti-neoplastic activity[100]. Strategies then shifted toward stroma modulation rather than depletion.

Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase protein that has been reported to be overexpressed and active in many solid tumors, including PDAC[101]. FAK is expressed by fibroblastic cells as well as tumoral, endothelial and immune cells[101], and its inhibition engenders pleiomorphic effects[102]. In preclinical models, FAK inhibition reduced fibrosis, decreased the amount of tumor-infiltrating immunosuppressive cells, and rendered the previously unresponsive KPC mouse models sensitive to PD-1 blockade[102]. Two phase I/II studies are underway to verify the benefit of this combination (NCT02546531 and NCT02758587). Other CAF-modulating or anti-fibrotic agents are also under investigation including TGFβ inhibitors (NCT02734160), PEGPH20 (NCT03193190) and vitamin D (NCT03331562) in combination with CPI. In addition, all-trans-retinoic acid (ATRA) (NCT03307148), and BET-inhibitors (NCT02711137) are being explored in combination with chemotherapy.

**CAR-T cells:** Adoptive cell therapy is a technology that has recently drawn increasing attention. T cells may be engineered to express a chimeric antigen receptor (CAR) in order to target specific tumor antigen[103]. This approach has already proven its effectiveness in B-cell hematological malignancies with T cells expressing CD19 CAR[104,105]. Similarly, mesothelin CAR-T therapy has been proposed in solid tumors[106]. In PDAC, this therapy led to the prolonged survival in a mouse model study[107]. Nevertheless, clinical development of this strategy in solid tumors is hampered by (1) its limited efficacy in comparison with the results seen in hematological malignancies, (2) high level of toxicity, including life-threatening immune adverse events (neurotoxicity and cytokine release syndrome) and (3) costs and logistics to be deployed on a large patient population. Next generation CAR T-cells are currently being developed to overcome these challenges[108].

**CONCLUSION**

***Rethink current clinical trial approaches***

Besides exploring new therapeutic avenues, it is also necessary to rethink the design of clinical immune therapy trials targeting PDAC. The clinical trial design tends to shift from traditional phase I to III development plan toward a signal detection strategy in multiple patient cohorts. In the context of an increasing number of clinical trials, there is a need to identify the most relevant combinations among the numerous candidate agents. Development of new preclinical models closer to the complex *in vivo* conditions should significantly improve the predictive value for therapeutic agent testing and guide the selection of the most active combinations for evaluation in clinical trials.

Second, the examples of MEK-i, vaccines, evofosfamide or TGFβ inhibitors show that it may be worth giving a second chance to some molecules that were found inactive as monotherapy.

In addition, patients with heavily pre-treated, progressive, advanced PDAC are not good candidates for immune therapy and this may partially account for failure of previous studies. These patients should possibly be excluded from immunotherapy clinical trials. Alternatively, positioning immune therapy as maintenance strategy following a course of induction chemotherapy (*e.g.,* with FOLFIRINOX) seems to present several advantages: (1) it allows the identification and exclusion of patients with rapid tumor progression; (2) such a treatment may have induced immunogenic cell death and sensitized the tumor to CPI; and (3) given that induction chemotherapy was not interrupted due to inefficacy, it could be reintroduced at disease progression. Taken together, these elements support the development of immune therapy as maintenance therapy in patients with controlled disease.

Finally, there is a critical need for predictive biomarker identification in order to guide patient selection for immune therapy and to stratify the randomization. Meanwhile, it is necessary to assess the predictive value of already available PDAC molecular classifications in the ancillary studies of ongoing clinical trials[109-112].

***Future directions***

PDAC is resistant to CPI monotherapy due to its unfavorable non-immune inflamed microenvironment. A better understanding of the biological mechanisms underlying PDAC immunosuppression may pave the way to innovative and promising strategies. Given the key role of the team hypoxia-TGFβ-CAF-M2/MDSC, the development of rational combinations of immunotherapy targeting these pathways and cell populations to increase intra-tumor recruitment and activation of T cells is coherent. To achieve this, we will have to reconsider inactive molecules in monotherapy, optimize the position of immunotherapy in the therapeutic sequence and develop new preclinical models to better predict therapeutic efficacy.

Furthermore, an improved understanding of the mechanisms of sensitivity and resistance to immunotherapy has revealed the increasing complexity in the tumor antigens, TIL, TREG, and MDSC landscape[113]. For instance, (1) anti-inflammatory and pro-inflammatory cytokines have counter balancing activities; (2) biological effects may be different between primary and metastatic tumor sites as illustrated by dissociated responses; (3) hypermutated tumors are more likely to respond to but also to develop resistance to CPI[114]; (4) the immune therapy response is also dependent on the patient microbiota[115,116] and genetics[117]. Mechanisms of action of CPI remain yet to be fully elucidated. The collaboration between clinicians and researchers will be the cornerstone of future progress in this field.

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Grade E (Poor): 0

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**Figure 1 Cytotoxic T lymphocyte-associated protein 4 and Programmed cell death-1 biological functions and therapeutic targeting**. Cells of the immune system express several surface molecules that are important for immune surveillance and regulation of the immune response. T cell receptor (TCR) is expressed by T cells; it is an antigen-specific molecule that is unique to each T cell clone. Major human compatibility (MHC) molecule is expressed by antigen-presenting cells (*e.g.,* dendritic cell) and display a potential tumor antigen for recognition by the specific TCR. Left panel: When an antigen presented in the context of MHC is recognized by the TCR, interaction of CD28 (expressed by T cell) with B7 (CD80/CD86) molecules provide a co-stimulatory signal leading to T-cell activation. However, depending on the conditions and microenvironment, these T cells can also express various levels of cytotoxic T lymphocyte-associated protein 4 (CTLA-4), a regulatory receptor (immune checkpoint) with a higher binding affinity for B7 than CD28. Therefore, when CTLA-4 is available at the cell surface, it successfully competes for binding with B7, removing the co-stimulatory signal and leading to T-cell downregulation. Tumor cells can then escape the T cell cytotoxic effect (immune evasion). CTLA-4 blockade affects the immune priming phase occurring in the lymph node, by supporting the activation and proliferation of a higher number of effector T cells, regardless of TCR specificity, and by reducing Treg-mediated suppression of T-cell responses. Right panel: T cells also express PD-1 receptor, which has the potential to induce a programmed-death cascade in T cells that mistakenly react to host cells and thereby maintaining self-tolerance. PD-1 ligand, PD-L1, is used by tumor cells to engage the PD-1 receptor and switch off the reaction, inducing immune tolerance to the MHC-presented antigen. PD-L1 can also be expressed by stromal cells (*e.g.,* M2 macrophages). PD-1 blockade works during the effector phase in peripheral tissues (tumor) to restore the immune function of “exhausted” T cells that have been turned off following extended or high levels of antigen exposure. CTLA-4: Cytotoxic T lymphocyte-associated protein 4; DC: Dendritic cell; MHC: Major human compatibility; PD-1: Programmed cell death-1; PD-L1: Programmed death-ligand 1; TCR: T cell receptor.



**Figure 2 Summary of the mechanisms responsible for pancreatic ductal adenocarcinoma resistance to immune therapy.** The circle outlines the three steps of the cancer-immunity cycle: (1) Immunogenicity (yellow); (2) T-cell recruitment and (3) activation. Pancreatic ductal adenocarcinoma resistance to immune therapy is due to the combination of several factors: (1) Low tumor immunogenicity, with a low mutation rate and low neaoantigen burden compared to other tumors (*e.g.,* melanoma); (2) low T-cell recruitment and (3) activation: the dense desmoplastic stroma generates high interstitial pressure; this results in poor tumor perfusion and intra-tumor hypoxia, which in turn activates fibroblasts to release immunosuppressive cytokines (*e.g.,* TGFβ, IL-6, CSF1 = “chemical barrier”) that lead to the recruitment of immunosuppressive cells (M2 macrophages, TREG, MDSC) and exclusion and anergy of effector T cells. CSF1: Colony stimulating factor 1; IL-6: Interleukin-6; MDSC: Myeloid-derived suppressive cells; TGFβ: Transforming growth factor β; TREG: T regulatory cells.

**Table 1 Summary of clinical trials of immune therapies (single agent or combination with gemcitabine) in patients with pancreatic ductal adenocarcinoma**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of immunotherapy** | **Molecules** | **Trial** | **Phase** | ***n*** | **Population** | **Main results** |
| Immune checkpoint inhibitors | PD-L1 (BMS-936559) | Brahmer *et al*[8] | I | 14 | Advanced PDACPre-treated | No objective response |
|  | PD-L1 (atezolizumab) | Herbst *et al*[12] | I | 1 | Advanced PDACPre-treated | No objective response |
|  | PD-1 (pembrolizumab) | Patnaik *et al*[13] | I | 1 | Advanced PDACPre-treated | No objective response |
|  | CTLA-4 (ipilimumab) | Royal *et al*[14] | II | 27 | Advanced PDACPre-treated | No objective response |
| Therapeutic vaccines | GVAX | Jaffee *et al*[118] | I | 14 | Resected PDACAdjuvantCombination with chemoradiotherapy | 3 patients remained disease-free for > 25 mo |
|  |  | Lutz *et al*[119] | II | 60 | Resected PDACAdjuvantCombination with chemoradiotherapy | Median disease-free survival: 17.3 mo Median overall survival: 24.8 mo |
|  |  | Laheru *et al*[120] | II | 50 | Advanced PDACPre-treatedCombination with cyclophosphamide | Median overall survival: 4.3 mo  |
|  |  | Lutz *et al*[30] | PilotRandomized | 54 | Resected PDACNeoadjuvant and adjuvantCombination with cyclophosphamide | Arm 1: GVAX aloneArm 2: Cyclophosphamide (intravenous) + GVAXArm 3: Cyclophosphamide (daily oral) + GVAXIntra-tumoral tertiary lymphoid aggregatesPD-1 and PDL-1 upregulation  |
|  | CRS 207 | Le *et al*[121] | I | 7 | Advanced PDACPre-treated | No objective response |
|  | GVAX + CRS 207 | Le *et al*[78] | IIRandomized | 90 | Advanced PDACPre-treated | Arm 1: Cyclophosphamide + GVAX + CRS-207Arm 2: Cyclophosphamide + GVAXNo objective response |
|  | Algenpantucel-L | Hardacre *et al*[122] | II | 70 | Resected PDACAdjuvantCombination with chemotherapy | Disease-free survival: 62% at 1 yearOverall survival: 86% at 1 year |
|  | Mutated *KRAS* peptide | Gjertsen *et al*[123] | I/II | 5 | Advanced PDACPre-treated | No objective response |
|  |  | Gjertsen *et al*[124] | I/II | 48 | Advanced PDACPre-treatedResected PDACAdjuvant | No objective responseMedian overall survival in resected PDAC: 25.6 mo |
|  |  | Abou-Alfa *et al*[125] | I | 24 | Resected PDACAdjuvant | Median disease-free survival: 8.6 moMedian overall survival: 20.3 mo |
|  | Telomerase peptide (GV1001) | Middleton *et al*[126] | IIIRandomized | 1062 | Advanced PDACFirst lineCombination with chemotherapy | Arm 1: chemotherapy aloneArm 2: sequential chemo-immunotherapyArm 3: concurrent chemo-immunotherapyNo benefit on overall survival of adding vaccination to chemotherapy |
| Oncolytic viruses | Mutated adenovirus (ONYX-15) | Hecht *et al*[127] | I/II | 21 | Advanced PDACPre-treated and first line Combination with chemotherapy | Two partial responses  |
|  |  | Mulvihill *et al*[128] | I | 23 | Advanced PDACPre-treated and first line | No objective response |
| Anti-transforming growth factor β (TGFβ) | Anti-TGFβ2 (trabedersen) | Oettle *et al*[129] | I/II | 37 | Advanced PDACPre-treated | One complete response  |
|  | TGFβ receptor inhibitor (galunisertib) | Melisi *et al*[130] | IIRandomized | 156 | Advanced PDACPre-treated and first lineCombination with chemotherapy | Arm 1: galunisertib + gemcitabineArm 2: gemcitabine +placeboNo benefit on overall survival of adding galunisertib to chemotherapy  |

CTLA-4: Cytotoxic T lymphocyte-associated protein 4; PD-1: Programmed cell death-1; PD-L1: Programmed death-ligand 1.