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Title: Immune Evasion in Pancreatic Cancer: from Mechanisms to Therapy

Neus Martinez-Bosch¹, Judith Vinaixa¹ and Pilar Navarro¹, ²*

¹ Cancer Research Program, IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain
² Institute of Biomedical Research of Barcelona (IIBB-CSIC), Barcelona, Spain
* Correspondence: pnavarro@imim.es; Tel.: +00 34 933160400

Abstract

Pancreatic ductal adenocarcinoma (PDA), the most frequent type of pancreatic cancer, is one of the main unfinished businesses in the biomedical and clinical fields, with still discouraging 5 year survival rates and poor therapy efficiency. PDA abundant desmoplasia has for long played the lead in the mechanisms involved in poor drug performance, being the main source of cytokines and chemokines orchestrating rapid and silent tumor progression and guilty of isolating tumor cells into a extensive fibrotic reaction resulting in inefficient drug delivery. However, since immunotherapy was proclaimed the breakthrough of the year back to 2013, the focus in the stroma of pancreatic cancer has interestingly moved from activated fibroblasts to the immune compartment, trying to understand the immunosuppressive factors that play part in the strong immune evasion that characterizes PDA. PDA microenvironment is highly immune-suppressive, being basically composed of T regulatory cells (Tregs), tumor-associated macrophages (TAMs) and myeloid-derived suppressive cells (MDSCs), which boycott CD8+ T-cell duties in tumor recognition and clearance. Interestingly, preclinical data have highlighted the importance of this immune evasion as the source of resistance to single checkpoint immunotherapies and cancer vaccines and point at pathways inhibiting the immune attack as the key to solve the therapy puzzle. Here, we will discuss the molecular mechanisms involved in PDA immune escape as well as the state of the art of the PDA immunotherapy.

Keywords: pancreatic cancer; immune surveillance; galectins; immunotherapy; immune checkpoints; stroma
Introduction

The immune system plays a key role in the regulation, both positively and negatively, of tumor development and progression and, for this reason, crosstalk between cancer cells and immune cells has been incorporated to the list of major hallmarks of cancer [1]. While immune surveillance [2,3] is the first filter to identify and eliminate aberrant or malignant cells, some tumor cells are able to develop several strategies to avoid the recognition by the host immune cells, escaping from immune control and continuing cancer progression. Different mechanisms are involved in tumor immune evasion. First, cancer cells can decrease immune recognition by downregulation of antigen presentation pathways, like the major histocompatibility complex (MHC) I proteins, TAP (transporter associated with antigen processing) protein or latent membrane proteins (LMP2 and LMP7) [4–9]. Moreover, genetic instability of tumors and constant cell division can result in the loss of tumor antigens recognized by effector T-cells (CD8+ or CD4+ T-cells). These changes in the immunogenicity of cancer cells leading to immune-resistant clones have been called tumor “immunoediting” [10]. Second, tumor cells and other cells from the tumor microenvironment can promote an immune privilege status by secretion of immunosuppressive cytokines – such as IL-1, IL-6, IL-10, TGFβ, TNFα or VEGF [11–18] - or modulation of the expression of immunoregulatory molecules to induce T-cell anergy or tolerance –like the immune checkpoints molecules of the B7 family (PD1/PDL1, B7-1 and B7-2/CTLA4, B7-H4, etc.), A2AR, LAG-3, galectin-9/TIM-3, IDO or VISTA [19–22]. Finally, overexpression of STAT3 or BCL-2 by tumor cells to increase their resistance to apoptosis also contributes to immune escape. Altogether these mechanisms orchestrate an immunosuppressive cancer microenvironment through inhibition of immune cells involved in tumor rejection – CD4+/CD8+ T lymphocytes and NK cells– together with the recruitment and/or activation of immune suppressive cells, like CD4+CD25+FoxP3+ T regulatory cells (Tregs), myeloid-derived suppressor cells (MDSCs) and pro-inflammatory M2 macrophages.

Importantly, although there is considerable knowledge on these immune evasion strategies and how to face them using immunotherapy in several tumors (i.e. melanoma, lung or lymphomas), little is known in pancreatic cancer. Here, we review the molecular mechanisms involved in the immunosuppressive nature of PDA and the current progress made in immunology-based therapy against this dismal disease.

PDA tumor heterogeneity and immune responses

Genomic analyses have revealed the biological complexity and high tumor heterogeneity of PDA. Whole-exome and whole-genome sequencing studies have revealed a high number of somatic copy number alterations and mutations in pancreatic cancer, leading to altered
expression of key oncogenes and tumor suppressor genes, such as KRAS, TP53, SMAD4 and CDKN2A [23–26]. More recent studies from the APGI/International Cancer Genome Consortium [27] and from The Cancer Genome Atlas (TCGA) network [28] have validated these results and have also added a long list of other less frequently mutated genes, confirming the complex molecular landscape of pancreatic cancer. These gene expression analyses have allowed classifying PDA patients according to their molecular signatures into different subtypes that associate with histopathological hallmarks and prognosis, providing new avenues for personalized medicine. The most recent subcategories proposed for PDA include four subtypes: 1) pancreatic progenitor or classical PDA, which express early pancreatic development genes (i.e. PDX1, FOXA2/3, HES1) 2) squamous or quasimesenchymal tumors, which are enriched in TP53 mutations, upregulation of TP63ΔN and activation of TGF-β signalling and MYC pathway, and have a poor prognosis; 3) aberrantly differentiated endocrine/exocrine tumors, which overexpress genes involved in KRAS activation, and endocrine (NR5A, RBPJL) and exocrine (NEUROD1, NXX2-2) markers; and 4) immunogenic PDA. The last subtype has molecular similarities to classical PDA but it also expresses genes associated to immune phenotypes, such as Toll-like receptors, antigen presentation molecules and genes related to infiltrating B and T cells, both T-cytotoxic (CD8+) and Tregs. Moreover, these tumors also showed upregulation of the immune checkpoint molecules CTLA4 and PD1, suggesting that immunogenic PDA subtype may be sensitive to immunotherapy. However, most of the pancreatic tumors do not belong to the immunogenic subtype and for this reason PDA is generally described as a poorly immunogenic tumor. PDA immunosuppressive microenvironment is mainly composed by Tregs, macrophages and MDSCs, which block the anti-tumoral activity of effector CD4+ and CD8+ T-cells [29–32]. These immunosuppressive cells are already present in preneoplastic lesions (PanINs) indicating that they may be key players in tumor initiation [33]. The important role of Tregs in PDA has been shown in a murine model where disruption of these cells correlated with tumor growth inhibition [34]. Tumor associated macrophages (TAMs) play also important functions in pancreatic tumor chronic inflammation, progression and metastasis. Tumor infiltrated macrophages express CCR2, a chemokine receptor that interacts with CCL2 and exerts a protumoral role mediating tumor proliferation, angiogenesis and chemotaxis of immune suppressive cells to the tumor stroma [35]. PDA also contributes to immunosuppression by upregulation of negative T-cell costimulatory molecules [36]. In this regard, PDL1 and PDL2 are overexpressed in PDA patients [37] and correlate with reduced tumor infiltrating leukocytes (TILs) and worse prognosis [38,39]. Accordingly, downregulation of PDL1 inhibits pancreatic tumor cell proliferation [40].
Furthermore, increased expression of inhibitory molecules on inactivated T-cells has been suggested as another way to induce pancreatic cancer immunosuppression. Thus, pancreatic tumors show a high expression of CD40, a cell membrane receptor of the tumor necrosis factor family that modulates immune response, and this overexpression is associated to higher TNM staging and metastasis [41].

Another reason to explain the PDA immunosuppressive phenotype might be its mutational signature. Indeed, recent cancer mutational analyses have demonstrated high variability among tumors, indicating that those tumors with increased mutation rate, like melanoma or lung, are highly immunogenic while those with low mutation rate, as PDA [23], are poorly antigenic [42–44]. Interestingly, very recent data have provided evidences for the presence of neoantigens in long term survivors of PDA [45]. This study suggests that immune response against neoantigens generated during pancreatic tumor evolution, such as MUC16, can lead to decreased relapse and best prognosis. Further studies to identify other PDA immunogenic hotspots could be encouraging for the development of neoantigen-targeted strategies for the treatment of checkpoint blockade-resistant patients.

### Immune checkpoints in PDA

Immune checkpoints refer to several costimulatory and inhibitory signals in immune cells that, in physiological conditions, prevent autoimmunity and constrict tissue damage during infections. However, these pathways can be hijacked by tumor cells to achieve immune evasion [46,47]. Among the many different immune checkpoints that regulate T-cell activation, cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD1) are the most well characterized and studied for cancer immunotherapy.

CTLA4 is expressed by activated T-cells and Tregs and binds to its ligands B7-1/CD80 and B7-2/CD86 on the surface of antigen-presenting cells, leading to downregulation of T helper cells and enhancement of Treg-mediated immune suppression [46–48]. Interestingly, blockade of CTLA4 in preclinical murine cancer models restores effector T-cells and decreases Tregs in melanoma [49,50] and colorectal cancer [51], demonstrating the important role of this molecule in tumor immune evasion. As previously mentioned, PDA is characterized for its highly immunosuppressive stroma rich in Tregs, therefore CTLA4 immune checkpoint emerges as a promising target also for PDA treatment. Indeed, recent data by Bengsch et al [52] using PDA mouse models have demonstrated that blockade of CTLA4 in Tregs induces CD4⁺ T-cell tumor infiltration, suggesting an important role for pancreatic cancer immunotherapy. However, PDA patients enrolled in clinical trials with α-CTLA4 immunotherapy failed to
respond to the treatment [53–55]. Similar results were obtained in a genetically engineered PDA murine model (KPC model), where treatment with α-CTLA4 monoclonal antibodies (mAbs) showed no effect in tumor growth or survival [56]. Nonetheless, T-cell stimulation with agonistic α-CD40 mAbs plus gemcitabine and nab-paclitaxel induced tumor regression and increased survival [56], suggesting that CD40-mediated induction of T-cell response can overcome PDA resistance to α-CTLA4 immunotherapy (see next sections). In contrast, other studies using PDA model after Panc02 subcutaneous injection, showed tumor rejection in 50% of mice after α-CTLA4 treatment [57]. These discrepancies in α-CTLA4 therapy efficacy can be a consequence of the origin of Panc02 cells, which are derived from a mouse tumor generated by carcinogen-induction [58] and therefore are probably hypermutated and display higher antigenicity than KPC tumors (and most human PDAs, see previous section).

Regarding PD1/PDL1 immune checkpoint, PDL1 overexpression has been reported in PDA tumors, both in tumor cells and in the scarce immune infiltrates [38,56] and increased PDL1 expression is associated with poor survival [38]. Controversial data regarding the correlation of PDL1 expression and presence of infiltrating CD8+ T-cells [38] have been reported [38,56]. PD1 has been found expressed in around half of pancreatic tumor cytotoxic infiltrates [59]. Overexpression of PDL1 is also found in the KPC mouse model of PDA, were moderate staining was observed in around 40% of tumor cells and also in stromal dendritic cells (DCs) and macrophages, which express higher PDL1 levels than their counterparts in the spleen. Moreover, tumor infiltrating T-lymphocytes, including Tregs and few CD4+ and CD8+ T-cells also expressed PD1 at higher level that the spleen populations [56]. These results point to a role of the PD1/PDL1 immune checkpoint in PDA immune evasion. Similarly to α-CTLA4 therapy, Pan02 tumors did provide objective responses to α-PD1 or α-PDL1 therapies [38,60]. However, KPC tumors were refractory to α-PD1 or α-PDL1 mAbs, either alone or in combination with α-CTLA4 therapy, and this resistance was reverted by combined treatment with α-CD40 and chemotherapy [56]. Importantly, Winograd et al. demonstrated that this combined regimen not only induces tumor rejection in a CD8+ T-cell dependent-way, but also increases the capability of mice to reject subsequent tumor insults, suggesting the generation of an anti-tumor immune memory with high therapeutic potential. Indeed ongoing clinical trials in patients with α-CD40 are giving promising results [61] (see below).

Role of Galectins in immune evasion in PDA

Among the different mechanisms involved in immune evasion, galectins – a family of proteins with high affinity for β-galactoside residues- have recently emerged as one of the key players
in the regulation of lymphoid and myeloid cells in cancer. This family is formed by 15 members, which share a consensus carbohydrate recognition domain (CRD) responsible for their glycan binding [62–64]. Some galectins contain only one CRD and are active as monomers (galectins-5, -7, -10) or dimers (galectin-1 (Gal-1), -2, -11, -13, -14 and -15), while galectin-4, -6, -8, -9 and -12 contain two CRD linked by a short peptide. Galectin-3 (Gal-3) is the only one which contain one CRD and a non-lectin domain, which allows its oligomerization [63,65]. Although galectins can act both via sugar-dependent and sugar-independent interactions, most of their biological functions are via interaction with glycosylated proteins on the cell surface or the extracellular matrix, playing key roles in cell-cell adhesion, migration and signaling. They can be localized in different cell compartments [66] and also secreted extracellularly through a non-classical secretory pathway [67].

Importantly, altered expression of different members of the galectin family has been reported in several cancer types. In particular, Gal-1 overexpression is frequently associated to poor prognosis of many tumors [68] while Gal-3 and Gal-9 expression are tumor type-dependent [66]. Overexpression of galectins in cancer induce pro-tumoral functions like proliferation, EMT, angiogenesis and, remarkably, tumor immune evasion [69–73].

The role of galectins in the regulation of immune system has been widely studied. Gal-1 is able to recognize a variety of glycoproteins on T-cell surface inhibiting transendothelial T-cell migration, T-cell activation and importantly promoting apoptosis of activated Th1 and Th17 CD8+ T-cells, tilting the immune balance towards a Th2 profile [74,75]. Besides Gal-1 can induce Treg and DCs differentiation, impairs NK cell recruitment to the tumor, induces M2 macrophage polarization and induces expansion of MDSCs and γδ-T cells [76,77]. Altogether, these modulatory functions on different immune cell populations lead to a key role for Gal-1 in immune evasion, both in physiology during self-recognition but also in pathology during cancer progression. Gal-1 contribution to tumor immunosuppression has also been reported in PDA. Pancreatic stellate cells (PSC) are responsible for Gal-1 secretion and overexpression in the tumor microenvironment. In this context, it has been described that extracellular Gal-1 secreted by PSC reduces viability of both CD4+ and CD8+ infiltrating activated T-cells [78]. Moreover, high presence of Gal-1 favors a significant anti-tumor Th2 cytokine secretion and a drastically decrease of Th1 cytokines production [78]. Importantly, Gal-1 is highly expressed in the stroma of human and murine pancreatic tumors, where it drives tumor progression through stroma activation, induced angiogenesis tumor cell proliferation and acinar to ductal metaplasia [79]. Of note, when genetically inhibiting Gal-1, tumors show significantly
enhanced number of T-cell infiltrates and neutrophils [79] suggesting that galectin-1 may be key in driving immune evasion in PDA [80,81].

Gal-3 regulates immune system responses acting as a chemoattractant for monocytes and macrophages [82] and impairing NK anti-tumor function. It also controls expansion of DCs [77] and is able to modulate T-cell responses through apoptosis, TCR downregulation and crosslinking, consequently, inhibiting T-cell activation [83]. Gal-3 also promotes reduced immune response by decreasing IL-5 production and blocking B lymphocytes differentiation [84]. Interestingly, in PDA, treatment of tumor infiltrating CD8⁺ T-cells with α-Gal-3 mAb boosts their activation by increased IFNγ secretion. In addition, on ex vivo restimulation, GCS-100, a polysaccharide in clinical development, has the ability to detach Gal-3 from TILs. This favors both CD8⁺ and CD4⁺ T-cell activation and anti-tumor cytokine secretion. Importantly, in vaccinated tumor-bearing mice, GCS-100 injections resulted in tumor regression [85]. Of note, Gal-3 binding to LAG-3 induces CD8⁺ Tcell suppression in vitro and Gal-3 KO mice show increased T effector functions upon GM-CSF vaccine administration [86]. Indeed, phase I clinical trials with still inconclusive results have already been designed with Gal-3 inhibitors in combination with peptide vaccines as well as with ipilimumab and pembrolizumab in metastatic melanoma. Importantly, Gal-3 has already been added to the list of “new generation” checkpoints in immunotherapy [47].

Finally, Gal-9 has also crucial roles in controlling immune regulatory circuits, both in immune cell homeostasis and during cancer immune surveillance. Gal-9 can induce Treg cell differentiation and promote expansion of immunosuppressive MDSCs. Importantly, it specifically triggers apoptosis interacting with TIM-3 in Th1 and CD8⁺ T-cells [77,87], what has also propelled Gal-9 to the “new generation” checkpoints list [47,88]. Interestingly, it has been recently discovered Gal-9 ability to modulate immune responses in PDA. This lectin is a ligand of dectin-1, which is an innate immune receptor highly expressed in macrophages in PDA. Dectin-1/Gal-9 axis plays a key role promoting differentiation of TAMs to a M2-like phenotype. Importantly Gal-9 inhibition could restore intratumoral T-cell infiltrates in PDA, but this was impaired in the context of dectin-1 deletion, suggesting that this interaction is key in reprogramming CD4⁺ ad CD8⁺ T-cells, regulating tumor progression and highlighting these players as targets in immunotherapy [89].
Other molecular mechanisms of immune escape in PDA

Preclinical evaluation of checkpoint inhibitors in PDA therapy has been well considered although, as PDA is known as a non-immunogenic tumor, monotherapy blocking the PD1/PDL1 axis or CTLA4 have not been successful [56,90]. Nevertheless, strategies stimulating the immune system in parallel have shed some light into the field and presented promising outcomes.

α-CD40 antibody plus chemotherapy altered the phenotype of TILs, and, in combination with α-PD1 or α-CTLA4 or both, led to regression of established tumors due to reduced number of Tregs and enhanced CD8:Treg ratio [56,91]. Interestingly, Feig et al reported that depletion of FAP+ cells in the tumor hampered tumor growth due to increased infiltrating CD4+ and CD8+ T-cells and synergized with checkpoint monotherapies. CXCL12 was found to be the chemokine responsible for recruiting effector T-cells and targeting its receptor (CXCR4) resulted in reduced pancreatic tumor growth in a T-cell dependent manner. In combination with α-PDL1, the number of proliferating cancer cells in tumors were greatly reduced [90]. Besides, in a more recent report [92], CXCR2 inhibitors, which increased the amount of infiltrating CD3+ T-cells in KPC mice, were administered in a priming phase to enhance pancreatic T-cell infiltration, and animals were subsequently treated with the combination of CXCR2 inhibitors + α-PD1 or directly α-PD1 as a control. Immune checkpoint blockade proved to be efficient when targeting CXCR2 in parallel, significantly increasing animal survival by reducing the proliferation tumor rates and enhancing the proportion of both CD4+ and CD8+ T-cells while diminishing Tregs. IL-6 blockade in combination with α-PDL1 also proved increased T-cell infiltration and synergistic outcomes in immunocompetent mouse models [93]. Ruxolitinib (a JAK-Stat inhibitor), which inhibits systemic inflammation in the stroma of pancreatic cancer also enhances CTL infiltration and boosts α-PD1 therapy in an orthotopic mouse model of PDA [94].

Multiple additional different designs have been proposed in preclinical studies to target PDA immunosuppressive microenvironment and increase immunotherapy efficiency, such as combination with chemotherapy, radiation, therapeutic vaccines or even several of them together [95]. For instance, addition of α-PDL1 to high radiotherapy doses improved response in KPC and Pan02 allografts, through shifting the balance towards increased CD8+ T-cell at expenses of reducing CD11b+Gr1+ myeloid infiltrations [96]. The combination of checkpoint inhibitors with therapeutic vaccines to prime the microenvironment with effector T-cells before repressing the inhibitory signals [97] has also shown impressive results. For example, GVAX together with α-PD1 therapy improved survival of tumor-bearing mice [98]. More sophisticated mechanisms have also provided alternatives to increase immunotherapy efficiency such as targeting the MLL1-H3K4me3 epigenetic axis [59] or using antiangiogenic
therapy to form intratumoral HEVs (high endothelial venules) to facilitate CTL tumor infiltration [99].

**PDA and immunotherapy**

Pancreatic cancer clearly remains in dire needs of efficient treatment. Considering the embarrassing improvement in survival rates of current regimens with chemotherapy, and understanding the profound immunosuppressive microenvironment as a feasible source of resistance, immunotherapy has raised high expectations in clinical trials. First studies with checkpoint inhibitors have not been up to the challenge and more sophisticated combinations are now being contemplated.

The first pathways to be approached in clinical trials concerned checkpoint inhibitions (Figure 1). α-CTLA4 ipilimumab was assessed in a phase II clinical trial [54] in patients with advanced disease. The RECIST (standard response evaluation criteria in solid tumors) reported absence of response although 1 out of 27 patients did improve condition due to the treatment. Several clinical trials with ipilimumab have been organized, combining it with chemotherapy and/or with stimulators of the immune response, such as GVAX, achieving mild improvements in overall survival (OS) and one year OS [55]. Most of these combinatory clinical trials have not yet published their results. A phase I dose escalation of another α-CTLA4 (tremelimumab) has been also performed in patients with metastatic pancreatic cancer [100], opening the door to further advanced clinical trials to evaluate its safety and efficacy, alone or in combination with radiation and other immune checkpoint inhibitors. First results on the α-PD1 pembrolizumab in pancreatic cancer have just been published. The PembroPlus phase Ib study [101] allowed studying safety combination of α-PD1 and different chemotherapy agents, although conclusions about efficiency require further data. A phase Ib/II study of gemcitabine, nab-paclitaxel and pembrolizumab in 17 metastatic PDA patients [102] was recently presented without meeting its primary endpoint. 20 clinical trials with pembrolizumab in pancreatic cancer are active at the moment. BMS-956559 α-PDL1 achieved good performance in phase I study with different solid tumors although no objective responses were observed in pancreatic cancer patients [53]. 11 clinical trials are running with the α-PDL1 durvalumab in pancreatic cancer in different combos. Although CTLA4 and PD1/PDL1 are the most well studied immune checkpoints, other molecules of this type such as LAG-3, TIM-3, A2AR also function to shut down the immune response and are also being considered for immunotherapy in clinical trials [103].

Therapeutic cancer vaccines have shown impressive results in several tumors and are also in the spotlight in pancreatic cancer clinical trials (Figure 1). For instance, GVAX (GM-CSF vaccine)
presented favorable results in a phase I study [104] and phase II studies in combination with cyclophosphamide (to reduce Tregs) [105] or chemoradiation [106], uncovering an interesting prognostic factor through the correlation between induction of mesothelin-specific T-cell responses with improved overall survival. Besides a phase II clinical trial with GVAX in combination with CRS-207 (a live-attenuated strain of *Listeria monocytogenes* encoding mesothelin) to enhance both the innate and adaptive immunity, showed improved overall survival [107]. Positive results on the vaccine Algenpantucel-L have also been published in a phase II study [108] in combination with gemcitabine or fluorouracil, leading to ongoing promising phase III clinical trials (IMPRESS and PILLAR trials). Clinical trials with peptide vaccines instead of whole-cell vaccines have also been organized. A phase I/II trial with K-Ras peptides in combo with GM-CSF was performed with partial positive responses [109]. Further evidence on the potential of Ras vaccine in pancreatic cancer was demonstrated by a subsequent trials [110–112] with encouraging results and even impressive 10-year follow ups. Interestingly the GI-4000 vaccine (which triggers an immune response against mutated Ras) has also gathered clinician’s attention [113]. The telomerase peptide vaccine (GV1001) showed successful results in a phase I/II trial [114] although phase III clinical trials with chemotherapy regimens have not been able to show a survival advantage [115]. Although different results have been achieved depending on combinations, other peptide-based vaccines have led to benefits for pancreatic cancer patients inducing an antibody response without achieving impressive results: MUC1 vaccines [116], anti-VEGFR vaccines [117,118], survivin [119], anti-gastrin [120], anti-heat shock protein vaccine [121] and anti-WT-1 [122]. Dendritic cell vaccines are also under the scope in pancreatic cancer clinical trials, highlighting CEA-loaded DCs [123], mutated p53 and K-Ras loaded DCs [124], MUC1 [125] and WT-1 pulsed DCS [126]. Interestingly it has been very recently published that therapy with DCs together with CIK (cytokine-induced killer cells) and chemotherapy associates with increased immune responses resulting in favorable progression free survival and OS [127].

Adoptive cell therapy and several chimeric antigen receptors (CARs) (anti-CEA, Her2, MUC1, mesothelin, CD24, PSCA and Natural Killer receptors) have performed pretty well in pancreatic cancer preclinical studies [128–134], however taking the plunge to the clinics has not been so successful probably due to tumor the immunosuppressive microenvironment, so neoadjuvant chemoradiation and combos with costimulators are now being explored (Figure 1). Interestingly strategies have been designed to enhance CAR T-cell therapy efficiency such as reducing tumor bulk before adoptive cell therapy, optimizing the Th2 to Th1 ratio of infused CAR T-cells, pre-conditioning to deplete Tregs (inhibiting CTLA4, CD-25 targeting, TNFα agonism or combining with rosiglitazone), increasing CD8+ TILS (with metformin or inhibiting
TGF-β), increasing macrophage activation (by targeting CSF1, CCR2 or Bruton tyrosine kinase (BTK)) and even co-expressing albumin on the surface of CAR T-cells to enhance accumulation in the tumor [134].

Considering the strong immunosuppressive barrier as a handicap in pancreatic cancer therapy, approaches with immune-modulating agents have been also considered in clinical trials (Figure 1). CD40 agonist antibody was combined with gemcitabine in 22 advanced PDA patients [61], achieving promising results and opening the door to a couple more clinical trials with CD40 agonist and chemotherapy, with results yet to be published. In a similar context, inhibition of CCR2 to target TAMs and monocytes in combination with folfirinox, lead to either stabilization of disease or partial response in all of the evaluable patients by RECIST [135].

An interesting meta-analysis gathering all clinical trials with immunotherapies in pancreatic cancer [136] found out that indeed this strategy does significantly increase the 3-, 6-, 12-month and 3-year OS of patients. Importantly immunotherapy significantly increased the immune response of patients with PDA and reduced CA19-9 levels.

In conclusion, a large effort to fit immunotherapy in pancreatic cancer therapies is being made by companies and clinicians but it is still far from being the panacea. The strong immunosuppressive PDA microenvironment becomes a double-edge sword: on one hand it makes immunotherapy a reasonable option as therapy but it is per se a stumbling block rather than a stepping stone that hinders very much its efficiency. More sophisticated combinations and personalized patient stratification will be necessary to achieve favorable outcomes and implement it as practical therapy in the clinics.

**Concluding remarks**

Pancreatic cancer has a complex molecular landscape that handicaps both research advances and therapy efficiency. PDA strong immunosuppressive microenvironment, mainly consisting of Tregs, macrophages and MDSCs, blocks T effector cells, leading to immune evasion and rapid tumor progression. Indeed checkpoint monotherapies have failed in clinical trials due to this strong immunological barrier. The main actor driving all this process to bypass immune surveillance is a tangled combination of chemokines and receptor-ligand signaling pathways, both at the tumor and the stroma, which understanding is critical to target the key regulators and allow immunotherapy to be efficient. In this direction, basic research and preclinical trials with immunocompetent mice are shedding some light on the exact molecular mechanisms involved but are also raising concerns on the complexity of the issue and the feasibility of coming up with a combination that may suit a high percentage of patients. Indeed, around a hundred clinical trials with immunotherapy in pancreatic cancer have been designed, none of
them achieving impressive results, although clearly some of the patients come up with objective responses and could benefit from therapies boosting the immune system. In this regard, new ultrasequencing data from cancer international consortiums have allowed to classify human pancreatic tumors according to their molecular signature, unveiling a new immunogenic subtype that can be more sensitive to immunotherapy. Once again, it seems that we are underestimating the heterogeneity of cancer disease and that although economic issues cannot be undervalued, the real solution will need to approach the issue through personalized medicine.

**Figure Legends**

Figure 1. Immunotherapy strategies that have been considered in PDA therapy. Targeting immune checkpoints, the use of vaccines, CAR T-cells or immune modulating molecules have been included in pancreatic cancer clinical trials.

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Conflicts of interests

No conflicts of interest to declare

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