Peer-reviewed version available at *Cancers* **2018**, *10*, 6; doi:10.3390/cancers10010006

Type of article: Review (by invitation)

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

Neus Martinez-Bosch[1], Judith Vinaixa[1] and Pilar Navarro[1, 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 extense 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 exocrine (*NR5A*, *RBPJL*) and endocrine (*NEUROD1*, *NKX2-2*) 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 pro-tumoral 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 assosicated 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. Importanly, 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]. Futher 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 pase 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 adoptivev 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 patiens [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

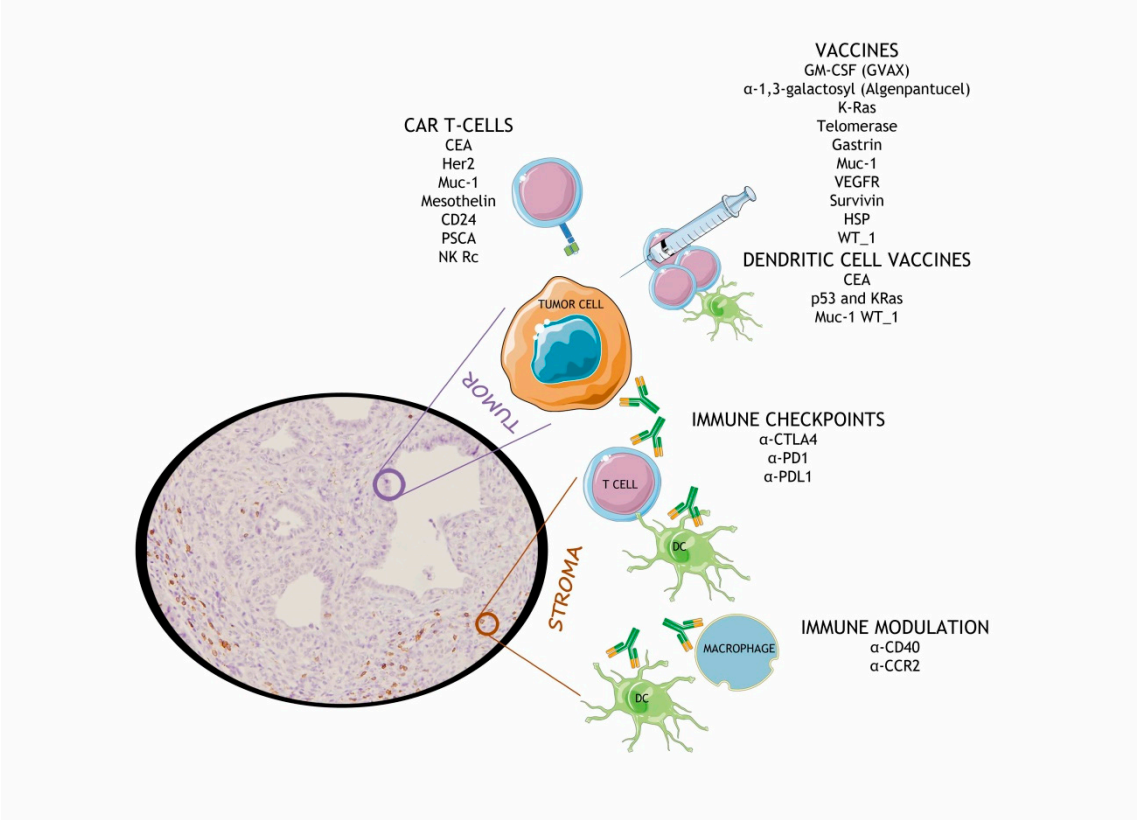

Figure 1. Immunotherapy strategies that have been considered in PDA therapy. Targeting immune checkpoints, the use of vaccines, CAR T-cells or immune modulating molecules have been included in pancreatic cancer clinical trials.

Acknowledgements

Authors would like to thank SMART (Servier Medical Art, http://smart.servier.com/) for providing images for figure preparation.

This work was supported by grants from the Spanish Ministerio de Economía y Competitividad/ ISCIII-FEDER (PI14/00125; PI17/00199), "1ª Beca Carmen Delgado/Miguel Pérez-Mateo contra el cáncer de páncreas" and the "Generalitat de Catalunya" (2014/SGR/143) to PN.

Author contributions

NM-B, JV and PN participated in the writing and revision of the manuscript.

Conflicts of interests

No conflicts of interest to declare

References

1. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011, 144: 646–674. doi:10.1016/j.cell.2011.02.013
2. Ehrlich P. Über den jetzigen stand der karzinomforschung. Ned Tijdschr Geneeskd. 1909;5: 273–290.
3. Burnet FM. The concept of immunological surveillance. Prog Exp Tumor Res. 1970;13: 1–27. Available: http://www.ncbi.nlm.nih.gov/pubmed/4921480
4. Garrido F, Ruiz-Cabello F, Cabrera T, Pérez-Villar JJ, López-Botet M, Duggan-Keen M, et al. Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. Immunol Today. 1997;18: 89–95. Available: http://www.ncbi.nlm.nih.gov/pubmed/9057360
5. Hicklin DJ, Marincola FM, Ferrone S. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. Mol Med Today. 1999;5: 178–86. Available: http://www.ncbi.nlm.nih.gov/pubmed/10203751
6. Johnsen AK, Templeton DJ, Sy M, Harding C V. Deficiency of transporter for antigen presentation (TAP) in tumor cells allows evasion of immune surveillance and increases tumorigenesis. J Immunol. 1999;163: 4224–31. Available: http://www.ncbi.nlm.nih.gov/pubmed/10510359
7. Restifo NP, Esquivel F, Kawakami Y, Yewdell JW, Mulé JJ, Rosenberg SA, et al. Identification of human cancers deficient in antigen processing. J Exp Med. 1993;177:

265–72. Available: http://www.ncbi.nlm.nih.gov/pubmed/8426105

8. Rotem-Yehudar R, Groettrup M, Soza A, Kloetzel PM, Ehrlich R. LMP-associated proteolytic activities and TAP-dependent peptide transport for class 1 MHC molecules are suppressed in cell lines transformed by the highly oncogenic adenovirus 12. J Exp Med. 1996;183: 499–514. Available: http://www.ncbi.nlm.nih.gov/pubmed/8627162
9. Seliger B, Maeurer MJ, Ferrone S. TAP off--tumors on. Immunol Today. 1997;18: 292–9. Available: http://www.ncbi.nlm.nih.gov/pubmed/9190116
10. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3: 991–8. doi:10.1038/ni1102-991
11. Pasche B. Role of transforming growth factor beta in cancer. J Cell Physiol. 2001;186: 153–68. doi:10.1002/1097-4652(200002)186:2<153::AID-JCP1016>3.0.CO;2-J
12. Lind MH, Rozell B, Wallin RPA, van Hogerlinden M, Ljunggren H-G, Toftgård R, et al. Tumor necrosis factor receptor 1-mediated signaling is required for skin cancer development induced by NF-kappaB inhibition. Proc Natl Acad Sci U S A. 2004;101: 4972–7. doi:10.1073/pnas.0307106101
13. Lin EY, Gouon-Evans V, Nguyen A V, Pollard JW. The macrophage growth factor CSF-1 in mammary gland development and tumor progression. J Mammary Gland Biol Neoplasia. 2002;7: 147–62. Available: http://www.ncbi.nlm.nih.gov/pubmed/12465600
14. Klein SC, Jücker M, Abts H, Tesch H. IL6 and IL6 receptor expression in Burkitt's lymphoma and lymphoblastoid cell lines: promotion of IL6 receptor expression by EBV. Hematol Oncol. 13: 121–30. Available: http://www.ncbi.nlm.nih.gov/pubmed/7622142
15. Matsuda M, Salazar F, Petersson M, Masucci G, Hansson J, Pisa P, et al. Interleukin 10 pretreatment protects target cells from tumor- and allo-specific cytotoxic T cells and downregulates HLA class I expression. J Exp Med. 1994;180: 2371–6. Available: http://www.ncbi.nlm.nih.gov/pubmed/7964510
16. Sotomayor EM, Fu YX, Lopez-Cepero M, Herbert L, Jimenez JJ, Albarracin C, et al. Role of tumor-derived cytokines on the immune system of mice bearing a mammary adenocarcinoma. II. Down-regulation of macrophage-mediated cytotoxicity by tumor-derived granulocyte-macrophage colony-stimulating factor. J Immunol. 1991;147: 2816–23. Available: http://www.ncbi.nlm.nih.gov/pubmed/1918995
17. Gabrilovich D. Mechanisms and functional significance of tumour-induced dendritic-cell defects. Nat Rev Immunol. 2004;4: 941–52. doi:10.1038/nri1498
18. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the

functional maturation of dendritic cells. Nat Med. 1996;2: 1096–103. Available: http://www.ncbi.nlm.nih.gov/pubmed/8837607

19. Driessens G, Kline J, Gajewski TF. Costimulatory and coinhibitory receptors in anti-tumor immunity. Immunol Rev. 2009;229: 126–44. doi:10.1111/j.1600-065X.2009.00771.x
20. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol. 2012;24: 207–12. doi:10.1016/j.coi.2011.12.009
21. French LE, Tschopp J. Defective death receptor signaling as a cause of tumor immune escape. Semin Cancer Biol. 2002;12: 51–5. doi:10.1006/scbi.2001.0405
22. Berraondo P, Minute L, Ajona D, Corrales L, Melero I, Pio R. Innate immune mediators in cancer: between defense and resistance. Immunol Rev. 2016;274: 290–306. doi:10.1111/imr.12464
23. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science (80-). Sol Goldman Pancreatic Cancer Research Center, Ludwig Center and Howard Hughes Medical Institute at the Johns Hopkins Kimmel Cancer Center, Baltimore, MD 21231, USA; 2008;321: 1801–1806.
24. Biankin A V., Waddell N, Kassahn KS, Gingras M-C, Muthuswamy LB, Johns AL, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature. 2012;491: 399–405. doi:10.1038/nature11547
25. Waddell N, Pajic M, Patch A-M, Chang DK, Kassahn KS, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015;518: 495–501. doi:10.1038/nature14169
26. Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin W-C, Mansour J, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun. 2015;6: 6744. doi:10.1038/ncomms7744
27. Bailey P, Chang DK, Nones K, Johns AL, Patch A-M, Gingras M-C, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature. 2016;531: 47–52. doi:10.1038/nature16965
28. Raphael BJ, Hruban RH, Aguirre AJ, Moffitt RA, Yeh JJ, Stewart C, et al. Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. Cancer Cell. 2017;32: 185–203.e13. doi:10.1016/j.ccell.2017.07.007
29. Liyanage UK, Moore TT, Joo H-G, Tanaka Y, Herrmann V, Doherty G, et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of

patients with pancreas or breast adenocarcinoma. J Immunol. 2002;169: 2756–61. Available: http://www.ncbi.nlm.nih.gov/pubmed/12193750

30. Zhao F, Obermann S, von Wasielewski R, Haile L, Manns MP, Korangy F, et al. Increase in frequency of myeloid-derived suppressor cells in mice with spontaneous pancreatic carcinoma. Immunology. 2009;128: 141–149. doi:10.1111/j.1365-2567.2009.03105.x
31. Amedei A, Niccolai E, Benagiano M, Della Bella C, Cianchi F, Bechi P, et al. Ex vivo analysis of pancreatic cancer-infiltrating T lymphocytes reveals that ENO-specific Tregs accumulate in tumor tissue and inhibit Th1/Th17 effector cell functions. Cancer Immunol Immunother. 2013;62: 1249–60. doi:10.1007/s00262-013-1429-3
32. Ino Y, Yamazaki-Itoh R, Shimada K, Iwasaki M, Kosuge T, Kanai Y, et al. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. Br J Cancer. 2013;108: 914–23. doi:10.1038/bjc.2013.32
33. Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, Vonderheide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. Cancer Res. 2007; doi:10.1158/0008-5472.CAN-07-0175
34. Tan MCB, Goedegebuure PS, Belt BA, Flaherty B, Sankpal N, Gillanders WE, et al. Disruption of CCR5-dependent homing of regulatory T cells inhibits tumor growth in a murine model of pancreatic cancer. J Immunol. 2009;182: 1746–55. Available: http://www.ncbi.nlm.nih.gov/pubmed/19155524
35. Mitchem JB, Brennan DJ, Knolhoff BL, Belt BA, Zhu Y, Sanford DE, et al. Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. Cancer Res. 2013;73: 1128–41. doi:10.1158/0008-5472.CAN-12-2731
36. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. NIH Public Access; 2013;13: 227–42. doi:10.1038/nri3405
37. Saqib Razzaque, Noman Ashraf, Julio C. Chavez, Mokenge Peter Malafa, Domenico Coppola, Gregory M. Springett, James Helm and RDK. Expression of programmed death ligand 1 (PD-L1) in malignant and nonmalignant pancreatic tissue. J Clin Oncol. 2013;31: 215.
38. Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res. 2007;13: 2151–7. doi:10.1158/1078-0432.CCR-06-2746
39. Geng L, Huang D, Liu J, Qian Y, Deng J, Li D, et al. B7-H1 up-regulated expression in human pancreatic carcinoma tissue associates with tumor progression. J Cancer Res

Clin Oncol. 2008;134: 1021–1027. doi:10.1007/s00432-008-0364-8

40. Song X, Liu J, Lu Y, Jin H, Huang D. Overexpression of B7-H1 correlates with malignant cell proliferation in pancreatic cancer. Oncol Rep. 2014;31: 1191–1198. doi:10.3892/or.2013.2955
41. Li D, Zhao H, Fei M, Wu Y, Wang L, Zhu X, et al. Expression of the co-signaling molecules CD40-CD40L and their growth inhibitory effect on pancreatic cancer in vitro. Oncol Rep. 2012;28: 262–8. doi:10.3892/or.2012.1790
42. Lawrence MS, Stojanov P, Polak P, Kryukov G V., Cibulskis K, Sivachenko A, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013;499: 214–218. doi:10.1038/nature12213
43. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin A V, et al. Signatures of mutational processes in human cancer. Nature. 2013;500: 415–21. doi:10.1038/nature12477
44. Beatty GL, Gladney WL. Immune escape mechanisms as a guide for cancer immunotherapy. Clinical Cancer Research. 2015. doi:10.1158/1078-0432.CCR-14-1860
45. Balachandran VP, Łuksza M, Zhao JN, Makarov V, Moral JA, Remark R, et al. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. Nature. 2017; doi:10.1038/nature24462
46. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12: 252–64. doi:10.1038/nrc3239
47. Melero I, Berman DM, Aznar MA, Korman AJ, Gracia JLP, Haanen J. Evolving synergistic combinations of targeted immunotherapies to combat cancer. Nat Rev Cancer. 2015;15: 457–472. doi:10.1038/nrc3973
48. Odorizzi PM, Wherry EJ. Inhibitory receptors on lymphocytes: insights from infections. J Immunol. 2012;188: 2957–65. doi:10.4049/jimmunol.1100038
49. Peggs KS, Quezada SA, Chambers CA, Korman AJ, Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med. 2009;206: 1717–25. doi:10.1084/jem.20082492
50. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med. 2013;210: 1695–710. doi:10.1084/jem.20130579
51. Selby MJ, Engelhardt JJ, Quigley M, Henning KA, Chen T, Srinivasan M, et al. Anti-CTLA-4 Antibodies of IgG2a Isotype Enhance Antitumor Activity through Reduction of Intratumoral Regulatory T Cells. Cancer Immunol Res. 2013;1: 32–42.

doi:10.1158/2326-6066.CIR-13-0013

52. Bengsch F, Knoblock DM, Liu A, McAllister F, Beatty GL. CTLA-4/CD80 pathway regulates T cell infiltration into pancreatic cancer. Cancer Immunol Immunother. 2017;66: 1609–1617. doi:10.1007/s00262-017-2053-4

53. Brahmer JR, Tykodi SS, Chow LQM, Hwu W-J, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366: 2455–65. doi:10.1056/NEJMoa1200694

54. Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, et al. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother. 2010;33: 828–33. doi:10.1097/CJI.0b013e3181eec14c

55. Le DT, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunother. 2013;36: 382–9. doi:10.1097/CJI.0b013e31829fb7a2

56. Winograd R, Byrne KT, Evans RA, Odorizzi PM, Meyer ARL, Bajor DL, et al. Induction of T-cell Immunity Overcomes Complete Resistance to PD-1 and CTLA-4 Blockade and Improves Survival in Pancreatic Carcinoma. Cancer Immunol Res. 2015; doi:10.1158/2326-6066.CIR-14-0215

57. Sandin LC, Eriksson F, Ellmark P, Loskog AS, Tötterman TH, Mangsbo SM. Local CTLA4 blockade effectively restrains experimental pancreatic adenocarcinoma growth in vivo. Oncoimmunology. 2014;3: e27614. doi:10.4161/onci.27614

58. Corbett TH, Roberts BJ, Leopold WR, Peckham JC, Wilkoff LJ, Griswold DP, et al. Induction and chemotherapeutic response of two transplantable ductal adenocarcinomas of the pancreas in C57BL/6 mice. Cancer Res. 1984;44: 717–26. Available: http://www.ncbi.nlm.nih.gov/pubmed/6692374

59. Lu C, Paschall A V, Shi H, Savage N, Waller JL, Sabbatini ME, et al. The MLL1-H3K4me3 Axis-Mediated PD-L1 Expression and Pancreatic Cancer Immune Evasion. J Natl Cancer Inst. 2017;109: djw283. doi:10.1093/jnci/djw283

60. Okudaira K, Hokari R, Tsuzuki Y, Okada Y, Komoto S, Watanabe C, et al. Blockade of B7-H1 or B7-DC induces an anti-tumor effect in a mouse pancreatic cancer model. Int J Oncol. 2009;35: 741–9. Available: http://www.ncbi.nlm.nih.gov/pubmed/19724910

61. Beatty GL, Torigian DA, Chiorean EG, Saboury B, Brothers A, Alavi A, et al. A Phase I Study of an Agonist CD40 Monoclonal Antibody (CP-870,893) in Combination with Gemcitabine in Patients with Advanced Pancreatic Ductal Adenocarcinoma. Clin Cancer Res. 2013;19: 6286–6295. doi:10.1158/1078-0432.CCR-13-1320

62. Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T, et al. Galectins: a family of animal beta-galactoside-binding lectins. Cell. 1994;76: 597–598.

63. Leffler H, Carlsson S, Hedlund M, Qian Y, Poirier F. Introduction to galectins. GlycoconjJ. Section MIG (Microbiology, Immunology, Glycobiology), Inst Laboratory Medicine, Lund University, Sweden. hakon.leffler@mig.lu.se; 2004;19: 433–440.

64. Cerliani JP, Blidner AG, Toscano MA, Croci DO, Rabinovich GA. Translating the "Sugar Code" into Immune and Vascular Signaling Programs. Trends Biochem Sci. 2017;42: 255–273. doi:10.1016/j.tibs.2016.11.003

65. Cooper DN. Galectinomics: finding themes in complexity. BiochimBiophysActa. Departments of Psychiatry and Anatomy, University of California, San Francisco, LPPI, Box F-0984, 401 Parnassus Ave., San Francisco, CA 94143-0984, USA. cooper@cgl.ucsf.edu; 2002;1572: 209–231.

66. Liu FT, Rabinovich GA. Galectins as modulators of tumour progression. NatRevCancer. Department of Dermatology, School of Medicine, University of California, Davis, Sacramento, California, USA. fliu@ucdavis.edu; 2005;5: 29–41.

67. Hughes RC. Secretion of the galectin family of mammalian carbohydrate-binding proteins. BiochimBiophysActa. National Institute for Medical Research, Mill Hill, London, UK. c-hughes@nimr.mrc.ac.uk; 1999;1473: 172–185.

68. Demydenko D, Berest I. Expression of galectin-1 in malignant tumors. ExpOncol. Department of Molecular Biology, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kiev 01601, Ukraine. d.v.demydenko@gmail.com; 2009;31: 74–79.

69. Croci DO, Salatino M, Rubinstein N, Cerliani JP, Cavallin LE, Leung HJ, et al. Disrupting galectin-1 interactions with N-glycans suppresses hypoxia-driven angiogenesis and tumorigenesis in Kaposi's sarcoma. J Exp Med. 2012;209: 1985–2000. doi:10.1084/jem.20111665

70. Croci DO, Cerliani JP, Dalotto-Moreno T, Méndez-Huergo SP, Mascanfroni ID, Dergan-Dylon S, et al. Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. Cell. 2014;156: 744–58. doi:10.1016/j.cell.2014.01.043

71. Espelt M V, Croci DO, Bacigalupo ML, Carabias P, Manzi M, Elola MT, et al. Novel roles of galectin-1 in hepatocellular carcinoma cell adhesion, polarization and in vivo tumor growth. Hepatology. Instituto de Quimica y Fisicoquimica Biologicas (IQUIFIB), CONICET, Departamento de Quimica Biologica, Facultad de Farmacia y Bioquimica, Buenos Aires, Argentina; 2011;

72. Thijssen VL, Barkan B, Shoji H, Aries IM, Mathieu V, Deltour L, et al. Tumor cells secrete galectin-1 to enhance endothelial cell activity. Cancer Res. 2010;70: 6216–24. doi:10.1158/0008-5472.CAN-09-4150

73. Bacigalupo ML, Manzi M, Espelt M V., Gentilini LD, Compagno D, Laderach DJ, et al. Galectin-1 Triggers Epithelial-Mesenchymal Transition in Human Hepatocellular Carcinoma Cells. J Cell Physiol. 2015;230: 1298–1309. doi:10.1002/jcp.24865

74. Rabinovich GA, Ramhorst RE, Rubinstein N, Corigliano A, Daroqui MC, Kier-Joffe EB, et al. Induction of allogenic T-cell hyporesponsiveness by galectin-1-mediated apoptotic and non-apoptotic mechanisms. Cell DeathDiffer. Division of Immunogenetics, Hospital de Clinicas Jose de San Martin, University of Buenos Aires, Argentina. gabyrabi@ciudad.com.ar; 2002;9: 661–670.

75. He J, Baum LG. Presentation of galectin-1 by extracellular matrix triggers T cell death. JBiolChem. Department of Pathology, UCLA School of Medicine, Los Angeles, California 90095, USA; 2004;279: 4705–4712.

76. Méndez-Huergo SP, Blidner AG, Rabinovich GA. Galectins: emerging regulatory checkpoints linking tumor immunity and angiogenesis. Curr Opin Immunol. 2017;45: 8–15. doi:10.1016/j.coi.2016.12.003

77. Rabinovich GA, Conejo-García JR. Shaping the Immune Landscape in Cancer by Galectin-Driven Regulatory Pathways. J Mol Biol. 2016;428: 3266–3281. doi:10.1016/j.jmb.2016.03.021

78. Tang D, Yuan Z, Xue X, Lu Z, Zhang Y, Wang H, et al. High expression of Galectin-1 in pancreatic stellate cells plays a role in the development and maintenance of an immunosuppressive microenvironment in pancreatic cancer. Int J Cancer. 2012;130: 2337–48. doi:10.1002/ijc.26290

79. Martinez-Bosch N, Fernandez-Barrena MG, Moreno M, Ortiz-Zapater E, Munne-Collado J, Iglesias M, et al. Galectin-1 Drives Pancreatic Carcinogenesis through Stroma Remodeling and Hedgehog Signaling Activation. Cancer Res. 2014;74: 3512–3524. doi:10.1158/0008-5472.CAN-13-3013

80. Martínez-Bosch N, Navarro P. Targeting Galectin-1 in pancreatic cancer: immune surveillance on guard. Oncoimmunology. 2014;3: e952201. doi:10.4161/21624011.2014.952201

81. Martínez-Bosch N, Navarro P. Galectin-1 as a therapeutic target in pancreatic cancer: the tumor stroma in the spotlight. Cancer Cell Microenviron. 2014;1: 1–6. doi:10.14800/ccm.296

82. Sano H, Hsu DK, Yu L, Apgar JR, Kuwabara I, Yamanaka T, et al. Human galectin-3 is a

novel chemoattractant for monocytes and macrophages. J Immunol. 2000;165: 2156–64. Available: http://www.ncbi.nlm.nih.gov/pubmed/10925302

83. Demetriou M, Granovsky M, Quaggin S, Dennis JW. Negative regulation of T-cell activation and autoimmunity by Mgat5 N-glycosylation. Nature. Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; 2001;409: 733–739.
84. Acosta-Rodríguez E V, Montes CL, Motrán CC, Zuniga EI, Liu F-T, Rabinovich GA, et al. Galectin-3 mediates IL-4-induced survival and differentiation of B cells: functional cross-talk and implications during Trypanosoma cruzi infection. J Immunol. 2004;172: 493–502. Available: http://www.ncbi.nlm.nih.gov/pubmed/14688359
85. Demotte N, Wieers G, Van Der Smissen P, Moser M, Schmidt C, Thielemans K, et al. A Galectin-3 Ligand Corrects the Impaired Function of Human CD4 and CD8 Tumor-Infiltrating Lymphocytes and Favors Tumor Rejection in Mice. Cancer Res. 2010;70: 7476–7488. doi:10.1158/0008-5472.CAN-10-0761
86. Kouo T, Huang L, Pucsek AB, Cao M, Solt S, Armstrong T, et al. Galectin-3 Shapes Antitumor Immune Responses by Suppressing CD8+ T Cells via LAG-3 and Inhibiting Expansion of Plasmacytoid Dendritic Cells. Cancer Immunol Res. 2015;3: 412–23. doi:10.1158/2326-6066.CIR-14-0150
87. Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol. 2005;6: 1245–1252. doi:10.1038/ni1271
88. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell. NIH Public Access; 2015;27: 450–61. doi:10.1016/j.ccell.2015.03.001
89. Daley D, Mani VR, Mohan N, Akkad N, Ochi A, Heindel DW, et al. Dectin 1 activation on macrophages by galectin 9 promotes pancreatic carcinoma and peritumoral immune tolerance. Nat Med. 2017; doi:10.1038/nm.4314
90. Feig C, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. Europe PMC Funders Group The pancreas cancer microenvironment. 2013;18: 4266–4276. doi:10.1158/1078-0432.CCR-11-3114.The
91. Luheshi NM, Coates-Ulrichsen J, Harper J, Mullins S, Sulikowski MG, Martin P, et al. Transformation of the tumour microenvironment by a CD40 agonist antibody correlates with improved responses to PD-L1 blockade in a mouse orthotopic pancreatic tumour model. Oncotarget. 2016;7: 18508–20. doi:10.18632/oncotarget.7610
92. Steele CW, Karim SA, Leach JDG, Bailey P, Upstill-Goddard R, Rishi L, et al. CXCR2 Inhibition Profoundly Suppresses Metastases and Augments Immunotherapy in

Pancreatic Ductal Adenocarcinoma. Cancer Cell. 2016; doi:10.1016/j.ccell.2016.04.014

93. Mace TA, Shakya R, Pitarresi JR, Swanson B, McQuinn CW, Loftus S, et al. IL-6 and PD-L1 antibody blockade combination therapy reduces tumour progression in murine models of pancreatic cancer. Gut. 2016; gutjnl-2016-311585. doi:10.1136/gutjnl-2016-311585

94. Lu C, Talukder A, Savage NM, Singh N, Liu K. JAK-STAT-mediated chronic inflammation impairs cytotoxic T lymphocyte activation to decrease anti-PD-1 immunotherapy efficacy in pancreatic cancer. doi.org. Informa UK Limited; 2017; e1291106. doi:10.1080/2162402x.2017.1291106

95. Zheng W, Skowron KB, Namm JP, Burnette B, Fernandez C, Arina A, et al. Combination of radiotherapy and vaccination overcomes checkpoint blockade resistance. Oncotarget. Impact Journals, LLC; 2016;7: 43039–43051. doi:10.18632/oncotarget.9915

96. Azad A, Yin Lim S, D'Costa Z, Jones K, Diana A, Sansom OJ, et al. PD-L1 blockade enhances response of pancreatic ductal adenocarcinoma to radiotherapy. EMBO Mol Med. 2017;9: 167–180. doi:10.15252/emmm.201606674

97. Mahoney KM, Rennert PD, Freeman GJ. Combination cancer immunotherapy and new immunomodulatory targets. Nat Rev Drug Discov. 2015;14: 561–84. doi:10.1038/nrd4591

98. Soares KC, Rucki AA, Wu AA, Olino K, Xiao Q, Chai Y, et al. PD-1/PD-L1 blockade together with vaccine therapy facilitates effector T-cell infiltration into pancreatic tumors. J Immunother. 2015;38: 1–11. doi:10.1097/CJI.0000000000000062

99. Allen E, Jabouille A, Rivera LB, Lodewijckx I, Missiaen R, Steri V, et al. Combined antiangiogenic and anti–PD-L1 therapy stimulates tumor immunity through HEV formation. Sci Transl Med. 2017;9: eaak9679. doi:10.1126/scitranslmed.aak9679

100. Aglietta M, Barone C, Sawyer MB, Moore MJ, Miller WH, Bagalà C, et al. A phase I dose escalation trial of tremelimumab (CP-675,206) in combination with gemcitabine in chemotherapy-naive patients with metastatic pancreatic cancer. Ann Oncol. 2014;25: 1750–1755. doi:10.1093/annonc/mdu205

101. Weiss GJ, Waypa J, Blaydorn L, Coats J, McGahey K, Sangal A, et al. A phase Ib study of pembrolizumab plus chemotherapy in patients with advanced cancer (PembroPlus). Br J Cancer. 2017;117: 33–40. doi:10.1038/bjc.2017.145

102. Weiss GJ, Blaydorn L, Beck J, Bornemann-Kolatzki K, Urnovitz H, Schütz E, et al. Phase Ib/II study of gemcitabine, nab-paclitaxel, and pembrolizumab in metastatic pancreatic adenocarcinoma. Invest New Drugs. 2017; doi:10.1007/s10637-017-0525-1

103. Li X, Hu W, Zheng X, Zhang C, Du P, Zheng Z, et al. Emerging immune checkpoints for cancer therapy. Acta Oncol. 2015;54: 1706–13. doi:10.3109/0284186X.2015.1071918

104. Jaffee EM, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol. 2001;19: 145–56. doi:10.1200/JCO.2001.19.1.145

105. Laheru D, Lutz E, Burke J, Biedrzycki B, Solt S, Onners B, et al. Allogeneic granulocyte macrophage colony-stimulating factor-secreting tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: a pilot study of safety, feasibility, and immune activation. Clin Cancer Res. 2008;14: 1455–63. doi:10.1158/1078-0432.CCR-07-0371

106. Lutz E, Yeo CJ, Lillemoe KD, Biedrzycki B, Kobrin B, Herman J, et al. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A Phase II trial of safety, efficacy, and immune activation. Ann Surg. 2011;253: 328–35. doi:10.1097/SLA.0b013e3181fd271c

107. Le DT, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G, et al. Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. J Clin Oncol. 2015;33: 1325–33. doi:10.1200/JCO.2014.57.4244

108. Hardacre JM, Mulcahy M, Small W, Talamonti M, Obel J, Krishnamurthi S, et al. Addition of Algenpantucel-L Immunotherapy to Standard Adjuvant Therapy for Pancreatic Cancer: a Phase 2 Study. J Gastrointest Surg. 2013;17: 94–101. doi:10.1007/s11605-012-2064-6

109. Gjertsen MK, Buanes T, Rosseland AR, Bakka A, Gladhaug I, Søreide O, et al. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant: Clinical and immunological responses in patients with pancreatic adenocarcinoma. Int J cancer. 2001;92: 441–50. Available: http://www.ncbi.nlm.nih.gov/pubmed/11291084

110. Toubaji A, Achtar M, Provenzano M, Herrin VE, Behrens R, Hamilton M, et al. Pilot study of mutant ras peptide-based vaccine as an adjuvant treatment in pancreatic and colorectal cancers. Cancer Immunol Immunother. 2008;57: 1413–20. doi:10.1007/s00262-008-0477-6

111. Abou-Alfa GK, Chapman PB, Feilchenfeldt J, Brennan MF, Capanu M, Gansukh B, et al. Targeting mutated K-ras in pancreatic adenocarcinoma using an adjuvant vaccine. Am J Clin Oncol. 2011;34: 321–5. doi:10.1097/COC.0b013e3181e84b1f

112. Wedén S, Klemp M, Gladhaug IP, Møller M, Eriksen JA, Gaudernack G, et al. Long-term follow-up of patients with resected pancreatic cancer following vaccination against

mutant K-ras. Int J cancer. 2011;128: 1120–8. doi:10.1002/ijc.25449

113. Hartley ML, Bade NA, Prins PA, Ampie L, Marshall JL. Pancreatic cancer, treatment options, and GI-4000. Hum Vaccin Immunother. 2015;11: 931–7. doi:10.1080/21645515.2015.1011017

114. Bernhardt SL, Gjertsen MK, Trachsel S, Møller M, Eriksen JA, Meo M, et al. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/II study. Br J Cancer. 2006;95: 1474–82. doi:10.1038/sj.bjc.6603437

115. Middleton G, Silcocks P, Cox T, Valle J, Wadsley J, Propper D, et al. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): an open-label, randomised, phase 3 trial. Lancet Oncol. 2014;15: 829–840. doi:10.1016/S1470-2045(14)70236-0

116. Lepisto AJ, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, et al. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. Cancer Ther. 2008;6: 955–964. Available: http://www.ncbi.nlm.nih.gov/pubmed/19129927

117. Miyazawa M, Ohsawa R, Tsunoda T, Hirono S, Kawai M, Tani M, et al. Phase I clinical trial using peptide vaccine for human vascular endothelial growth factor receptor 2 in combination with gemcitabine for patients with advanced pancreatic cancer. Cancer Sci. 2010;101: 433–9. doi:10.1111/j.1349-7006.2009.01416.x

118. Suzuki N, Hazama S, Iguchi H, Uesugi K, Tanaka H, Hirakawa K, et al. Phase II clinical trial of peptide cocktail therapy for patients with advanced pancreatic cancer: VENUS-PC study. Cancer Sci. 2017;108: 73–80. doi:10.1111/cas.13113

119. Mehrotra S, Britten CD, Chin S, Garrett-Mayer E, Cloud CA, Li M, et al. Vaccination with poly(IC:LC) and peptide-pulsed autologous dendritic cells in patients with pancreatic cancer. J Hematol Oncol. 2017;10: 82. doi:10.1186/s13045-017-0459-2

120. Gilliam AD, Broome P, Topuzov EG, Garin AM, Pulay I, Humphreys J, et al. An international multicenter randomized controlled trial of G17DT in patients with pancreatic cancer. Pancreas. 2012;41: 374–9. doi:10.1097/MPA.0b013e31822ade7e

121. Maki RG, Livingston PO, Lewis JJ, Janetzki S, Klimstra D, Desantis D, et al. A phase I pilot study of autologous heat shock protein vaccine HSPPC-96 in patients with resected pancreatic adenocarcinoma. Dig Dis Sci. 2007;52: 1964–72. doi:10.1007/s10620-006-9205-2

122. Koido S, Okamoto M, Shimodaira S, Sugiyama H. Wilms' tumor 1 (WT1)-targeted cancer vaccines to extend survival for patients with pancreatic cancer. Immunotherapy. 2016;8: 1309–1320. doi:10.2217/imt-2016-0031

123. Morse MA, Nair SK, Boczkowski D, Tyler D, Hurwitz HI, Proia A, et al. The feasibility and safety of immunotherapy with dendritic cells loaded with CEA mRNA following neoadjuvant chemoradiotherapy and resection of pancreatic cancer. Int J Gastrointest Cancer. 2002;32: 1–6. doi:10.1385/IJGC:32:1:1

124. Carbone DP, Ciernik IF, Kelley MJ, Smith MC, Nadaf S, Kavanaugh D, et al. Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune response and clinical outcome. J Clin Oncol. 2005;23: 5099–107. doi:10.1200/JCO.2005.03.158

125. Kondo H, Hazama S, Kawaoka T, Yoshino S, Yoshida S, Tokuno K, et al. Adoptive immunotherapy for pancreatic cancer using MUC1 peptide-pulsed dendritic cells and activated T lymphocytes. Anticancer Res. 28: 379–87. Available: http://www.ncbi.nlm.nih.gov/pubmed/18383873

126. Koido S, Homma S, Okamoto M, Takakura K, Mori M, Yoshizaki S, et al. Treatment with chemotherapy and dendritic cells pulsed with multiple Wilms' tumor 1 (WT1)-specific MHC class I/II-restricted epitopes for pancreatic cancer. Clin Cancer Res. 2014;20: 4228–39. doi:10.1158/1078-0432.CCR-14-0314

127. Jiang N, Qiao G, Wang X, Morse MA, Gwin WR, Zhou L, et al. Dendritic Cell/Cytokine-Induced Killer Cell Immunotherapy Combined with S-1 in Patients with Advanced Pancreatic Cancer: A Prospective Study. Clin Cancer Res. 2017;23: 5066–5073. doi:10.1158/1078-0432.CCR-17-0492

128. Chmielewski M, Hahn O, Rappl G, Nowak M, Schmidt–Wolf IH, Hombach AA, et al. T Cells That Target Carcinoembryonic Antigen Eradicate Orthotopic Pancreatic Carcinomas Without Inducing Autoimmune Colitis in Mice. Gastroenterology. 2012;143: 1095–1107.e2. doi:10.1053/j.gastro.2012.06.037

129. Maliar A, Servais C, Waks T, Chmielewski M, Lavy R, Altevogt P, et al. Redirected T cells that target pancreatic adenocarcinoma antigens eliminate tumors and metastases in mice. Gastroenterology. 2012;143: 1375-84–5. doi:10.1053/j.gastro.2012.07.017

130. Posey AD, Schwab RD, Boesteanu AC, Steentoft C, Mandel U, Engels B, et al. Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma. Immunity. 2016;44: 1444–1454. doi:10.1016/j.immuni.2016.05.014

131. Zhao Y, Moon E, Carpenito C, Paulos CM, Liu X, Brennan AL, et al. Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor. Cancer Res. 2010;70: 9053–61. doi:10.1158/0008-5472.CAN-10-2880

132. Abate-Daga D, Lagisetty KH, Tran E, Zheng Z, Gattinoni L, Yu Z, et al. A novel chimeric

antigen receptor against prostate stem cell antigen mediates tumor destruction in a humanized mouse model of pancreatic cancer. Hum Gene Ther. 2014;25: 1003–12. doi:10.1089/hum.2013.209

133. Tal Y, Yaakobi S, Horovitz-Fried M, Safyon E, Rosental B, Porgador A, et al. An NCR1-based chimeric receptor endows T-cells with multiple anti-tumor specificities. Oncotarget. 2014;5: 10949–58. doi:10.18632/oncotarget.1919

134. DeSelm CJ, Tano ZE, Varghese AM, Adusumilli PS. CAR T-cell therapy for pancreatic cancer. Journal of Surgical Oncology. 2017. doi:10.1002/jso.24627

135. Nywening TM, Wang-Gillam A, Sanford DE, Belt BA, Panni RZ, Cusworth BM, et al. Targeting tumour-associated macrophages with CCR2 inhibition in combination with FOLFIRINOX in patients with borderline resectable and locally advanced pancreatic cancer: a single-centre, open-label, dose-finding, non-randomised, phase 1b trial. Lancet Oncol. 2016;17: 651–62. doi:10.1016/S1470-2045(16)00078-4

136. Chen J, Xiao-Zhong G, Qi X-S. Clinical Outcomes of Specific Immunotherapy in Advanced Pancreatic Cancer: A Systematic Review and Meta-Analysis. doi:10.1155/2017/8282391