Advanced Drug Delivery Reviews 225 (2025) 115671

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

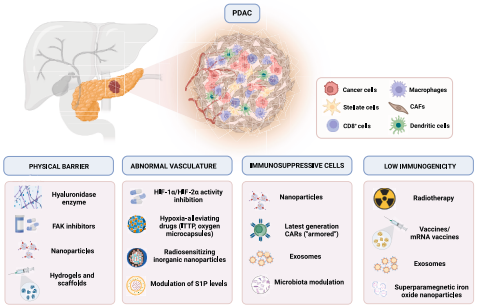
journal homepage: www.elsevier.com/locate/adr

Classic versus innovative strategies for immuno-therapy in pancreatic cancer☆

Maria Giovanna Formelli [a], Andrea Palloni [b], Simona Tavolari [b], Chiara Deiana [a], Elisa Andrini [a], Mariacristina Di Marco [a,b], Davide Campana [a,b], Giuseppe Lamberti [a,*], Giovanni Brandi [a,b]

[a] Department of Medical and Surgical Sciences (DIMEC) – Alma Mater Studiorum, University of Bologna, Italy
[b] Medical Oncology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Pancreatic ductal adenocarcinoma
Immunotherapy
Immune checkpoint inhibitors
Adoptive cell therapy
Vaccine
Microbiota
Tumor microenvironment

ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with a dismal prognosis. Immunotherapy with immune checkpoint inhibitors (ICIs), either as monotherapy, in combination with other ICIs, or alongside chemotherapy, has significantly improved outcomes in several solid tumors. However, its efficacy in PDAC remains limited due to multiple resistance mechanisms.

Key determinants of immunotherapy resistance in PDAC include physical barriers that hinder immune cells infiltration, such as aberrant vasculature, cancer-associated fibroblasts (CAFs), and excessive hyaluronic acid deposition in the tumor microenvironment (TME). Additionally, PDAC is characterized by an immunosuppressive TME enriched with regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), and by low immunogenicity of tumor cells due to KRAS mutations, MYC overexpression, and a low tumor mutational burden, further impairing antitumor immunity.

This review discusses advanced drug delivery systems to overcome determinants of immunotherapy resistance and to improve outcomes, explores emerging immunotherapy strategies, including adoptive cell therapies, cancer vaccines, and the potential role of microbiota as modulator of TME through fecal microbiota

☆ This article is part of a special issue entitled: 'Pancreatic cancer' published in Advanced Drug Delivery Reviews.

* Corresponding author at: Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum, University of Bologna, Via P. Albertoni, 15, 40138 Bologna, Italy.
E-mail address: giuseppe.lamberti8@unibo.it (G. Lamberti).

https://doi.org/10.1016/j.addr.2025.115671
Received 3 March 2025; Received in revised form 3 July 2025; Accepted 6 August 2025
Available online 7 August 2025

transplantation or intratumoral bacterial inoculation. Given the ambivalent role of microbiota in PDAC, the need for a clear definition of favorable strains and their selection is highlighted. Emerging approaches involving engineered bacteria and artificial intelligence applications are also explored.

Finally, we propose a hypothetical conceptual framework for an innovative multimodal immunotherapy approach to overcome resistance and improve clinical outcomes in PDAC.

1. Introduction

Pancreatic cancer (PC) is an aggressive tumor with dismal prognosis and represents the seventh cause of cancer related death in both sexes, worldwide [1]. Incidence and deaths rates from this cancer are projected to increase in both sexes, leading PC to become the second cause of cancer-related death in the United States, by 2040 [2]. Pancreatic ductal adenocarcinomas (PDAC) is the most frequent form of PC, accounting for about 90 % of cases [3].

PDAC is one of the solid tumors with the most dismal prognosis with around 20 % of patients alive 5 years from diagnosis of resected disease and adjuvant therapy [4] which falls as low as 3.1 % in case of metastatic disease [5]. Currently, the mainstay of PDAC treatment is cytotoxic chemotherapy, which in first-line setting yields a median overall survival (OS) of less than 1 year [6,7]. Subsequent treatment options are limited and associated with poor outcomes. Main reasons for treatment failure in PDAC are its unique biology and peculiar tumor microenvironment (TME), which are still incompletely understood. Given the scarce advancement in treatment and outcomes, in 2012 the US Congress defined PDAC as one of the "recalcitrant cancers", together with small-cell lung cancer, by the Recalcitrant Cancer Research Act to foster research in these settings.

Because of the dismal prognosis and the paucity of treatment alternatives, the availability of effective, tolerable, and modern therapies for PDAC is a highly unmet need. This review analyses PDAC microenvironment biology and immunity to understand how modern immunotherapy with immune checkpoint inhibitors (ICIs) failed PDAC and envision the future application of immunotherapy strategies in this cancer.

2. Immunotherapy with ICIs in solid tumors

The advent of immunotherapy with ICIs has radically changed the therapeutic scenario in several solid tumors. Tumor cells evade the immune system anti-tumoral response by up-regulating the inhibitory signals and reducing the co-stimulatory ones, thus promoting an immunosuppressive TME [8]. Immunotherapy with ICIs exerts its anti-tumor activity by blocking inhibitory checkpoints to restore T-cells immune response [8]. Currently available ICIs are directed against cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4) and the programmed death-1 (PD-1) protein and its ligand (PDL-1).

T-cell receptors (TCR) recognize the tumor antigen linked to the class II major histocompatibility complex (MHC) on antigen presenting cells (APCs), but T-cell requires a second co-stimulation signal to become activated [9,10]. CTLA-4 (on T-cells), due to its higher affinity, binds the ligand B7 competing with the co-stimulatory molecule CD28, and so blocks T-cells activation [10]. The other co-inhibitory receptor PD-1 on T-cells interacts with PD-L1, expressed on APCs and tumor cells, triggering an inhibitory signal [8,11].

Due to their efficacy and long-lasting responses, monoclonal antibodies against PD-1, PD-L1 and CTLA-4 have been approved by the US Food and Drug Administration (FDA) for several types of gastrointestinal (GI) and non-GI tumors [8,12]. Nonetheless, disappointing results have been observed with ICIs in patients with PDAC, except for the small proportion carrying high microsatellite instability (MSI high) or mismatch repair deficiency (dMMR) [13]. Indeed, a great number of infiltrating lymphocytes, which express high levels of immune checkpoints, such as PD-1, CTLA-4 and lymphocyte activation gene-3 (LAG-3), are found in tumors with MSI-high or dMMR due to their high tumor mutational burden (TMB), and consequent high rate of potentially immunogenic neoantigens, thus explaining the high sensitivity of these tumors to ICIs [14–16]. As such, pembrolizumab (an anti PD-1 antibody) received the agnostic approval for the treatment of previously treated metastatic or unresectable MSI-high/dMMR solid tumors without other satisfactory therapeutic options, regardless of the anatomic location, thus including PDAC, following the results of KEYNOTE-158 study [14]. Among the 22 patients with PDAC included in the study, one complete response and three partial responses (objective response rate [ORR]: 18.2 %) to pembrolizumab have been observed, which was among the lowest in the study, suggesting an intrinsic resistance to immunotherapy of PDAC [13].

3. PDAC biology and microenvironment

The aggressive biology and related resistance to therapy in PDAC is mostly driven by the non-cancerous components of the TME, given that neoplastic cells represent the smallest part of the entire tumor cellularity in PDAC (Fig. 1) [17]. The TME consists mainly of different types of cancer-associated fibroblasts (CAFs) and suppressive immune cells, dense extracellular matrix, and a poorly formed vascular system [17]. Indeed, PDAC can be considered as a scar of fibrotic tissue with few tumor cells scattered in it.

The aberrant tumor vasculature and associated hypoxia impair the delivery of therapeutic agents and limit immune cells infiltration, thereby promoting treatment resistance in PDAC and adversely influencing the patient's prognosis [18]. Nonetheless, previous attempts to normalize the tumor vasculature and enhance perfusion and drug delivery using the anti-vascular endothelial growth factor (VEGF) monoclonal antibody bevacizumab in combination with chemotherapy, have failed to improve outcomes in patients with PDAC [19,20]. Targeting VEGF has pleiotropic effects, not only on vasculature, but also on the immune TME which could potentially synergize with ICIs and other immunotherapy treatments [21–23]. These approaches may be more effective for tumors with hyperpermeable but uncompressed vessels, while stress alleviation is beneficial for compressed but less permeable vessels, such as in most of PDACs [24]. Hypoxia can be targeted downstream the aberrant vasculature. For instance, inhibition of hypoxia-inducible factor (HIF)-1α, produced in response to reduced oxygen tension in tissues, by PX-478 enhanced the antitumor efficacy of gemcitabine and promoted immunogenic cells death in immunocompetent mouse models of PDAC [25]. Novel approaches to vascular normalization include modulating levels of the bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P), which reduced hypoxia and enhanced chemotherapy efficacy in PDAC models [26], or the use of myo-inositol trispyrophosphate, which improved chemotherapy and radiotherapy activity in PDAC models [27,28] and entered clinical evaluation [29]. In a separate approach, direct alleviation of hypoxia through polydopamine-nanoparticle-stabilized oxygen microcapsules increased intratumoral oxygen levels and induced antitumor immunophenotypic shifts in the TME of PDAC mouse models, ultimately improving the *in vivo* efficacy of ICIs [30]. To bypass the hypoxic TME of PDAC which also severely limits the efficacy of conventional radiotherapy, radiosensitizing inorganic nanoparticles, such as AGuIX, have shown considerable promise in enhancing localized radiation absorption and amplifying DNA damage selectively within irradiated tissues (NCT04881032) [31]. Furthermore, other gadolinium-based theranostic

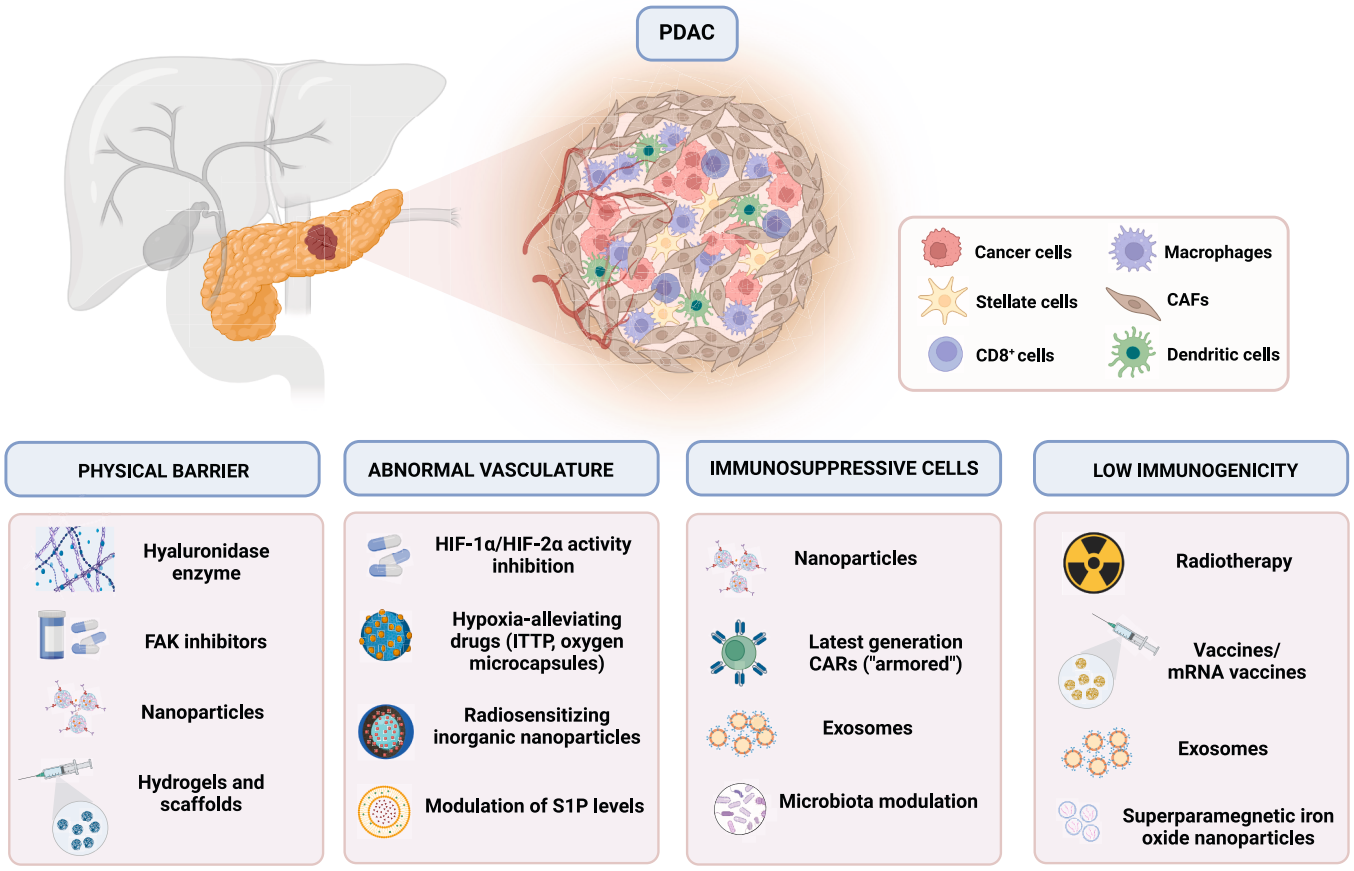


Fig. 1. Determinants of resistance to immunotherapy in pancreatic ductal adenocarcinoma (PDAC) and strategies to overcome them. Created in https://BioRender.com. PDAC is characterized by abnormal vasculature, dense extracellular matrix (ECM) and abundant cancer-associated fibroblasts (CAFs), an immunosuppressive tumor microenvironment (TME), and scattered cancer cells. Physical barrier: cancer-associated fibroblasts (CAFs) deposit a dense extracellular matrix, mainly composed of collagen and hyaluronic acid, which retains water, leading to increased extrinsic fluid pressure and consequent vascular collapse. This process hinders drug delivery and prevents the migration of immune cells with antitumor activity. Delivery of the hyaluronidase enzyme disrupts hyaluronic acid relieving extracellular pressure; focal adhesion kinase (FAK) inhibitors act directly on CAFs reducing their ECM deposition and their immune-suppressive and tumor promoting effects. Nanoparticles, either organic or inorganic, improve drug delivery within the tumor, increasing drug concentration within the tumor. Abnormal vasculature: hypoxia induced by abnormal vasculature hinders anti-tumoral immune cells activity, drug penetration into tumor, and radiotherapy efficacy. Hypoxia-inducible factors 1α (HIF-1α) and 2α (HIF-2α) are the main effectors of the hypoxic microenvironment and could effectively be inhibited by drugs or by hypoxia-modulating agents like myo-inositol trispyrophosphate (ITPP) or sphingosine-1-phosphate (S1P). Hypoxia-alleviating compounds act downstream by increasing oxygen levels within the tumor, like polydopamine-nanoparticle-stabilized oxygen microcapsules. Radiosensitizing inorganic nanoparticles, like gadolinium-based nanoparticles, can enhance radiotherapy efficacy and provide increased diagnostic accuracy (theranostic). Immunosuppressive cells: PDAC TME is enriched with cells exerting immunosuppressive effects. Regulatory T cells (Tregs) directly eliminate effector T cells, compete with them for access to antigen-presenting cells (APCs), and release immunosuppressive cytokines. Myeloid-derived suppressor cells (MDSCs) produce reactive oxygen species (ROS) that inhibit T cell proliferation and induce immune tolerance in effector T cells. CAF-specific HIF-2α further promotes immunosuppression through paracrine signaling, recruiting M2 tumor-associated macrophages (TAMs), with anti-inflammatory pro-tumoral phenotype compared to pro-inflammatory antitumoral M1 TAMS, and Tregs into the TME. Immunotherapy strategies, including immune checkpoint inhibitors (ICIs), adoptive cell therapies and vaccines, aim at reverting the immunosuppressive TME. Nanoparticles, exosomes, and injectable hydrogels deliver different compounds to favorably condition TME. These compounds include chemokines, combinations of ICIs and chemotherapy, vaccine adjuvants, and small interfering RNA (siRNA) or messenger RNA (mRNA) to target APCs.. Some bacteria strains have been shown to induce a pro-inflammatory TME reshaping. Low immunogenicity: PDAC cancer cells do not elicit a strong immune response due to their intrinsic features. KRAS-activating mutations mediate granulocyte–macrophage colony-stimulating factor (GM-CSF) production, which recruits MDSCs and promotes class I major histocompatibility complex (MHC-I) degradation. MYC overexpression induces programmed death-ligand 1 (PD-L1) and CD47 expression. Additionally, the low tumor mutational burden (TMB) of PDAC tumor cells is associated with a reduced neoantigen load. Nanoparticles-based delivery systems allow effective targeting of KRAS-mutant cells. Radiation and superparamagnetic iron oxide nanoparticles (SPIONs) can induce immunogenic cell death and provide increased diagnostic accuracy (theranostic).

nanoparticles can also more precisely delineate cancer tissues at non-contrast magnetic resonance imaging to allow a more accurate radiation therapy planning and delivery (NCT04682847) [32].

CAFs are an heterogenous group of cells in PDAC stroma, which include αSMA^{+} myofibroblasts (myCAF), inflammatory CAFs (iCAFs), antigen-presenting CAFs (apCAFs) among others [33]. CAFs are mainly derived from pancreatic stellate cells (PSCs) and partly from mesenchymal stem cells [34,35]. Different types of CAFs can exert opposite functions within PDAC, that can be either tumor promoting, e.g. extracellular matrix (ECM) deposition, enhancement of tumor proliferation and immune evasion, or tumor-suppressing, e.g. antigen presentation and vessel formation [33,36]. CAFs are the main responsible for the

deposition of dense ECM, mostly composed of collagen, fibronectin, and hyaluronic acid, that creates a physical barrier that hinders drug delivery and cells migration [17,37,38]. Indeed, hyaluronic acid retains water causing increased interstitial fluid pressure and consequent vascular collapse compromising drug penetration [38]. Likely because of the ambivalent role of CAFs in PDAC, unselective stroma ablation strategies have yielded mostly disappointing results to date. Indeed, preclinical studies in mouse models suggested that targeting hyaluronic acid reduces vascular collapse and increase chemotherapy delivery in PDAC [17,37], but randomized HALO-109–301 and SWOG S1313 trials combining pegylated human recombinant PH20 hyaluronidase (PEGPH20) to chemotherapy in patients with advanced PDAC failed to demonstrate a clinical benefit [39,40]. Interestingly, the local injection of the hyaluronidase-expressing oncolytic adenovirus VCN-01 in combination with gemcitabine was well tolerated and resulted in decreased tumor stiffness in a proof-of-concept phase I trial [41]. These results prompted the phase II VIRAGE trial in which VCN-01 is administered intravenously with gemcitabine and nab-paclitaxel (NCT05673811). On the other hand, vitamin D analog paricalcitol and hydroxychloroquine with gemcitabine demonstrated selective reduction of tumor-promoting CAFs and induced antitumor immune reshaping of TME (increase in $CD4^+$ and $CD8^+$ T cells, M1 macrophages polarization, and decrease in regulatory T cells) in orthotopic PDAC mouse models and patient-derived xenografts [42]. Furthermore, it was found to increase tumor vascularity in post-treatment samples [43]. Clinical trials are ongoing to investigate the incorporation of paricalcitol and/or hydroxychloroquine in chemotherapy regimens for the treatment of patients with PDAC (NCT03883919, NCT04524702) [43].

The TME of PDAC is enriched with immunosuppressive cell populations that exert distinct functions in dampening antitumor immune responses. The contribution of each cell type to immunosuppression varies across different stages of tumor progression [44,45]. In the early stages, regulatory T cells (T-regs) represent the predominant immunosuppressive population. At later stages, myeloid-derived suppressor cells (MDSCs) become the major contributors, promoting oxidative stress through the release of reactive oxygen species (ROS), thereby inhibiting T-cells proliferation and contributing to the progressive decline of effector T cells over time.

T-regs in the tumor stroma strongly inhibit antitumor immunity through direct elimination of effector T cells, competition with these cells for the access to APCs, and release immune-suppressive cytokines [46]. On the other hand, MSDCs represent the greatest part of PDAC cell population since these cells are recruited thanks to mutant KRAS-mediated expression of granulocyte–macrophage colony-stimulating factor (GM-CSF) by PDAC cells [17,47,48]. MDSCs are immature cells with immunosuppressive effect exerted in an antigen non-specific manner, through ROS production, and in an antigen-specific way, by altering antigen processing and inducing immune tolerance in effector T cells [46,49]. MDSCs also promote PD-L1 expression on tumor cells via EGFR-MAPK signaling pathways [24] and deplete key amino acids such as cysteine through arginase-1 and the Xc^- transporter, thereby impairing T-cell receptor signaling and effector function [50], and fostering the expansion of T-regs through direct cell–cell interactions [51]. Experimental depletion of granulocytic MDSCs has been associated with enhanced infiltration of $CD8^+$ cytotoxic T cells and increased tumor cell apoptosis in preclinical models [28]. Inhibition of CXCR2 to block MDSC trafficking has been shown to reduce metastatic spread and enhance the response to anti-PD-1 therapy [52]. Moreover, combined inhibition of CXCR1/2, in conjunction with a 4-1BB agonist and a LAG3 antagonist, effectively reprogrammed the immunosuppressive TME and elicited durable antitumor responses in murine models of PDAC, supporting the rationale for combinatorial strategies targeting MDSCs alongside immune checkpoint modulation [53].

Nonetheless, many other cells contribute to the immunosuppressive TME of PDAC. Tumor-associated macrophages (TAMs) are able to present antigens to immune cells, in order to activate adaptive immunity [46,54]. TAMs are classified as M1, with a pro-inflammatory phenotype and more frequent expression in early stage tumor, and M2, with an anti-inflammatory phenotype, more abundant in more advanced stages of disease [46]. The blockade of the receptor-interacting serine/threonine protein kinase 1 (RIP1) can reprogram TAMs, thereby promoting a shift toward an anti-tumoral immune TME [55]. Thus, an interesting approach involves the reversal of the M2 TAM phenotype and enhancement of anti-tumor immunity in PDAC mouse models using a dual-delivery biosystem, based on exosomes loaded via electroporation with galectin-9 siRNA and surface-modified with an oxaliplatin prodrug [56].

Furthermore, hypoxia-inducible factor 2α (HIF-2α) produced by CAFs promotes immunosuppression within the TME via paracrine signaling, facilitating the recruitment of M2 TAMs and Tregs. Conversely, CAF-specific HIF-2α knockout in murine models of PDAC is associated with reduced fibrosis and diminished recruitment of immunosuppressive cell populations. [57].

Immune cells are also critical determinants of metastases development in PDAC. Primary tumor cells secrete soluble factors and extracellular vesicles, cytokines, and chemokines to create the so-called “pre-metastatic niche” (PMN) in secondary organs and prepare the distant site microenvironment to host tumor cells colonization [45,58]. Tumor-mobilized bone marrow derived cells (BMDCs) migrate in the PMN and modify the local microenvironment by secretion of inflammatory cytokines, growth factors, and proangiogenic molecules, thus supporting cancer cell colonization and proliferation [58,59]. Immune and stromal cells such as CAFs, stellate cells, metastasis-associated-macrophages (MAMs), neutrophils, as well as T-regs and MDSCs are recruited in the PMN and establish an immunosuppressive microenvironment [45,59]. However, the degree of immunosuppression and the immune infiltrate is different for each PMN [45]. The liver is the most common metastatic site for PDAC cells, followed by peritoneum and lung [60]. Immunosuppressive monocytes have been identified as the first cells to migrate into the liver and drive the PMN creation, while neutrophils are the first cell type to prepare the PMN creation in the lung [45]. The different biological characteristics of PMN in metastatic sites are in agreement with the worse prognosis in patients with PDAC and liver-only metastases than in those with lung-only metastases observed in population-based analyses [61].

Lastly, PDAC tumor cell itself has low immunogenicity and is a poor target for anti-tumor immune response. A transformed cell to survive must acquire an immunosuppressive phenotype, including the down-regulation of class I MHC and the up-regulation of PD-L1 and Cluster of differentiation 47 (CD47), to evade the immune system response against the tumor [45]. In PDAC cells, *KRAS* regulates degradation of class I MHC by autophagy [62,63], while PD-L1 and CD47 expression is regulated by MYC overexpression, which is observed in 32.3 % and 29.4 % of primary and metastatic tumors, respectively [64,65]. Furthermore, PDAC cells have low TMB, defined as the number of non-synonymous mutation per unit of transcribed genome, which determines low immunogenicity [45]. Indeed, a high-TMB is associated with potential benefit from immunotherapy as TMB is a proxy for a high neoantigen load [16]. However only 1.1 % of PDACs have high TMB, which is most commonly associated with other molecular alterations such as dMMR and MSI-H status [66].

The poor immunogenicity of PDAC cause cells to escape immune surveillance, take the lymph-vascular or perineural stream to migrate to distant organs leading to early metastases development [45]. Also pancreatic intraepithelial neoplasms (PanINs), the premalignant precursors of PDAC, which harbour only mutations in *KRAS*, can undergo epithelial to mesenchymal transition (EMT) and disseminate, thus suggesting that cellular spread may precede the development of invasive carcinoma and thereby contribute to early immune evasion [67].

Table 1
Classic immunotherapy strategies with immune checkpoint inhibitors in advanced PDAC.

Study	Phase	Setting	N	Drug	ORR (%)	DCR (%)	mOS (months)		mPFS (months)	
Non-randomized trials										
Royal et al. [68]	II	Locally advanced/metastatic	27	Ipi	0	NR	NR		NR	
Sharma et al. [69]	II	Metastatic,≥II line	20	T	0	0	4		NR	
Brahmer et al. [70]	I	Metastatic,≥II line	14 PDAC	BMS-936559	0	NR	NR		NR	
KN-158 *Maio et al.* [13]	II	Pretreated, MSI-H	22 PDAC	Pembro	18.2	46.7	3.7		2.1	
Gang et al. [80]	II	Metastatic,≥II line	21	KN046	11.1	44.4	2.1		7.5	
Aglietta et al. [72]	I	Metastatic, no prior cht	34	T + gem	7.1	25.0	7.4		NR	
Kalyan et al. [73]	Ib	Metastatic, no prior gem	16	Ipi + gem	12.5	43.0	8.5		2.5	
Kamath et al. [74]	Ib	Metastatic, no prior gem	21	Ipi + gem	14.3	33.3	6.9		2.8	
Weiss et al. [77]	Ib/II	Metastatic, no prior cht	12	Pembro + GnP	27.3*	100.0*	15.0*		9.1*	
Wainberg et al. [75]	I	Metastatic, no prior cht	50	Nivo + GnP	18.0	64.0	9.9		5.5	
Cheng et al. [76]	Ib/II	Metastatic, untreated	72	Tori + Gnp	33.3	90.3	8.9		5.6	
Morizane et al. [78]	II	Metastatic, untreated	31	Nivo + FOLFIRINOX	32.3	75.9	13.4		7.4	
Gang et al. [81]	II	Advanced, untreated	17	KN046 + GnP	55.6	88.9	NR		NR	
Randomized trials										
O'Reilly et al. [71]	II	Metastatic, II line	32	D + T	3.1	9.4	3.1		1.5	
			33	D	0	6.1	3.6		1.5	
CCTG PA.7 *Renouf et al.* [79]	II	Metastatic, untreated	119	D + T + GnP	30.3	70.6	9.8	p = 0.72	5.5	p = 0.91
			61	GnP	23	57.4	8.8		5.4	
Padrón et al. [84]	Ib/II	Advanced, untreated	34	Nivo + GnP	50	74	16.7	p = 0.006[+]	6.4	
			36	Soti + GnP	33	78	11.4	p = 0.062[+]	7.3	
			35	Nivo + soti + GnP	31	69	10.1	p = 0.223[+]	6.7	

* data from N = 12 treatment-naïve patients.
[+] There were no comparisons between arms, p-value for the primary endpoint of 1-year OS rate (1y-OS) was tested independently for each arm against a null hypothesis of 1y-OS = 35 % (historical control of GnP alone). Cht: chemotherapy; D: durvalumab; DCR: disease control rate; FOLFIRINOX: 5 fluorouracil + oxaliplatin + leucovorin + irinotecan; Gem: gemcitabine; GnP: gemcitabine + nab-paclitaxel; Ipi: ipilimumab; mOS: median overall survival; mPFS: median progression-free survival; MSI-H: microsatellite; Nivo: nivolumab; ORR: objective response rate; PDAC: pancreatic ductal adenocarcinoma; Pembro: pembrolizumab; T: tremelimumab; Tori: toripalimab; Soti: sotigalimab.

4. Immunotherapy in PDAC

Immunotherapy with ICIs in PDAC.

Studies involving ICI-based immunotherapy in PDAC are summarized in Table 1.

First studies investigated the activity of ICIs directed against CTLA-4 and PD-1 as single agents in patients with PDAC. These studies yielded overall poor results, as no objective response observed, likely because of the abovementioned immunosuppressive TME and the low immunogenicity typical of PDAC, which could not be overcome by single-agent ICIs [68–70].

Trials investigating ICI monotherapy or dual ICI combinations (e.g., durvalumab ± tremelimumab) in metastatic PDAC have shown limited efficacy [71]. Objective response rates (ORR) were low or absent (0–3.1 %), with disease control rates (DCR) also modest (up to 9.4 %). Median progression-free survival (PFS) and overall survival (OS) were generally poor (PFS ~ 1.5 months, OS ~ 3–3.6 months). A small subset of patients (e.g., MSI-H or long-term survivors) derived prolonged benefit, but overall results were disappointing.

Several early-phase studies combining CTLA-4 inhibitors (e.g., tremelimumab or ipilimumab) with gemcitabine showed acceptable safety profiles but no meaningful survival improvement compared to chemotherapy alone. Reported ORRs ranged from 7.1 % to 14 %, with median OS between 6.7 and 8.5 months [72–74]. Interestingly, correlative analyses in one of these studies showed that $CD8^+$ T cells underwent universal reduction in the inhibitory molecule LAG-3 and of $FoxP3^{high}CD45RA^-$ T regs (FrII), while $FoxP3^{low}CD45RA^+$ T regs (FrI) increased, after treatment with ipilimumab [73].

Subsequent studies added anti-PD-1/PD-L1 agents (e.g., pembrolizumab, nivolumab, toripalimab) to standard chemotherapy regimens such as gemcitabine/nab-paclitaxel or mFOLFIRINOX. These combinations were generally well tolerated and showed some activity signs, particularly in treatment-naïve patients, with ORRs up to 33 %, DCRs up to 100 %, median PFS of 5.5–9.1 months, and median OS up to 15.0 months [75–78]. Also, in patients with PD-L1 positive tumors, toripalimab yielded an ORR of 56 % compared to 25 % in those with PD-L1 negative ones [76]. Nonetheless, the clinical benefit was marginal overall, with no significant survival advantage compared to historical controls, except for few responders [77]. In these patients, exploratory analyses found increased peak levels of CD8 + and $CD4^+$ T cells (with higher levels of CD8 +) and higher peak of on treatment Ki-67^+ $CD8^+$ T cells [75].

In light of these results, the combination of PD-1/PD-L1 and CTLA-4 blockade added to standard chemotherapy has been also investigated. A phase II trial testing the combination of durvalumab and tremelimumab with gemcitabine/nab-paclitaxel in unselected metastatic PDAC patients did not demonstrate added benefit over chemotherapy alone (median OS: 9.8 vs. 8.8 months). Circulating tumor DNA (ctDNA) analysis confirmed the poor prognosis associated with KRAS mutations (77 % of patients), irrespective of treatment [79]. KN046, a bispecific antibody targeting both PD-L1 and CTLA-4, showed modest activity as monotherapy (ORR 11.1 %, OS 7.5 months) in previously treated patients [80]. However, in combination with gemcitabine/nab-paclitaxel in the first-line setting, it yielded promising preliminary outcomes (ORR 55.6 %, DCR 88.9 %) [81], prompting a phase III trial currently underway (NCT05149326) [82].

Because of the unsatisfactory results of ICI alone or in combination and with chemotherapy, further studies investigated the combination of ICI with other immunostimulatory agents. CD40 is a surface molecule expressed on APC cells that binds its ligand (CD40-L) on activated T cells to initiate a specific immune response [83]. Based on the encouraging preclinical data for the antitumor activity of CD40 agonist antibodies [83], the phase II PRINCE trial evaluated the addition of nivolumab with or without sotigalimab (CD40 agonist) to gemcitabine plus nab-paclitaxel chemotherapy as first-line treatment patients with metastatic PDAC [84]. Patients were randomized in three non-comparative arms and the primary endpoint of the study was 1 year OS rate (1y-OS). The primary endpoint was met in the nivolumab plus chemotherapy group

Table 2
Novel immunotherapy combination strategies with immune checkpoint inhibitors in advanced pancreatic ductal adenocarcinoma (PDAC).

Study	Phase	Setting	N	Drug	ORR (%)	DCR (%)	mOS (months)	mPFS (months)
Non-randomized trials								
Naing et al. [96]	IIa	Pretreated PDAC, ICI naïve	32	Pembro + NT-I7	8	NR	NR	NR
Zhen et al. [99]	II	Metastatic, ≤2 prior lines, HA-high	8	Pembro + PEGPH20	0	25	7.2	1.5
Algaze et al. [100]	Ib	Metastatic, ≥1 prior line	24	D + guadecitabine	5	33	4.4	2.1
Randomized trials								
Reiss et al. [98]	Ib/II	Maintenance I line	46	Nivo + niraparib	7.1	NR	13.2	1.9
			45	Ipi + niraparib	15.4	NR	17.3	8.1
COMBAT *Bockorny et al.* [88]	IIa	Metastatic, ≥1 prior line	37	Pembro + motixafortide	3.4	34.5	3.3 ITT, 7.5 2nd line	NR
			22	Pembro + motixafortide + cht	32	77	NR	NR

Cht: chemotherapy; D: durvalumab; DCR: disease control rate; HA: hyaluronic acid; ipi: ipilimumab: nivo: nivolumab; mOS: median overall survival; mPFS: median progression-free survival; NT-I7: long-acting interleukin-7; ORR: objective response rate; PDAC: pancreatic ductal adenocarcinoma; PEGPH20: pegylated, human recombinant PH20 hyaluronidase; Pembro: pembrolizumab.

(1y-OS: 57.7 %), but in the sotigalimab plus chemotherapy (48.1 %) or in the triple combination arm (41.3 %) [84].

The desmoplastic reaction typical of PDAC TME is predominantly mediated by the activation of quiescent PSCs into CAFs which also reduce the migration of CD8 + T cells in the tumor stroma through CXCL12-CXCR4 axis [85,86]. Pre-clinical data suggested that the combination of a C-X-C chemokine receptor type 4 (CXCR4) blockade to an anti PD-1 increases $CD8^+$ T cells in PDAC [87]. Based on these data, the phase IIa COMBAT trial demonstrated that the addition of CXCR4 antagonist BL-8040 (motixafortide) and pembrolizumab reinforces the benefit of chemotherapy in pre-treated advanced PDAC patients (Table 2) [88]. In cohort 1, 37 patients were randomized to receive motixafortide plus pembrolizumab, while the cohort 2 enrolled 22 patients who received motixafortide plus pembrolizumab and chemotherapy [88]. The median ORR and DCR were 3.4 % (1 PR) and 34.5 % (9 SD + 1 PR) in cohort 1, and 32 % and 77 % in cohort 2. Although in the motixafortide plus pembrolizumab group the median OS was only 3.3 months, this was up to 7.5 months in patients who received these drug combination as second-line treatment [88], which is numerically longer than what observed with the current second-line chemotherapy treatments [89]. Moreover, motixafortide has shown to increase tumor infiltration of $CD8^+$ T cells, and to decrease MDSCs and Tregs, consistent with the supposed mechanism of action [88]. Moreover, focal adhesion kinase (FAK) expressed on CAFs upregulates intratumoral Tregs through expression of cytokine transforming growth factor β2 (TGF-β2) and CC-chemokine ligand 5 (CCL5) [90]. Thus, the coadministration of the FAK inhibitor defactinib with gemcitabine and pembrolizumab has been tested in phase I study and is being further evaluated in PDAC [91]. Interestingly, defactinib improves drug penetration by affecting TME and has gained the orphan drug designation by the American Food and Drug Administration (FDA) in combination with avutometinib, an oral RAF/MEK clamp active in KRAS-mutant tumors, following the results of the phase Ib/II RAMP205 study (NCT05669482) [92].

Because of the immunomodulatory role of the Bruton kinase in PDAC TME [93], some studies have focused on the addition of the Bruton kinase inhibitors acalabrutinib [94] and ibrutinib [95] to ICIs, failing to demonstrate a significant antitumor activity. Also, the combination of NT-I7, a long-acting interleukin (IL)-7 formulation, with pembrolizumab in patients with relapsed/refractory PDAC who have not received ICIs, yielded a deep response in 2 out of 26 cases, with tumor shrinkage of 100 % and 72 %, and a duration of response (DOR) of over 1.35 months and 6.64 months, respectively [96].

Other targets not directly related to the immune system have been investigated for their potential to enhance antitumoral immune response and, overall, treatment outcomes. Given the potential synergistic antitumoral effect of combined DNA damage repair (DDR) machinery and PD-1/PD-L1 inhibition [97], an open-label phase Ib/II trial investigated the activity of two non-comparative arms of nivolumab (N = 46) or ipilimumab (N = 45) plus niraparib (a PARP inhibitor) as maintenance in patients with PDAC who had not progressed after ≥ 16 weeks of platinum-based chemotherapy [98]. The primary endpoint of PFS rate at 6 months > 44 % has been met in the niraparib plus ipilimumab arm, which yielded a median PFS of 8.1 months, a median OS of 17.3 months, and an ORR of 15.4 % [98]. Notably, the better outcomes observed in the niraparib plus ipilimumab arm were consistent also after excluding patients with known mutations in analyzed DDR genes in a pre-specified analysis, suggesting that the benefit of combined PARP and CTLA-4 inhibition is independent on DDR mutational status. As aforementioned, hyperproduction of hyaluronic acid in TME hampers drug delivery and immune cell migration to the tumor [37,38]. To overcome this, a phase II clinical trial investigated the efficacy of the PEGPH20 and pembrolizumab in patients with metastatic PDAC with high hyaluronan expression levels treated with ≤ 2 prior therapies [99]. The trial was discontinued early due to the negative results of the phase III randomized HALO-109–301 trial of the addition of PEGPH20 to chemotherapy [39]. Indeed, despite an encouraging median OS of 7.2 months, a disappointing median PFS has been observed.

Deregulated epigenetic modifications have pleiotropic effects on tumor cells and TME in solid tumors [100], including CAFs differentiation from PSCs and shift between different CAFs types [101]. As such, inhibition of DNA methyl transferase upregulates interferon pathways facilitating immune response [100]. A phase IB trial evaluated the DNA methyltransferase inhibitor guadecitabine in addition to durvalumab in advanced hepatocellular, biliary cancers and patients with PDAC progressing to ≥ 1 prior line of therapy for advanced disease [100]. Among patients with PDAC, only 1 PR has been observed, but it lasted for > 24 months, while the median PFS was 2.1 and the median OS was 4.4 months [100].

Furthermore, trials on local radiotherapy with ICIs in order to enhance immunotherapy efficacy by leveraging on the abscopal effect [102], failed to demonstrate a significant benefit from this strategy [102–104]. Indeed, a phase II trial evaluated the addition of radiation therapy to ipilimumab and nivolumab treatment in 25 patients with metastatic PDAC, with a median number of prior lines of treatment of 2, and demonstrated DCR 20 % in the intention-to-treat (ITT) population and 29 % in the per-protocol analysis (patients who received radiation) [102]. Similar results have been shown in the phase II CheckPAC study evaluating the addition of stereotactic body radiotherapy (SBRT) to nivolumab with or without ipilimumab, in 84 patients with refractory metastatic PDAC: the DCR was 37.2 % in the dual ICI arm (ORR of 14 %) compared to 17.1 % in the nivolumab monotherapy arm, but this did not result in any survival improvement (mOS 3.8 months in both arms). Notably, a durable clinical benefit has been demonstrated in the small subset of patients who achieved PR (median DOR 5.4 months) [103]. Further studies have confirmed the limited efficacy of combining radiotherapy with ICIs: the addition of SBRT to durvalumab with or

without tremelimumab showed 5.1 % ORR with mOS ranging from 2.1 to 9 months across cohorts [104]. Similarly, the TRIPLE-R phase II trial evaluating the efficacy of a triple immunotherapy (ipilimumab, nivolumab and tocilizumab) with SBRT showed no responses with only 19 % of patients with stable disease and mOS of 5.3 months; notably, the study revealed high incidence (73 %) of treatment-related adverse events (AE), 8 % with grade 3 (G3) or higher, thus suggesting that intensifying immune stimulation, even if combined with radiotherapy treatment, not only is insufficient to overcome the highly suppressive PDAC TME, but is also associated with increased toxicity, further limiting its clinical applicability [105]. Moreover, a pooled analysis of three phase I trials aiming to assess the safety of combining radiotherapy with ICIs has been performed including metastatic cancer patients, among witch those with PDAC; the analysis demonstrated more severe toxicities in patients receiving a dual-agent immunotherapy with AE $\geq$ G3 occurring in 27 % of cases; however the increased toxicity was not associated with survival benefit [106].

Overall, the failure of ICI-based strategies in PDAC depended on multiple factors, including the lack of patient selection and mostly delivery failure due to the physical and biological barrier of TME against drugs and immune cells. Artificial intelligence (AI) and machine learning (ML) algorithms are increasingly being leveraged to refine biomarker discovery and patient stratification strategies in immune-oncology [107]. AI-based approaches applied to radiomic features or hematoxylin/eosin (H/E)-stained histopathology slides offer novel avenues for ICI response prediction in PDAC [108]. Specifically, MRI-based radiomic models have been shown to identify PDAC tumors with high densities of $CD8^+$ tumor-infiltrating lymphocytes (TILs), a phenotype associated with improved prognosis [109]. A similar strategy could be useful for identifying $CD4^+$ TILs which were associated with improved outcomes in the nivolumab + chemotherapy arm in the PRINCE study [84]. In parallel, convolutional neural networks trained on digitized H/E slides are being explored to screen for MSI with high throughput and cost-efficiency, potentially enabling scalable pre-selection of ICI-eligible cases in the absence of comprehensive molecular profiling or to reduce turnaround time of molecular analyses [110].

From a drug-delivery point of view, nanoparticle-based systems, like nano-albumin bound paclitaxel and nanoliposomal irinotecan (nal-IRI), demonstrated to partially overcome TME-mediated delivery failure and to effectively improve drug penetration and clinical activity compared to the respective drug solutions [6,35,111]. Indeed, nal-IRI exhibited intensified systemic circulation and increased AUC compared to free irinotecan; moreover computational pharmacokinetic (PK) modelling predicted that nal-IRI could meet the same tumor exposure to SN-38 (the irinotecan active drug) at 1/5 dose of free irinotecan [112].

Nanoparticle-based systems can be exploited in different ways to improve ICI penetration and favorably modify TME [113]. Nanocarriers such as poly(lactic-co-glycolic) acid (PLGA), liposomes, iron-dextran particles, and lipid-protamine-DNA (LPD) complexes have been used to deliver anti-PD-1/PD-L1 antibodies or small-interfering RNA (siRNA) selectively to tumor sites, improving T cell-mediated anti-tumor responses. LNP-based nanoparticles exhibit high drug-loading capacity especially for mRNA payloads, but limited stromal penetration ability [114,115], however, new-generation liposomes functionalized with aptamers have shown promising results in other solid tumors. For example, PLGA-based polymeric and lipid-coated calcium phosphate nanoparticles have shown effective delivery of PD-L1 siRNA to tumor-infiltrating lymphocytes, improving T cell responses in breast cancer models [116–118], while iron-dextran nanoparticles conjugated with PD-L1 and 4-1BB antibodies enabled dual immune checkpoint modulation with enhanced tumor T cells infiltration [119]. In addition, co-delivery of ICIs with chemotherapy agents (e.g., paclitaxel, doxorubicin, or cisplatin), immunostimulatory cytokines, or other immune adjuvants within a single nanoparticle system synergizes immune responses and overcomes tumor immune evasion. For instance, a system co-delivering paclitaxel and anti-PD-1 via pH-sensitive micelles enhanced immunogenic cell death and tumor suppression [120]. In breast cancer, a modified ROS-responsive nanoparticles have been used to deliver PD-L1 siRNA and doxorubicin boosting T cells activity and chemotherapy cytotoxicity *in vitro* and *in vivo* [121]. Nanoparticles co-delivering ICIs with chemokine or cytokine inhibitors (e.g., CXCL12 antagonists) remodel the TME, enhancing T cells infiltration and reducing immunosuppressive signals. In a preclinical PDAC model, LPD nanoparticles delivering plasmids encoding PD-L1 and CXCL12 traps improved T cells recruitment and reduced metastasis compared to systemic ICIs administration [122].

Compared to other nanoparticle systems, superparamagnetic iron oxide nanoparticles (SPIONs) offer distinct advantages, including superior biocompatibility, controlled biodegradability, and the ability to provide real-time, high-resolution MRI-based monitoring of biodistribution and therapeutic response, while also enabling magnetic field–mediated targeting and hyperthermia functionalities (referred to as “nanotheranostic”) [123,124]. SPIONs are nanoparticles composed of iron oxide that have been extensively investigated as multifunctional theranostic platforms for both imaging and treatment of PDAC [125,126]. In the context of immunotherapy, SPIONs have demonstrated the capacity to activate robust antitumor immune responses through the induction of immunogenic cell death, such as ferroptosis, via intracellular ROS generation [127], and the reprogramming of M2 TAMs to a pro-inflammatory M1 phenotype [128]. SPIONs also enhance infiltration and activation of $CD8^+$ T cells in tumors, contributing to their conversion from an immunologically “cold” to “hot” state [129]. Furthermore, SPION-based magnetic hyperthermia and photothermal therapies have been combined with ICIs, such as PD-L1 and CTLA-4 inhibitors, achieving enhanced T cell–mediated responses and inhibition of primary and metastatic tumor growth in preclinical models [130–132].

Exosomes represent a promising platform for immunotherapy-based drug delivery in PDAC, owing to their natural biocompatibility, low immunogenicity, and intrinsic capacity to carry immunomodulatory molecules, compared to synthetic nanoparticles [133]. Tumor-derived exosomes have prolonged persistence in circulation due to the expression of CD47 to avoid phagocytosis and can effectively target tumor cells with signaling molecules on their surface [134]. Nonetheless, because of the incomplete understanding of their structure and function, there is concern that PDAC-derived exosomes might promote tumor progression and metastases making mesenchymal stem cell (MSC)-derived exosomes the safest and most explored approach to date [133]. Exosomes are potentially a potent and flexible tool given the possibility to be charged with several different types of molecules, including peptides and proteins, cytokines, chemotherapy, nucleic acids (DNA, RNA, siRNA, miRNA), tumor antigens and immune adjuvants, showing potential in the delivery of ICIs or their genetic regulators, such as siRNA or miRNA targeting PD-L1 or other checkpoint pathways [133]. Several studies have demonstrated that exosomes can be engineered to deliver cytokines, such as tumor necrosis factor-α (TNF-α), directly to tumor sites, thereby enhancing local immune activation while minimizing systemic toxicity. For instance, MSC-derived exosomes loaded with TNF-α and modified with cell-penetrating peptides and magnetic nanoparticles achieved targeted delivery and effective tumor regression in melanoma models [135], suggesting a potential translational pathway for PDAC. TNF-α may exert various biological effects, including the increase of vascular permeability by acting on tumor-associated endothelial cells. This property is harnessed by PEGylated inorganic gold nanoparticles CYT-6091, which exploit enhanced tumor vascular permeabilization to achieve selective accumulation within the tumor microenvironment and to promote immune activation; the pharmacokinetic analysis demonstrated a 5-fold prolonged half-life of CYT-6091 with a 4-fold greater AUC compared to free recombinant human TNF, thereby representing a platform of potential interest for co-administration with ICIs (NCT00356980) [136]. Since peritumoral administration of anti-CTLA-4 monoclonal antibodies using direct injection achieved significant

tumor growth suppression and regulatory T cell depletion [137], local delivery of ICIs in implantable platforms provides a suggestive strategy to bypass the TME barrier [138].

Locally injected hydrogels or scaffolds can be leveraged to deliver various classes of agents and are particularly useful for the sequential release of cytokines, chemotherapy, and immune-modulating agents including ICIs, bypassing systemic administration barriers [139–141]. Nanofluidic drug-eluting seeds (NDES) allow for sustained localized delivery of ICIs directly within or adjacent to the tumor, thereby maximizing local immunomodulation while minimizing systemic exposure [142]. These devices are engineered to release antibodies in a controlled manner over days to weeks, enabling temporal coordination with the immune cycle. Moreover, emerging bioresponsive scaffolds that enable phase-specific release of anti-PD-1 or anti-CTLA-4 antibodies are being designed to match the immune activation state and to exploit windows of antigen presentation [143]. These strategies could be particularly useful in the setting of localized or locally advanced PDAC, i.e. in absence of clinically evident nodal or distant metastases.

In the context of metastatic or adjuvant micrometastatic disease, strategies with the capacity to trigger an immune abscopal effect may hold greater translational relevance. Particularly, the GEM-STING@Gel, a ROS-responsive hydrogel system designed to co-deliver gemcitabine and the STING agonist DMXAA, enhanced antitumor immunity through immunogenic cell death, dendritic cell (DC) maturation, and demonstrated abscopal effects in murine PDAC models [144]. Similarly, a dual-crosslinked hyaluronic acid hydrogel was engineered to deliver polydopamine-encapsulated IL-15 and platelet-conjugated anti-TIGIT antibodies into the resection cavity of orthotopic PDAC models. This strategy suppressed local recurrence and metastasis by promoting CD8 + T cell and NK cell activation, with controlled release kinetics sustained over several weeks [145]. These implantable or injectable platforms facilitate spatially confined, durable, and immune-potentiating delivery of cytokine-based therapies, especially in adjuvant and perioperative contexts. In the advanced setting, the local administration of the oncolytic adenovirus LOAd703, encoding for the trimerized membrane-bound extracellular CD40L (TMZ-CD40L) and 4-1BB ligand, in combination with intravenous gemcitabine and nab-paclitaxel chemotherapy was feasible and safe, and yielded a potentially interesting ORR of 44 %, in the phase I/II LOKON001 study [146]. Subsequently, an arm which also added the PD-L1 inhibitor atezolizumab is currently ongoing (NCT02705196).

Immunotherapy with Adoptive cellular therapy in PDAC.

Adoptive cellular therapy (ACT) is a novel immunotherapy strategy that gained increased interest because of its specificity against tumor cells and its potential to overcome resistance to ICIs [147]. Unlike ICIs mechanism of action, ACT involves the collection of T-cells from patient's peripheral blood, the subsequent genetic engineering modification and expansion, and the final reinfusion back into the patient [147]. T cells genetically modified can express T-cell receptors (TCR) or chimeric antigen receptors (CARs), in order to target a tumor-specific antigen [148]. Differently from TCR, which binds tumor antigens presented by HLA complexes, CARs target tumor cell proteins, carbohydrates and glycolipids independently of HLA-presentation, as a way to overcome HLA down-regulation often observed in solid tumors [148,149]. Given its high prevalence in PDAC, mutant KRAS is an appealing target for such an approach. In a case report, ACT treatment by a TCR targeting mutant KRAS G12D has been offered to a patient with progressive metastatic PDAC who achieved a PR with a shrinkage of 72 % of the visceral metastasis at 6 months after a single infusion, durable for the following 6 months [150]. However, another PDAC patient carrying the same mutation did not benefit from the TCR infusion [150].

There are different generations of CARs, with structural modifications in the intracellular domain (endodomain) from one generation to the next, leading to progressive functional improvements. CARs comprise an extracellular domain, a transmembrane domain and an intracellular signaling domain. The extracellular domain consists of the

Table 3
Ongoing CAR-T trials targeting mesothelin and claudin 18.2 in PDAC (clinicaltrials.gov: "pancreatic cancer", "claudin 18.2 CAR-T", "CLDN18.2 CAR-T"; last access: 28/02/25).

Study	Phase	Condition	Intervention	Est. Study completion
Mesothelin				
NCT06760364	I	Advanced PDAC, mesothelin+	Meso-UCAR-T (CHT102)	12/2039
NCT06256055	I	Advanced solid tuomors, mesothelin+	Circular mRNA encoding anti-meso CAR-T (UCMYM802)	04/2025
NCT03323944	I	Unresectable or metastatic PDAC	Intravenous and local administration of huCART-meso cells	09/2025
NCT06051695	I/II	Unresectable, locally advanced, or metastatic PDAC or other solid tumors, mesothelin+, HLA-A*02 lost	autologous logic-gated CAR-T (A2B694)	06/2029
NCT05779917	I	Advanced PDAC (or other cancers), mesothelin+	Mesothelin/GPC3/GUCY2C: *meso*-CAR-T cell secreting a fusion protein of IL21 and scfv against PD1 (+/- PD1/PDL1/CTLA4 antibodies)	03/2036
NCT06196294	I	Advanced solid cancers, GPC3 or mesothelin+	GPC3/Mesothelin-CAR-γδT cells, (+/- PD1/PDL1/CTLA4 antibodies)	12/2036
Claudin				
NCT06134960	I	Advanced NKG2DL+/CLDN18.2 + solid tumors	NKG2D/CLDN18.2 CAR-T (KD-496)	11/2026
NCT04404595	Ib/II	Advanced solid digestive tract tumors, CLDN18.2+	CLDN18.2 CAR-T (CT041)	09/2035
NCT05472857	I	Advanced solid tumors, CLDN18.2+ (≥ 1+, positive rate ≥ 10 %)	CLDN18.2 CAR-T (IMC002)	12/2024
NCT05393986	I	Advanced solid tumors, CLDN18.2+ (≥1 prior line)	CLDN18.2 CAR-T (CT048)	12/2024
NCT05277987	I	Advanced gastric and GEJ (≥2 prior line), PDAC (≥1 prior line), CLDN18.2+	CLDN18.2 CAR-T (HEC-016)	03/2025
NCT05539430	I	Advanced gastric, GEJ, esophageal, PDAC cancer, CLDN18.2+ (≥1 in > 50 % tumour cells)	CLDN18.2 CAR-T (LB1908)	12/2025
NCT05583201	I	Advanced NKG2DL+/CLDN18.2 + solid tumors	NKG2D/CLDN18.2 CAR-T (KD-496)	06/2026

(*continued on next page*)

Table 3 (*continued*)

Study	Phase	Condition	Intervention	Est. Study completion
NCT05911217	Ib	PDAC, CLDN18.2+	CLDN18.2 CAR-T (CT041), after adjuvant CHT	12/2026
NCT05620732	I	Advanced PDAC and gastric cancer, CLDN18.2+ (high or moderate expression)	CLDN18.2 CAR-T	10/2028
NCT04581473	Ib/II	Advanced gastric and GEJ (≥2 prior line) and PDAC (≥1 prior line), CLDN18.2+	CLDN18.2 CAR-T (CT041)	06/2038

CHT: chemotherapy; CLDN18.2: claudin 18.2; CTLA-4: cytotoxic-T-lymphocyte-antigen 4; GEJ: gastroesophageal junction; GPC3: glypican-3; GUCY2C: Guanylyl cyclase 2C; HLA-A*02: human leukocyte antigen A-02; IL21: interleukin-21; NKG2D: Natural Killer Group 2 Member D; PDAC: pancreatic ductal adenocarcinoma; PD-1: programmed death protein-1; PD-L1: programmed death ligand-1; scfv: single chain variable fragment; UCAR-T: universal CAR-T; huCAR-T: humanized CAR-T.

signal peptide and the single-chain variable fragment (scFv) of an antigen-specific antibody which is responsible for the antigen recognition and binding [151]. The transmembrane domain is made up of a hydrophobic α-helix that crosses the membrane ensuring surface expression and receptor stability [151]. The intracellular domain, whose most typical component is represented by CD3ζ domain, provides conformational changes activating downstream protein signaling [151].

Second-generation CARs also include co-stimulatory receptors such as CD28 or CD137 to give T cells cytolytic ability, while third generation ones involve an additional intracellular domain such as CD134 or CD137 [148,151,152]. Later generations CARs were developed to circumvent immunosuppressive TME by including domains to enhance T-cell response and CARs efficacy. T cells redirected for universal cytokine-mediated killing (TRUCKs) or "armored" CARs are engineered to induce interleukin expression, like IL-12 (fourth-generation CARs), while fifth-generation of CARs include receptors to respond to specific cytokines, like IL-12 receptor beta (IL-12RB) domain [151].

The most common targets of CAR-T cells in patients with PDAC are mesothelin, claudin 18.2 (CLDN18.2), HER-2, CD24, CD133, carcinoembryonic antigen (CEA), prostate stem cell antigen (PSCA), MUC-1, and the epidermal growth factor receptor (EGFR) [8].

Mesothelin is highly expressed on mesothelioma, lung, pancreas, breast and ovarian tumor cells, but lowly expressed on mesothelial cells [153]. Because of its overexpression on PDAC cells, mesothelin is the most common CAR-T cell target [153]. Despite a manageable safety profile, results from phase I trials were disappointing [154], partly due to short CARs persistence. Neither lymphodepletion [155] nor the co-administration of anti-CD19 CAR-T cells, aimed at depleting B cells potentially responsible for CAR T-cell clearance, proved effective in enhancing CAR T-cell persistence [156]. Strategies to extend CARs persistence include the use of human anti-mesothelin CAR-T in lieu of those with ScFv of murine origin to avoid immune-mediated elimination of CARs (NCT03323944) [157].

CLDN18.2 is a protein normally expressed in tight junctions of epithelial cells, thus not accessible on the cell surface, which is abnormally activated during malignant transformation of pancreatic cells and expressed in up to 94 % of PDACs [158–160]. CLDN18.2 is the target of the anti-CLDN18.2 antibody, zolbetuximab, which is being investigated in association with gemcitabine and nab-paclitaxel as first-line treatment in patients with CLDN18.2-positive metastatic PDAC (NCT03816163) [161]. In the context of CAR-T therapy, a study evaluated two patients with metastatic PDAC treated with CLDN18.2 CAR-T cells after failure of standard therapies: one patient achieved PR and the other CR of the lung metastases, with a registered increase of $CD8^+$ and Tregs and reduction of $CD4^+$ and B cells in the peripheral blood [162]. Given the potential of ACT directed against these targets, several other trials of CAR-T cells targeting mesothelin and CLDN18.2 are currently ongoing (Table 3).

CD24 is a marker of pancreatic cancer stem cells which are responsible for the tumor growth, are able to self-renew and to create a differentiated progeny. Hence it is another appealing target for CAR-T cell-based therapies [163]. Based on these findings, a pre-clinical study in pancreatic cancer xenograft models has shown that CAR-T cells redirected to HER-2 or CD24 cause tumor shrinkage and prolongation of mice survival [164]. T cells targeting CD24 slow down the tumor growth and prolong the survival of mice even if CD24 is not expressed by all cells, differently from HER-2 CAR-T cells which prolong the survival of mice when most cells express the target. Moreover, in a phase I trial enrolling patients with advanced biliary tract or pancreatic cancer who received CAR-T cells targeting HER-2, 2 out of 2 patients with PDAC achieved SD, with median PFS of 5.3 and 8.3 months [165]. In a phase I trial of an anti-CD133 (another stem cells marker, overexpressed in various solid tumors) CAR-T cells, one of the 7 PDAC patients enrolled with metastatic progressive disease experienced a 40 % tumor reduction after first infusion, which lasted for 4 months [166].

As EGFR is overexpressed in advanced PDAC and is further related to poor prognosis [167], a phase I trial of anti-EGFR CAR-T cells was led in 14 patients with metastatic PDAC, and has demonstrated a DCR of 85.7 %, 4 PRs and 8 SDs (lasting 2–4 months), but with an overall median PFS of 3 months and median OS of 4.9 months [168].

Unlike hematologic malignancies, ACTs have failed to date to demonstrate breakthrough results in solid tumors. This can be related to several reasons which include: a) the limited expansion and persistence of CAR-T cells *in vivo*; b) the greater heterogeneity of solid tumors when compared to hematologic malignancies; c) the unfavorable TME of solid tumors, including PDAC, because of the hampered migration of immune cells, including CAR-T cells, to come in contact with tumor cells to exert their anti-tumor activity; d) the depletion of necessary substances to the CAR-T cells function; e) the hypoxic environment hostile to immune cells survival; f) the expression of inhibitory molecules like PD-1 [169]. Hence, to increase activity of these treatments, CAR-T cells against PD-1/PD-L1 have been developed, demonstrating increased CAR-T cells persistence, tumor regression, and reduced Ki-67 tumor cells index in treated pre-clinical models [170].

Intratumoral delivery via implantable scaffolds of CARs can be leveraged to overcome the limited tumor infiltration and persistence of adoptively transferred T cells following systemic administration. In a preclinical model using orthotopic *LSL-Kras* $^{G12D/+}$*Trp53* $^{fl/+}$*Pdx1-Cre* (KPC) allografts, a disk-shaped scaffold composed of alginate and lipid-coated silica microspheres was surgically implanted to deliver CAR-T cells in combination with a STING agonist [171]. This platform enabled high local concentrations of tumor-reactive T cells and immunostimulants, transforming the immunosuppressive tumor microenvironment and resulting in complete tumor clearance in a subset of treated mice. The device functioned as a self-contained immune activation site, using the tumor-derived debris as an *in situ* source of antigens, thereby enhancing both innate and adaptive immune responses. Such implantable scaffolds improve CAR-T cell viability, localization, and activation, offering a modality for treating otherwise non-immunogenic tumors like PDAC worth of further investigation. CARs equipped with enzymes, like hyaluronidase, are of particular interest for PDAC, as they demonstrated deeper infiltration also within solid tumors [172]. Furthermore, SPIONs have been functionalized with MHC-I/peptide complexes and anti-CD28 antibodies to act as artificial APCs capable of expanding antigen-specific $CD8^+$ and $CD4^+$ T cells *ex vivo*, with enhanced efficacy upon magnetic field-induced clustering [173].

Immunotherapy with Vaccines in PDAC.

Tumor vaccines represent another widely studied strategy of immunotherapy aimed at disrupting the immune tolerance and increasing the anti-tumor immune response in PDAC. Compared to CAR-T cells which target antigens on the cell surface (either private or shared), vaccines act through the recognition of tumor associated antigens (TAAs). First, patient's tumor cells are cultured and specific antigens are selected, then peptides are artificially synthesized and combined with adjuvants to increase the vaccine immunogenicity [174]. After the administration of a vaccine, selected TAAs are presented by APCs to activate cytotoxic T lymphocytes against the tumor [174].

Clinical trials regarding vaccine therapies in PDAC did not show encouraging results, but studies demonstrated an increase in the specific T cells response against the tumor [8].

Based on previous interesting data regarding PDAC allogeneic vaccine [175], a neoadjuvant trial has been performed with the initial randomization of PDAC patients to receive GM-CSF-secreting whole cell pancreatic cancer vaccine (GVAX) with low dose of cyclophosphamide (arm A), or the same combination in addition to nivolumab (arm B) [176]. The immune analyses on tumor samples demonstrated that the increase in $CD137^+$ activated T cells in the tertiary lymphoid structures (TLSs) of the TME, correlated with cytotoxic effector cells signature and was associated with improved OS rate at 2 years [177]. However, $CD137^+$ T cells had low density and did not infiltrate the tumor [176]. Based on the potentially interesting results in pre-clinical models [178], in the arm C of the study the CD137 agonist urelumab was added to GVAX with low-dose of cyclophosphamide and nivolumab. Patients in arm C showed a significant increase in $CD8^+$ and $CD137^+$ T cells in resected tumor specimens (compared to arm B), and demonstrated a numerically longer, though not statistically significant likely because of the small sample size, median OS (35.6 months in arm C vs 23.6 months in arm A and 27.0 in arm B) and DFS (33.5 months in arm C vs 13.9 months in arm A and 15.0 months in arm B) [176]. Interestingly, median DFS (but not OS) of arm C resulted increased compared to standard of care [179]. However, it should be noted that 70 % of patients in arm C have received FOLFIRINOX as adjuvant therapy, while most of patients in arm A and arm B have received gemcitabine plus capecitabine, suggesting a potential imbalance between arms [176].

Exosomes ability to carry TAAs also makes them attractive as platforms for cancer vaccines, as they can enhance antigen presentation and prime $CD8^+$ T cells responses via DCs [180]. A recent *in vivo* study demonstrated that combining conventional agents, like all-trans retinoic acid (ATRA), sunitinib, and gemcitabine, with DC-based vaccines loaded with PDAC-derived exosomes significantly inhibited metastatic progression and prolonged survival in murine models, due to a more robust recruitment and activation of effector T cells [181].

An innovative and promising tailored strategy recently investigated regards the use of mRNA vaccines. A recent phase I study evaluated the administration of cevumeran, an individualized uridine-based mRNA lipoplex vaccine targeting patient's specific PDAC somatic mutations, in combination with atezolizumab and mFOLFIRINOX as adjuvant treatment in unselected patients with resectable PDAC [182]. The study demonstrated that 50 % of vaccinated patients developed a high amount of long-lasting tumor-specific CD8 + T cells. The immunologic responders exhibited a longer median recurrence-free survival (mRFS) at 18 months follow-up, compared to non-responder patients without CD8 + T cells detection (mRFS not reached versus 13.4 months, $p = 0.003$) [182]. A subsequent longitudinal analysis at 3.2 years revealed that almost all $CD8^+$ T cell clones were newly generated after vaccination and the PhenoTrack plot detected that 86 % of them persisted at 3 years after vaccination [183]. Moreover, vaccine-induced CD8 + T cells presented an estimated life-span of 7.7 years and may target PDAC recurrences [183]. Crucially, the follow-up analysis confirmed the better mRFS in patients who developed vaccine-induced T cells compared to those who did not ($p = 0.007$). Indeed, out of eight responder patients, the two who recurred exhibited reduced clonal T cell expansion after vaccination [183]. A randomized clinical trial evaluating cevumeran plus atezolizumab and mFOLFIRINOX vs mFOLFIRINOX as adjuvant treatment in patients with resected PDAC is currently ongoing (NCT05968326).

These findings provide evidence that the combination of mRNA neoantigen vaccines combined with ICIs may generate active and durable immunity. To further explore safety, immunogenicity and clinical outcomes, a phase I trial (NCT06496373) is currently ongoing evaluating mRNA personalized vaccines in combination with anti PD-1 as adjuvant treatment for patients with resected PDAC who are ineligible for surgery.

Nanoparticles can be employed to facilitate the targeted delivery and intratumoral penetration of mRNA vaccines, thereby enhancing their local immunogenic effects (nanovaccines) [184]. A prominent example is V941, a nanoliposomal mRNA-based vaccine specifically designed to target common KRAS mutations (G12D, G12V, G13D, G12C). Through liposomal encapsulation, V941 enables efficient delivery of mRNA encoding mutant KRAS epitopes, which are subsequently processed by APCs to prime cytotoxic $CD8^+$ and helper $CD4^+$ T cells, thereby initiating a mutation-specific antitumor immune response. V941 is currently being evaluated in combination with pembrolizumab to enhance immunogenicity in KRAS-driven malignancies, including PDAC (NCT03948763).

Effective conditioning of the TME is critical to enhancing the efficacy of cancer vaccines in solid tumors, particularly in PDAC. In this context, smart delivery systems can be exploited to favorably modulate the TME [185]. Nanoparticles-mediated siRNA knockdown of ICIs like PD-1/PD-L1 or indoleamine 2,3-dioxygenase (IDO) significantly enhanced the anti-tumor effect of co-administered vaccines and restored CD8 + T cells function [186,187]. Injectable devices, such as hydrogels, can locally deliver cytokines to modulate immune cells activity in the TME, avoiding toxicity associated with systemic administration. One example is the injection of thermosensitive hydrogels releasing GM-CSF to recruit DC and increase vaccine efficacy [188]. In addition, SPIONs have been used as carriers of immune adjuvants such as CpG oligodeoxynucleotides, STING agonists, and PD-L1 siRNA, improving DC activation and priming tumor-specific CD8 + T cell responses [189–191].

Overall, vaccines demonstrated some preliminary but encouraging signal of activity in PDAC and shed some light on the obstacles to an effective anti-tumoral immune response in PDAC. By exploiting mRNA technology, combined with modern delivery technologies and TME conditioning strategies, neoantigen vaccines could represent a groundbreaking personalized approach to stimulate CD8 + T cells specific for patient's tumor antigens, even in a poorly immunogenic tumor such as PDAC.

5. The ambivalent role of microbiota in PDAC

The microbiota has been recently extensively studied for its role in tumorigenesis and response to cancer therapies, and for its interaction with immune host cells on the mucosal surfaces [192–194].

There is evidence to suggest that germ-free or antibiotic-induced bacteria depletion can impair response to cancer therapies, while the microbial modulation through responsive fecal microbiota transplantation (FMT) could enhance the antitumor response [195–199].

Compared to healthy controls, the gut microbiota diversity is lower in patients with colorectal, breast and pancreatic cancer [200–202]. In immunotherapy-treated melanoma patients, responders had a greater diversity in their fecal microbiota samples than non-responders [203]. In patients with advanced melanoma who have lost response to immunotherapy, FMT from responders, in association to anti PD-1 reinduction therapy, restores responses through changes in the gut microbiota and reprogramming of TME [204,205]. The analysis of fecal metagenome of 1,359 patients with melanoma, NSCLC, renal cell and hepatocellular carcinoma has identified that selected microorganisms were associated to ICI responses independently of clinical features, thus representing a potential predictive factor [206]. Conversely, the use of antibiotics in

cancer patients treated with ICIs was associated with a detrimental effect in OS and PFS, possibly due to reduction of alpha-diversity or ablation of favorable microbiota [207–210].

In recent years human microbiota has emerged as a dual modulator of tumor behavior, both suppressing and promoting tumor growth. The ambivalent role of gut microbiota could be detectable even within a single species. *Fusobacterium nucleatum* (*Fn*) indeed plays a role in progression, metastatisation and chemoresistance in colorectal cancer (CRC) [211–213], but it enhances responses to anti-PD-1 therapy in germ-free MSS CRC mice receiving FMT from patients with *Fn*-high MSS CRC [214]. The improved efficacy of anti-PD-1 therapy has been attributed to *Fn*-mediated production of butyric acid, which inhibits histone deacetylases (HDACs) 3 and 8 in CD8 + T cells. This inhibition leads to the epigenetic activation of the Tbx21 transcription factor, resulting in reduced PD-1 expression on CD8 + tumor-infiltrating lymphocytes (TILs) which finally reactivate intratumoral cytotoxic CD8 + TILs. Conversely, mice colonized with an *Fn* strain lacking a key gene involved in butyric acid production did not exhibit improved responses to anti-PD-1 therapy [214].

The ambivalent role of microbiota is evident in PDAC: some bacteria, particularly in the oral cavity, have been associated with increased risk, such as higher antibody titers against *Porphyromonas gingivalis* linked to a two-fold greater PDAC risk [215], whereas others, especially intratumoral species, may correlate with improved survival [216].

An unfavorable intra-tumoral microbiota contributes to the immunosuppressive TME, which is the most relevant cause of immunotherapy resistance in PDAC. In keeping with the detrimental effect of pancreatic microbiota, germ-free models of PDAC did not show tumor spread and the use of antibiotics delayed the disease progression, whilst the bacteria transplant from mice with PDAC promoted tumorigenesis [216]. The ablation of unfavorable microbiota resulted in MDSCs reduction and polarization of tumor macrophages into M1 phenotype, it enhanced the differentiation of Th1 cells into CD4 + and CD8 + and promoted the efficacy of ICIs through the up-regulation of PD-1 expression [216]. The immunosuppressive environment in PDAC is triggered by components (lipopolysaccharides and flagellins) of the microbiome which bind toll-like receptor (TLR) 2 and 5 in monocytic cells promoting tolerogenic macrophages activity. Conversely, the immunosuppressive feature is lacking in macrophages with TLR signaling deficiency [216], suggesting that targeting TLR or modulating microbiome could represent a strategy to enhance the efficacy of treatments, including immunotherapy, in PDAC.

Similarly, the administration of oral antibiotics with consequent depletion of gut microbiota led to a reduction in subcutaneous tumor burden in murine PDAC implants [217]. Notably, the anti-tumor effect mediated by the gut microbiota ablation was absent in mice lacking mature T lymphocytes (Rag1-knockout), thus suggesting that the anti-tumor response requires the adaptive immunity involvement [217]. Gut microbiota depletion increases Th1 and Tc1 cells in TME, and interferon-gamma (IFN-ɣ) secreting T-cells, resulting in decrease of IL-17a and IL-10 originating pro-tumorigenic T-cells [217]. IL-17 plays a role in PDAC development, progression, and resistance to immunotherapy by promoting an immunosuppressive tumor microenvironment [218–221]. While IL-17RA signaling helps maintain gut homeostasis, its disruption leads to dysbiosis [222]. In preclinical models, genetic deletion of IL-17RA reduced tumor growth, but pharmacological blockade of IL-17/IL-17RA alone showed limited efficacy. However, when combined with immune checkpoint inhibitors, it produced a synergistic antitumor effect [221]. IL-17RA deletion also caused microbial imbalance, which enhanced Th17 cell activation and IL-17 production in distant organs such as the pancreas [223]. IL-17 promotes tumor growth by increasing DUOX expression in tumor cells, leading to ROS production [223]. Notably, combining IL-17RA inhibition with antibiotics more effectively suppressed tumor progression, suggesting that microbiota dysregulation, rather than IL-17 signaling alone, is a key driver of tumor promotion [223].

Oral and gut microbiota in PDAC.

The role of oral microbiota is well recognized in PDAC tumorigenesis [224]. In particular, high levels of anti-*Porphyromonas gingivalis* antibodies were related to a 2-fold increase in PDAC risk (compared to patients with lower levels) and to higher orodigestive cancer mortality [215,224,225]. Indeed, oral bacteria are able to degrade arginine through peptidyl arginine deiminase production thereby leading to *TP53* and *KRAS* mutations, which are the most common mutated genes in PDAC and are associated with poor prognosis [226]. Moreover, an enrichment of oral bacteria (including *Fn*) has been found in intraductal papillary mucinous neoplasms with high grade dysplasia and the detection of *Fn* in PDAC samples (approximately 8.8 %) was independently associated with worse prognosis, potentially representing a prognostic biomarker in PDAC [227,228].

Given its rising relevance in various aspects of solid tumors, some studies have focused on characterizing also gut microbiota in patients with PDAC [202,229]. In both Asiatic and Western patients there is a low alpha diversity of gut microbiota of PDAC patients compared to healthy controls, with a reduction in beneficial bacteria and an increase in pathobionts. *Firmicutes* and butyrate-producing bacteria (e.g., *Lachnospiraceae*, *Ruminococcaceae*, *Clostridiaceae*) decrease, while pro-inflammatory genera like *Veillonella*, *Akkermansia*, and *Klebsiella* increase [202,229]. In addition, PDAC cases exhibit an increase in lipopolysaccharide (LPS)-producing bacteria and depletion of Firmicutes and Proteobacteria, as well as *Bifidobacteria* and butyrate-producing bacteria (e.g., *Lachnospiraceae*, *Ruminococcaceae*, *Clostridiaceae*), suggesting a role in chronic inflammation and tumor progression.

Intratumoral microbiota and mycobiota as PDAC risk factors.

Among the pancreatic lesions, the proportion of bacterial DNA seems to be related to the pathological degree: it increases from 33 % in pancreatic cyst to 59.6 % in IPMN and 81.5 % in PDAC, with *Porphyromonas gingivalis, Treponema denticola, P. stomatis, P. acidifaciens, P. endodontalis*, and *Filifactor alocis* increasing as PDAC progresses, whereas putative probiotic bacteria strains decline.

[227,230]. The gut microbiome of patients with PDAC is enriched in *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* compared to those of healthy subjects. High levels of *Proteobacteria* are also detected in the intra-pancreatic microbiota and are associated with advanced stage of PDAC [216]. *Proteobacteria* are present in the duodenum [231], where the outflow of the pancreatic ducts converges, suggesting a potential source for translocation. In support to this hypothesis it has been demonstrated that patients who undergo instrumental procedures of the pancreatic duct have higher levels of bacteria [232].

Furthermore, fungal mycobiota is also implicated in PDAC development through the bond of mannose binding lectin (MBL) to the glycans of the fungal surface, which leads to the activation of the complement cascade. The fungi migrate from the gut lumen to the pancreas, with a concentration in tumor tissue that is 3,000 times higher than in healthy tissue, both in pre-clinical models and humans. Indeed, the mycobiota of PDAC is enriched of *Malassezia* species. Mycobiome depletion prevented tumor growth while the reintegration of *Malassezia* increased oncogenesis, an effect which was not observed with other fungal species [233].

Microbiota as a modulator of therapeutic response in PDAC.

Available evidence points to a pro-tumorigenic role for *Proteobacteria* in PDAC. *Proteobacteria* have been hypothesized to be implicated in resistance to gemcitabine being able to metabolize it into its inactive form through cytidine deaminase [232]. Nonetheless, since there were a few *Proteobacteria* scattered within the tumor, the potential contribution to gemcitabine degradation seems not sufficient to produce a clinically relevant effect in humans. Despite this, two retrospective studies demonstrated that the addition of antibiotics to gemcitabine-based chemotherapy treatment improves OS and PFS in advanced PDAC patients as well as in patients with advanced solid tumors of different histology types [234,235]. Of interest, another retrospective study on

patients with PDAC, has shown that the benefit of adding antibiotics to chemotherapy was found only in advanced disease, as opposed to resectable disease, and only in patients receiving gemcitabine, not in those who received 5-Fluorouracil (5-FU)-based chemotherapy [236].

Further contributing to therapeutic resistance, *Proteobacteria* seem to be capable of adapting under gemcitabine pressure, modulating its efficacy through enhancing (thus protecting cancer cells) or reducing (making cancer cells more sensitive) drug degradation depending on specific genetic mutations involved. Notably, the most frequent mutation concerns NupC, a nucleoside transporter responsible for mediating drug entry within the bacterium, thus enhancing antitumor cytotoxic activity by increasing extracellular concentration of gemcitabine [237].

Furthermore, the indole-3-acetic acid (3-IAA) produced by specific gut bacteria (*Bacteroides*) may influence patients' response to chemotherapy, being higher levels of 3-IAA detected in responders to chemotherapy compared to non-responder patients. In PDAC models, FMT from responder patients, oral administration of 3-IAA, and specific dietary intervention with tryptophan (a 3-IAA precursor), increase efficacy of chemotherapy. A crucial role in this effect is played by myeloperoxidase produced by neutrophils, which oxidises 3-IAA, that in combination with chemotherapy results in ROS production and autophagy down-regulation in cancer cells, thus further sensitizing cells to the action of cytotoxic drugs. In clinical setting, a correlation between the 3-IAA concentration and the efficacy of chemotherapy in two independent cohorts of patients with PDAC, support specific dietary intervention during chemotherapy treatment as a complementary strategy [238].

Despite the global dismal prognosis, a small subset of patients with PDAC are long-term survival (LTS), surviving longer than 5 years after diagnosis [239]. LTS patients show a higher alpha diversity of intratumoral microbiota and immune activation compared to short-term survival patients (STS), and longer OS (median OS: 9.66 years vs 1.66 years). In particular, *Pseudoxantomonas*, *Saccharopolyspora*, *Streptomyces*, and *Bacillus Clausii* are specifically enriched in the tumor microbiome of LTS patients. The intratumoral microbiota landscape in mice undergoing FMT change according to the donor received (LTS, STS or healthy controls) and tumor growth is specifically reduced only in mice who receive FMT from LTS donors. Despite only a small fraction of the species from the donor directly translocate in the tumor (<5%), a shift in intratumoral microbiota after FTM is observed. Mice treated with antibiotics following FMT from LTS donors show larger tumors than untreated mice, thus suggesting that intratumoral bacteria sustain and mediates the anti-tumoral activity [240]. Indeed, mice receiving transplant from LTS exhibit higher IFN-ɣ and IL-2 levels and increased CD8 + T cells infiltration and T cells activation compared to those with FTM from STS and healthy donors. Moreover, CD8 + T cells depletion using antibodies in mice transplanted from LTS samples, revert the antitumoral effect of FMT from LTS, thus supporting the CD8 + T cells role. Conversely, an increase in CD4 + FOXP3 + cells and MDSCs infiltration has been found in mice transplanted with STS samples [240]. All taken together, available evidence suggests that the composition of gut microbiota may influence PDAC progression by immune effects.

Supporting the rationale to accurate discriminate between protective and tumor-promoting bacteria in PDAC, recent findings highlight the potential risk of tumor progression through FMT. Indeed, mutant KRAS G12D transgenic mice with induced pancreatitis, undergoing FMT from PDAC patients, exhibited acceleration of invasive cancer development from premalignant lesions [241]. Microbiota analyses detected altered composition with overall reduction in microbial diversity with a significative enrichment of *Actinobacteriota* and *Bifidobacterium* and a depletion of short chain fatty acid (SCFA) producing bacteria, usually associated with anti-inflammatory functions [241]. Microbiota derived from PDAC patients promote tumorigenesis in susceptible host, therefore, despite the theoretically intriguing preclinical evidence of FMT to be able to modulate the microbial ecosystem potentially restoring a favorable TME in PDAC, its clinical applicability currently remains entirely experimental. The recent findings, in fact, underscore the need not only to profile and carefully select donors but also to further investigate microbiota-host interactions before transplantation.

Table 4
Ongoing studies about microbiome in pancreatic ductal adenocarcinoma. (Clinicaltrials.gov "pancreatic cancer", "microbiota", "microbiome"; last access: 28/02/25).

Study	Intervention	Est. Study completion
NCT06319755	Profiling gut microbiota following pancreatic surgery	02/2025
NCT06800469	Microbiota analysis in patients undergoing surgery for PC	07/2025
NCT06655233	Intratumoral microbiome profile in PDAC	05/2026
NCT06411470	Analyze GI microbiota and serum metabolites in patients with PDAC and chronic pancreatitis	12/2025
NCT04638751	Analyze stool and blood samples of advanced NSCLC, TNBC, CRC and PDAC to evaluate the effect of gut microbiomes on treatment response	12/2025
NCT06436976	The effect of probiotics in advanced colorectal or PDAC undergoing Oxaliplatin-based CHT	12/2025
NCT06381882	Human microbiome after pancreatic resection for presumed PC or periampullary malignancy	11/2026
NCT04975217	FMT in resectable PDAC	12/2026
NCT06595160	Association of plant-based diet and gut microbiome in PDAC response to neoadjuvant therapy	08/2027
NCT06393400	FMT and gem + nab-paclitaxel in untreated advanced PDAC	02/2028
NCT05462496	Antibiotics and pembro following CHT in resectable PDAC	04/2028

CHT: chemotherapy; FMT: fecal microbiome transplantation; gem: gemcitabine; pembro: pembrolizumab; mPDAC: metastatic pancreatic ductal adenocarcinoma.

Considering the relevance and the implications of gut microbiota for PDAC treatment, several studies about microbiome profiling, FMT, and dietary interventions are currently ongoing in patients with PDAC (Table 4).

A recently completed (yet unpublished) prospective study analyzed gut and oral microbiome in addition to the whole transcriptome in gastrointestinal cancer patients (including PDAC) to establish a correlation between the biological profile and tumor type, treatment efficacy and toxicities (NCT05462314).

Currently available evidence suggests that the oral, intratumoral and intestinal microbiome plays a strong ambivalent role in PDAC pathogenesis and progression, and it may represent a predictor of survival. The interplay between the tumor microbiome and immune cells also contributes to immune evasion and resistance to therapies. To this end, emerging approaches such as FMT, oral administration of selective antibiotics, and dietary modifications and supplements, represent investigational strategies that warrant further investigations concerning microbial profiling.

Innovative therapeutic modulation of microbiota through genetically engineered systems.

A pioneristic approach to overcome the therapeutic resistance in PDAC putatively involves the delivery of engineered bacteria directly in the intratumoral space to localize their therapeutic action, limiting systemic toxicity. Promising results were obtained with *Clostridium novyi*-NT (*C. novyi*-NT), an attenuated anaerobic bacterium derived from wild-type *Clostridium novyi* by the removal of the α-toxin gene, thereby eliminating its virulence while preserving its ability to germinate in hypoxic environments [242]. It was administered as spores via intratumoral injection and evaluated in both preclinical models as well as in a human patient with advanced non-pancreatic cancer, demonstrating tumor reduction [242]. An innovative specific tumor-targeting approach involves the probiotic strain *E.Coli* Nissle (EcN) 1917 engineered to

deliver pore-forming toxin directly in cancer cells, thus sparing normal tissues. The expression of Theta toxin is induced by acyl-homoserine lactone (AHL) inducible promoter, and the gene is encoded on a plasmid stabilized by a toxin-antitoxin system (AxeTxe) to prevent plasmid loss due to its cytotoxic burden. Additionally, a luminescent operon (luxCDABE) was integrated to enable *in vivo* imaging of bacteria localization. In preclinical models of PDAC this approach, by modulating immune cells activity in TME, increases OS compared to standard chemotherapy [243]. Furthermore, an emerging strategy with great potential to overcome the dense stromal barrier typical of PDAC is exemplified by *Salmonella typhimurium* engineered to express the pore-forming cytolysin A (ClyA). Once localized in tumor tissues it releases ClyA causing stroma disruption and immune cells enhancement, thus resulting in reduced tumor growth in subcutaneous xenograft and orthotopic PDAC models. The *S. typhimurium* strain is engineered with ΔppGpp mutation, which disables ppGpp synthesis and attenuates pathogenicity. This genetic modification increases the 50 % lethal dose (LD_{50}) by 10,000- to 1,000,000-fold, while preserving bacteria's ability to selectively colonize tumor tissues [244].

The recent advancements in synthetic biology have also led to the development of biohybrid-engineered bacteria which represent a promising therapeutic strategy combining bacteria with engineered payloads (such as drugs or targeting ligands) to enhance targeted therapeutic efficacy.

The intraperitoneal administration of *Listeria monocytogenes* engineered to deliver 32-Phosphorus and the injection of conjugating aptamer-drug complexes to *Salmonella typhimurium* VNP20009 in PDAC mice resulted in marked tumor reduction and immune cells infiltration [245,246]. Moreover, a novel approach involves the conjugation of photosensitizer croconium molecules with *Escherichia Coli* MG1655 modified with Cadherin-17 (CDH17) nanobodies. CDH17 is a cell adhesion molecule which is overexpressed in gastrointestinal tumors, including PDAC. The biohybrid bacterial system, homed to CDH17 positive tumors, enables photothermal therapy (PTT) limiting tumor growth. In a syngeneic murine colorectal model, PTT acts reprogramming TME activating STING pathway in TAMs through releasing of bacterial nucleic acids, leading to macrophages infiltration and production of Type I IFNs, thus promoting the anti-tumor immunity, without significant T-cells recall. This study also performed the potential of synergistic therapies by the combination of bacteria-mediated PTT with a CD47 nanobody resulting in tumor eradication and increased survival of a pancreatic cancer model in immunocompetent mice; in a contest where the addition of PD-1 antibody is failing probably due to the lack of T-cells infiltration [247]. Another intriguing approach integrates PDAC TME reshaping to microbiota modulation, based on an engineered probiotic nanosystem. *Clostridium Butyricum* (CB), a probiotic bacterium suitable for hypoxic environments, drug-loaded liposomes via a matrix metalloproteinase-2 (MMP)-responsive peptide, ensures stimulation within the PDAC TME where MMP-2 is overexpressed. This probiotic nanosystem releases vactosertib, a TGF-beta receptor inhibitor, which inactivates PSCs thus reducing ECM production, so the disrupted stroma barrier provides easier penetration of Gemcitabine within the tumor. Moreover, CB reshapes intratumoral microbiota by suppressing ɣ-*Proteobacteria*, mitigating their drug degradation. Furthermore, this probiotic nanosystem, besides promoting immunogenic cells death, increases effector immune cells infiltration [248]. A further promising role of biohybrid systems is their ability to potentiate immunotherapy response. Upon oral administration, the probiotic Lactobacillus rhamnosus GG functionalized with a gallium-polyphenol network (LGG@Ga-Poly), disrupts tumor-promoting *Proteobacteria* and microbiota-derived LPS in PDAC, through the interference of bacteria metabolism gallium-mediated. The chitosan nanocoating represents the outer protective layer which shields the probiotic system from acid exposure degradation and stabilizes Ga^{3+} preventing premature dissociation in GI tract, thereby ensuring that LGG@Ga-Poly reaches PDAC TME intact via gut-pancreas axis. The intratumoral microbiota modulation leads to TLR signaling reduction which in turns downregulate PD-L1 and IL-1β. In addition, the expansion of cytotoxic CD8 + T cells enhances the tumor sensitivity to ICIs. Notably, in preclinical models, the combination of LGG@Ga-Poly to ICIs restrains tumor development and boosts the therapeutic efficacy of immunotherapy [249].

AI and ML applications for microbiome profiling in PDAC.

AI-based technologies, including ML, have provided new frameworks to examine PDAC associated microbial composition and its implications for cancer diagnosis and prognosis. A recently developed ML model is indeed capable of diagnosing PDAC through the analysis of serum microbiome-derived extracellular vesicles [250]. Among the models developed, the deep neural networks (DNN) demonstrated high diagnostic performance achieving an area under the curve (AUC) of 0.959 at the phylum level (3 markers: *Verrucomicrobia, Actinobacteria and Proteobacteria)* and 0.961 at genus level (11 markers) [250]. An additional approach leveraging ML integrating gut microbiome profiling and CA 19–9 biomarker has been recently developed to improve diagnostic accuracy in PDAC [251]. Expanding this strategy, an integrative model called Multi-Omics Co-training Graph Convolutional Networks (MOCO-GCN) combining gut microbiota and exposome, meaning as all exposures including lifestyle factors and host diseases, achieved high diagnostic accuracy in PDAC development. Mediation analyses also highlighted the causal association between microbiome and exposome in PDAC development, revealing *Fusobacterium hwasookii nucleatum*, known for its pro-inflammatory properties, as a potential microbial mediator linking rheumatoid arthritis and PDAC onset [252].

In parallel ML techniques have been also exploited to uncover whether specific gut microbiota patterns correlate with metastatic and non-metastatic PDAC. The analysis of fecal microbiota samples performed through the penalized logistic regression analysis (PELORA) and the random Forest algorithms revealed different microbial species according to disease stages. An overall enrichment in Gram-negative bacteria was found in metastatic compared to non-metastatic PDAC patients [253]. In particular, *Anaerostipes hadrus*, *Coprobacter secundus*, *Clostridium* sp. 619, *Roseburia inulinivorans*, *Porphyromonas* and *Odoribacter* had the most effective discriminatory power at genus level, while *Rhodospirillaceae, Clostridiaceae and Peptococcaceae* at the family level [253]. Notably, the link between *Fusobacterium* and *Porphyromonas* enrichment and risk of metastasis has been investigated, considering their previous mentioned association with PDAC. Consistent with prior findings, lower abundance of *Fusobacterium* correlates with decreased risk of metastasis, but unexpectedly higher abundance of *Porphyromonas* is associated with reduced risk [253]. Complementing this, a multi-omics ML framework integrating microbiome biomarkers and transcriptomic data demonstrated robust performance (AUC 0.815) in predicting recurrence and metastasis in PDAC patients, demonstrating strong prognostic potential [254].

Mounting evidence supports the dual role of microbiota as both a therapeutic target and an active modulator in reshaping PDAC TME. This dichotomy highlights the need to profile microbiota and investigate its role in tumorigenesis and cancer progression. The integration of the engineered microbial systems and ML-based microbial profiling further provides innovative therapeutic strategies and prognostic insights, offering new perspectives for risk stratification and outcome prediction in PDAC. The possibility of manipulating the microbiome to harness a specific intratumoral PDAC microbial profile associated with increased intratumoral CD8 + T cells and long-term survival in patients, represents an intriguing and putative innovative therapeutic avenue.

Theoretically, a favorable microbiota could be injected intratumorally via endoscopy with the aim of reshaping the TME, promoting the infiltration of effector T cells and tumor cell engagement, and thereby stimulating an antitumor immune response. Because currently available data suggest that there is a specific intratumoral microbiota composition associated with LTS condition in patients with PDAC [240], benefic bacteria strains selected by culturomics to modulate

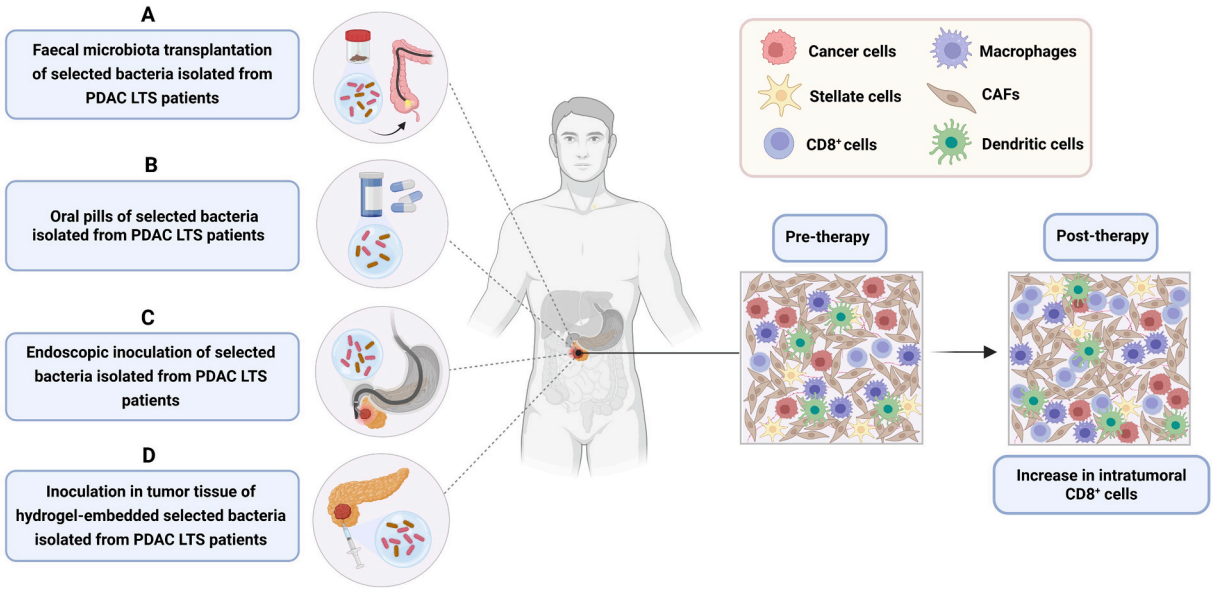


Fig. 2. Schema of a putative innovative immunotherapy strategy in pancreatic ductal adenocarcinoma (PDAC) to condition tumor microenvironment (TME). After selection of favorable bacteria strains, leveraging on data of long term survivors (LTS) microbiota can be transplanted or inoculated in different ways: canonical like faecal microbiota transplantation (**A**) or putative like selected bacteria encapsulated for oral administration (**B**), endoscopic intratumoral inoculation (**C**), and direct inoculation of selected bacteria embedded in hydrogels for controlled release (**D**).

Table 5
Comparative assessment of liposomes, exosomes, and engineered bacteria as drug delivery systems for pancreatic ductal adenocarcinoma. The table summarizes penetration capacity within the tumor microenvironment, drug loading strategies, and stage of clinical development based on preclinical and clinical studies in PDAC.

Delivery System	Penetration Efficiency	Drug Loading Capacity	Clinical Development Stage	References
Liposomes	- Moderate - Passive accumulation via EPR effect - Limited by PDAC stromal density	- High - Encapsulate both hydrophilic (core) and hydrophobic (bilayer) drugs	- Liposomal irinotecan (nal-IRI) approved for metastatic PDAC (phase III) - Additional phase I/II trials ongoing (NCT02551991, NCT03483038)	[89,111,112]
Exosomes	- Efficient uptake and biodistribution in preclinical PDAC models - Leverage endogenous vesicle trafficking with potential to access tumor cells across stromal barriers	- Moderate to low - Achieved via electroporation or endogenous sorting - Supports siRNA/protein delivery with favorable stability and low immunogenicity	- Preclinical and phase I (NCT03608631; iExosomes targeting KRASˆG12D)	[133,134]
Engineered Bacteria	- Very high - Actively penetrate and colonize hypoxic and necrotic tumor regions (e.g., *Clostridium novyi-NT*, *E. coli Nissle*)	- Variable - Genetically programmable for in situ drug production or secretion of therapeutic payloads	- Preclinical (e.g., *Listeria, Salmonella, Clostridium, E. coli Nissle 1917*) - No approved PDAC applications - Early clinical studies (e.g., LOAd703) ongoing	[146,242–246,248]

PDAC: pancreatic ductal adenocarcinoma, EPR: enhanced permeability and retention

intratumoral microbioma either indirectly through FMT or by direct intratumoral inoculation could be leveraged to actively promote immune TME shaping (Fig. 2). Also, local delivery devices, such as timed-release hydrogels or scaffolds, engineered to regularly release selective antibiotics, proteins or nucleic acids could foster favorable microbiota colonization, together with smart delivery systems, such as nanoparticles charged with ICIs siRNA or chemokines, that promote the TME reshaping. In this context, a wide range of delivery platforms, including synthetic nanoparticles, biological nanocarriers and engineered microbial systems, are being investigated to enhance therapeutic payload delivery and immune activation within the TME. Among these, liposomes, exosomes, and genetically engineered bacteria stand out as promising platforms with their key characteristics summarized in Table 5. Nonetheless, accurate preclinical investigations are required to confirm the validity of these approaches, which appear challenging but highlight the need for multimodal, multistrategy and multitechnology approaches in PDAC.

6. Conclusion

PDAC remains one of the most challenging cancers, given the limited and ineffective therapeutic strategies available to date. Immunotherapy, especially ICIs, have indeed failed to demonstrate survival benefit in PDAC, with the resistance primarily attributed to the extremely immunosuppressive TME consisting of a desmoplastic structure, acting as a steric barrier to drug penetration, and of immunosuppressive immune cells, which impair an effective immune response, in addition to a low immunogenicity mainly driven by a low TMB.

A deeper understanding of tumor biology, the development of intratumoral and systemic smart drug delivery systems to overcome delivery barriers, and targeted manipulation of the microbiota may represent key strategies to finally achieve the long-awaited breakthrough in PDAC outcomes.

Funding

PON “Ricerca e Innovazione” 2014–2020 (DM 1062 del 10/8/2021) (G.L.).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries - PubMed.
[2] L. Rahib, M.R. Wehner, L.M. Matrisian, K.T. Nead, Estimated Projection of US Cancer Incidence and death to 2040, JAMA Netw. Open 4 (4) (2021 Apr 7) e214708.
[3] S. Wang, Y. Zheng, F. Yang, L. Zhu, X.Q. Zhu, Z.F. Wang, et al., The molecular biology of pancreatic adenocarcinoma: translational challenges and clinical perspectives, Signal Transduct. Target. Ther. 5 (6) (2021 Jul) 249.
[4] O. Strobel, P. Lorenz, U. Hinz, M. Gaida, A.K. König, T. Hank, et al., Actual Five-year Survival after Upfront Resection for Pancreatic Ductal Adenocarcinoma: who Beats the odds? Ann. Surg. 275 (5) (2022 May) 962.
[5] Cancer of the Pancreas - Cancer Stat Facts. SEER.
[6] D.D. Von Hoff, T. Ervin, F.P. Arena, E.G. Chiorean, J. Infante, M. Moore, et al., Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine, N. Engl. J. Med. 369 (18) (2013 Oct 31) 1691–1703.
[7] T. Conroy, F. Desseigne, M. Ychou, O. Bouché, R. Guimbaud, Y. Bécouarn, et al., FOLFIRINOX versus Gemcitabine for Metastatic Pancreatic Cancer, N. Engl. J. Med. 364 (19) (2011 May 12) 1817–1825.
[8] R. Mukherji, D. Debnath, M.L. Hartley, M.S. Noel, The Role of Immunotherapy in Pancreatic Cancer, Curr. Oncol. 29 (10) (2022 Sep 23) 6864–6892.
[9] S. Bagchi, R. Yuan, E.G. Engleman, Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance, Annu. Rev. Pathol. 24 (16) (2021 Jan) 223–249.
[10] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Cancer 12 (4) (2012 Apr) 252–264.
[11] E. Hui, J. Cheung, J. Zhu, X. Su, M.J. Taylor, H.A. Wallweber, et al., T cell costimulatory receptor CD28 is a primary target for PD-1–mediated inhibition, Science 355 (6332) (2017 Mar 31) 1428–1433.
[12] T.U. Marron, A.E. Ryan, S.M. Reddy, S. Kaczanowska, R.H. Younis, D. Thakkar, et al., Considerations for treatment duration in responders to immune checkpoint inhibitors, J. Immunother. Cancer 9 (3) (2021 Mar 2) e001901.
[13] M. Maio, P.A. Ascierto, L. Manzyuk, D. Motola-Kuba, N. Penel, P.A. Cassier, et al., Pembrolizumab in microsatellite instability high or mismatch repair deficient cancers: updated analysis from the phase II KEYNOTE-158 study, Ann. Oncol. 33 (9) (2022 Sep) 929–938.
[14] A. Marabelle, D.T. Le, P.A. Ascierto, A.M. Di Giacomo, A. De Jesus-Acosta, J. P. Delord, et al., Efficacy of Pembrolizumab in patients with Noncolorectal High Microsatellite Instability/Mismatch Repair–Deficient Cancer: results from the phase II KEYNOTE-158 Study, J. Clin. Oncol. 38 (1) (2020 Jan 1) 1–10.
[15] V. Lee, A. Murphy, D.T. Le, L.A. Diaz, Mismatch Repair Deficiency and Response to Immune Checkpoint Blockade, Oncologist 21 (10) (2016 Oct) 1200–1211.
[16] X. Wang, G. Lamberti, A.D. Federico, J. Alessi, R. Ferrara, M.L. Sholl, et al., Tumor mutational burden for the prediction of PD-(L)1 blockade efficacy in cancer: challenges and opportunities, Ann. Oncol. 35 (6) (2024 Jun 1) 508–522.
[17] C.J. Halbrook, C.A. Lyssiotis, Pasca di Magliano M, Maitra a, Pancreatic Cancer: Advances and Challenges. Cell. 186 (8) (2023 Apr 13) 1729–1754.
[18] R. Abou Khouzam, J.M. Lehn, H. Mayr, P.A. Clavien, M.B. Wallace, M. Ducreux, et al., Hypoxia, a Targetable Culprit to Counter Pancreatic Cancer Resistance to Therapy, Cancers 15 (4) (2023 Jan) 1235.
[19] H.L. Kindler, D. Niedzwiecki, D. Hollis, S. Sutherland, D. Schrag, H. Hurwitz, et al., Gemcitabine Plus Bevacizumab Compared with Gemcitabine Plus Placebo in patients with Advanced Pancreatic Cancer: phase III Trial of the Cancer and Leukemia Group B (CALGB 80303), J. Clin. Oncol. 28 (22) (2010 Aug) 3617–3622.
[20] E. Van Cutsem, W.L. Vervenne, J. Bennouna, Y. Humblet, S. Gill, J.L. Van Laethem, et al., Phase III Trial of Bevacizumab in Combination with Gemcitabine and Erlotinib in patients with Metastatic Pancreatic Cancer, J. Clin. Oncol. 27 (13) (2009 May) 2231–2237.
[21] W.S. Lee, H. Yang, H.J. Chon, C. Kim, Combination of anti-angiogenic therapy and immune checkpoint blockade normalizes vascular-immune crosstalk to potentiate cancer immunity, Exp. Mol. Med. 52 (9) (2020 Sep) 1475–1485.
[22] J.H. Sussman, N. Kim, S.B. Kemp, D. Traum, T. Katsuda, B.M. Kahn, et al., Multiplexed Imaging Mass Cytometry Analysis Characterizes the Vascular Niche in Pancreatic Cancer, Cancer Res. 84 (14) (2024 Jul 15) 2364–2376.
[23] Y. Choi, K. Jung, Normalization of the tumor microenvironment by harnessing vascular and immune modulation to achieve enhanced cancer therapy, Exp. Mol. Med. 55 (11) (2023 Nov) 2308–2319.
[24] T. Stylianopoulos, R.K. Jain, Combining two strategies to improve perfusion and drug delivery in solid tumors, Proc. Natl. Acad. Sci. 110 (46) (2013 Nov 12) 18632–18637.
[25] T. Zhao, H. Ren, L. Jia, J. Chen, W. Xin, F. Yan, et al., Inhibition of HIF-1α by PX-478 enhances the anti-tumor effect of gemcitabine by inducing immunogenic cell death in pancreatic ductal adenocarcinoma, Oncotarget 6 (4) (2014 Dec 10) 2250–2262.
[26] M. Karam, A. Ives, C. Auclair, Is Sphingosine-1-Phosphate a Regulator of Tumor Vascular Functionality? Cancers 14 (5) (2022 Jan) 1302.
[27] Z. Raykov, S.P. Grekova, G. Bour, J.M. Lehn, N. Giese, A. Nicolau, et al., Myo-inositol trispyrophosphate-mediated hypoxia reversion controls pancreatic cancer in rodents and enhances gemcitabine efficacy, Int. J. Cancer 134 (11) (2014) 2572–2582.
[28] I. Grgic, F. Tschanz, N. Borgeaud, A. Gupta, P.A. Clavien, M. Guckenberger, et al., Tumor Oxygenation by Myo-Inositol Trispyrophosphate Enhances Radiation Response, Int. J. Radiat. Oncol. Biol. Phys. 110 (4) (2021 Jul 15) 1222–1233.
[29] M.A. Schneider, M. Linecker, R. Fritsch, U.J. Muehlematter, D. Stocker, B. Pestalozzi, et al., Phase Ib dose-escalation study of the hypoxia-modifier Myo-inositol trispyrophosphate in patients with hepatopancreatobiliary tumors, Nat. Commun. 12 (1) (2021 Jun 21) 3807.
[30] J. Wu, X. Wang, L. Chen, J. Wang, J. Zhang, J. Tang, et al., Oxygen microcapsules improve immune checkpoint blockade by ameliorating hypoxia condition in pancreatic ductal adenocarcinoma, Bioact. Mater. 1 (20) (2023 Feb) 259–270.
[31] E. Thivat, M. Casile, J. Moreau, I. Molnar, S. Dufort, K. Seddik, et al., Phase I/II study testing the combination of AGuIX nanoparticles with radiochemotherapy and concomitant temozolomide in patients with newly diagnosed glioblastoma (NANO-GBM trial protocol), BMC Cancer 23 (1) (2023 Apr 15) 344.
[32] S. Bennett, S.H. Hsu, C. Verry, E. Kaza, X. Miao, M.B. Keerthivasan, et al., Quantifying Gadolinium-based, Theranostic Nanoparticle Uptake in MR Images of Pancreatic Cancer, Int. J. Radiat. Oncol. Biol. Phys. 114 (3) (2022 Nov 1) e189.
[33] T. Zhang, Y. Ren, P. Yang, J. Wang, H. Zhou, Cancer-associated fibroblasts in pancreatic ductal adenocarcinoma, Cell Death Dis. 13 (10) (2022 Oct 25) 1–11.
[34] E. Sahai, I. Astsaturov, E. Cukierman, D.G. DeNardo, M. Egeblad, R.M. Evans, et al., A framework for advancing our understanding of cancer-associated fibroblasts, Nat. Rev. Cancer 20 (3) (2020 Mar) 174–186.
[35] H. Liu, Y. Shi, F. Qian, Opportunities and delusions regarding drug delivery targeting pancreatic cancer-associated fibroblasts, Adv. Drug Deliv. Rev. 1 (172) (2021 May) 37–51.
[36] A. Brichkina, P. Polo, S.D. Sharma, N. Visestamkul, M. Lauth, A Quick Guide to CAF Subtypes in Pancreatic Cancer, Cancers 15 (9) (2023 May 4) 2614.
[37] M.A. Jacobetz, D.S. Chan, A. Neesse, T.E. Bapiro, N. Cook, K.K. Frese, et al., Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer, Gut 62 (1) (2013 Jan) 112–120.
[38] P.P. Provenzano, C. Cuevas, A.E. Chang, V.K. Goel, D.D. Von Hoff, S.R. Hingorani, Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma, Cancer Cell 21 (3) (2012 Mar 20) 418–429.
[39] E. Van Cutsem, M.A. Tempero, D. Sigal, D.Y. Oh, N. Fazio, T. Macarulla, et al., Randomized phase III Trial of Pegvorhyaluronidase Alfa with Nab-Paclitaxel Plus Gemcitabine for patients with Hyaluronan-High Metastatic Pancreatic Adenocarcinoma, J. Clin. Oncol. 38 (27) (2020 Sep 20) 3185–3194.
[40] R.K. Ramanathan, S.L. McDonough, P.A. Philip, S.R. Hingorani, J. Lacy, J. S. Kortmansky, et al., Phase IB/II Randomized Study of FOLFIRINOX Plus Pegylated Recombinant Human Hyaluronidase Versus FOLFIRINOX Alone in patients with Metastatic Pancreatic Adenocarcinoma: SWOG S1313, J. Clin. Oncol. 37 (13) (2019 May 1) 1062–1069.
[41] M. Bazan-Peregrino, R. Garcia-Carbonero, B. Laquente, R. Álvarez, A. Mato-Berciano, M. Gimenez-Alejandre, et al., VCN-01 disrupts pancreatic cancer stroma and exerts antitumor effects, J. Immunother. Cancer 9 (11) (2021 Nov 1) e003254.
[42] G.P. Nagaraju, M.S. Saddala, J.B. Foote, A.M. Khaliq, A. Masood, Y. Golivi, et al., Mechanism of enhancing chemotherapy efficacy in pancreatic ductal adenocarcinoma with paricalcitol and hydroxychloroquine, Cell Rep. Med. 6 (1) (2025 Jan 21) 101881.
[43] P.M. Grierson, R. Suresh, B. Tan, K.S. Pedersen, M. Amin, H. Park, et al., A pilot Study of Paricalcitol plus Nanoliposomal Irinotecan and 5-FU/LV in Advanced Pancreatic Cancer patients after Progression on Gemcitabine-based Therapy, Clin Cancer Res off J Am Assoc Cancer Res. 29 (23) (2023 Dec 1) 4733–4739.
[44] J. Yang, Q. Zhang, J. Wang, Y. Lou, Z. Hong, S. Wei, et al., Dynamic profiling of immune microenvironment during pancreatic cancer development suggests early intervention and combination strategy of immunotherapy, EBioMedicine 19 (78) (2022 Mar) 103958.
[45] S.K. Gautam, S.K. Batra, M. Jain, Molecular and metabolic regulation of immunosuppression in metastatic pancreatic ductal adenocarcinoma, Mol. Cancer 24 (22) (2023 Jul) 118.
[46] A. Padoan, M. Plebani, D. Basso, Inflammation and Pancreatic Cancer: Focus on Metabolism, Cytokines, and Immunity, Int. J. Mol. Sci. 20 (3) (2019 Feb 5) 676.
[47] Y. Pylayeva-Gupta, K.E. Lee, C.H. Hajdu, G. Miller, D. Bar-Sagi, Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia, Cancer Cell 21 (6) (2012 Jun 12) 836–847.
[48] L.J. Bayne, G.L. Beatty, N. Jhala, C.E. Clark, A.D. Rhim, B.Z. Stanger, et al., Tumor-derived granulocyte-macrophage colony stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer, Cancer Cell 21 (6) (2012 Jun 12) 822–835.

[49] M. Pergamo, G. Miller, Myeloid-derived suppressor cells and their role in pancreatic cancer, Cancer Gene Ther. 24 (3) (2017 Mar) 100–105.
[50] N.A. Ullman, P.R. Burchard, R.F. Dunne, D.C. Linehan, Immunologic strategies in Pancreatic Cancer: making Cold Tumors Hot, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 40 (24) (2022 Aug 20) 2789–2805.
[51] C. Siret, A. Collignon, F. Silvy, S. Robert, T. Cheyrol, P. André, et al., Deciphering the Crosstalk between Myeloid-Derived Suppressor Cells and Regulatory T Cells in Pancreatic Ductal Adenocarcinoma, Front. Immunol. 10 (2019) 3070.
[52] Highfill SL, Cui Y, Giles AJ, Smith JP, Zhang H, Morse E, et al. Disruption of CXCR2-mediated MDSC tumor trafficking enhances anti-PD1 efficacy. Sci Transl Med. 2014 May 21;6(237):237ra67.
[53] P. Gulhati, A. Schalck, S. Jiang, X. Shang, C.J. Wu, P. Hou, et al., Targeting T cell checkpoints 41BB and LAG3 and myeloid cell CXCR1/CXCR2 results in antitumor immunity and durable response in pancreatic cancer, Nat Cancer. 4 (1) (2023 Jan) 62–80.
[54] R. Noy, J.W. Pollard, Tumor-associated macrophages: from mechanisms to therapy, Immunity 41 (1) (2014 Jul 17) 49–61.
[55] W. Wang, J.M. Marinis, A.M. Beal, S. Savadkar, Y. Wu, M. Khan, et al., RIP1 Kinase Drives Macrophage-Mediated Adaptive Immune Tolerance in Pancreatic Cancer, Cancer Cell 34 (5) (2018 Nov 12) 757–774.
[56] W. Zhou, Y. Zhou, X. Chen, T. Ning, H. Chen, Q. Guo, et al., Pancreatic cancer-targeting exosomes for enhancing immunotherapy and reprogramming tumor microenvironment, Biomaterials 1 (268) (2021 Jan) 120546.
[57] C.J. Garcia Garcia, Y. Huang, N.R. Fuentes, M.C. Turner, M.E. Monberg, D. Lin, et al., Stromal HIF2 Regulates Immune suppression in the Pancreatic Cancer Microenvironment, Gastroenterology 162 (7) (2022 Jun) 2018–2031.
[58] Y. Liu, X. Cao, Characteristics and significance of the Pre-metastatic Niche, Cancer Cell 30 (5) (2016 Nov 14) 668–681.
[59] T. Kitamura, B.Z. Qian, J.W. Pollard, Immune cell promotion of metastasis, Nat. Rev. Immunol. 15 (2) (2015 Feb) 73–86.
[60] S. Yachida, C.A. Iacobuzio-Donahue, The Pathology and Genetics of Metastatic Pancreatic Cancer, Arch. Pathol. Lab. Med. 133 (3) (2009 Mar 1) 413–422.
[61] H. Oweira, U. Petrausch, D. Helbling, J. Schmidt, M. Mannhart, A. Mehrabi, et al., Prognostic value of site-specific metastases in pancreatic adenocarcinoma: a Surveillance Epidemiology and end results database analysis, World J. Gastroenterol. 23 (10) (2017 Mar 14) 1872–1880.
[62] K. Yamamoto, A. Venida, J. Yano, D.E. Biancur, M. Kakiuchi, S. Gupta, et al., Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I, Nature 581 (7806) (2020 May) 100–105.
[63] J.J. El-Jawhari, Y.M. El-Sherbiny, G.B. Scott, R.S.M. Morgan, R. Prestwich, P. A. Bowles, et al., Blocking oncogenic RAS enhances tumour cell surface MHC class I expression but does not alter susceptibility to cytotoxic lymphocytes, Mol. Immunol. 58 (2) (2014 Apr 1) 160–168.
[64] S.C. Casey, L. Tong, Y. Li, R. Do, S. Walz, K.N. Fitzgerald, et al., MYC Regulates the Anti-Tumor Immune Response through CD47 and PD-L1, Science 352 (6282) (2016 Apr 8) 227–231.
[65] Schleger C, Verbeke C, Hildenbrand R, Zentgraf H, Bleyl U. c-MYC activation in primary and metastatic ductal adenocarcinoma of the pancreas: incidence, mechanisms, and clinical significance. Mod Pathol Off J U S Can Acad Pathol Inc. 2002 Apr;15(4):462–9.
[66] R.T. Lawlor, P. Mattiolo, A. Mafficini, S.M. Hong, M.L. Piredda, S.V. Taormina, et al., Tumor Mutational Burden as a potential Biomarker for Immunotherapy in Pancreatic Cancer: Systematic Review and Still-Open questions, Cancers 13 (13) (2021 Jun 22) 3119.
[67] A.D. Rhim, E.T. Mirek, N.M. Aiello, A. Maitra, J.M. Bailey, F. McCallister, et al., EMT and dissemination precede pancreatic tumor formation, Cell 148 (1–2) (2012 Jan 20) 349–361.
[68] R.E. Royal, C. Levy, K. Turner, A. Mathur, M. Hughes, U.S. Kammula, et al., Phase 2 Trial of Single Agent Ipilimumab (Anti-CTLA-4) for locally Advanced or Metastatic Pancreatic Adenocarcinoma, J Immunother Hagerstown Md 33 (8) (1997. 2010 Oct,) 828–833.
[69] P. Sharma, L. Dirix, F.Y.F.L. De Vos, J.P. Allison, L. Decoster, R. Zaucha, et al., Efficacy and tolerability of tremelimumab in patients with metastatic pancreatic ductal adenocarcinoma, J. Clin. Oncol. 36(4_suppl):470–470 (2018 Feb).
[70] J.R. Brahmer, S.S. Tykodi, L.Q.M. Chow, W.J. Hwu, S.L. Topalian, P. Hwu, et al., Safety and activity of Anti–PD-L1 Antibody in patients with Advanced Cancer, N. Engl. J. Med. 366 (26) (2012 Jun 28) 2455–2465.
[71] E.M. O'Reilly, D.Y. Oh, N. Dhani, D.J. Renouf, M.A. Lee, W. Sun, et al., Durvalumab with or without Tremelimumab for patients with Metastatic Pancreatic Ductal Adenocarcinoma, JAMA Oncol. 5 (10) (2019 Oct) 1431–1438.
[72] M. Aglietta, C. Barone, M.B. Sawyer, M.J. Moore, W.H. Miller, C. Bagalà, 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. 25 (9) (2014 Sep 1) 1750–1755.
[73] Kalyan A, Kircher SM, Mohindra NA, Nimeiri HS, Maurer V, Rademaker A, et al. Ipilimumab and gemcitabine for advanced pancreas cancer: A phase Ib study . J Clin Oncol. 2016 May 20;34(15_suppl):e15747–e15747.
[74] S.D. Kamath, A. Kalyan, S. Kircher, H. Nimeiri, A.J. Fought, A. Benson, et al., Ipilimumab and Gemcitabine for Advanced Pancreatic Cancer: a phase Ib Study, Oncologist 25 (5) (2020 May) e808–e815.
[75] Z.A. Wainberg, H.S. Hochster, E.J. Kim, B. George, A. Kaylan, E.G. Chiorean, et al., Open-label, phase I Study of Nivolumab combined with nab-Paclitaxel Plus Gemcitabine in Advanced Pancreatic Cancer, Clin. Cancer Res. 26 (18) (2020 Sep 15) 4814–4822.
[76] K. Cheng, X. Li, W. Lv, G. Zhao, R. Zhou, C. Chang, et al., Spatial interactions of immune cells as potential predictors to efficacy of toripalimab plus chemotherapy in locally advanced or metastatic pancreatic ductal adenocarcinoma: a phase Ib/II trial, Signal Transduct. Target. Ther. 9 (1) (2024 Nov 25) 1–14.
[77] G.J. Weiss, L. Blaydorn, J. Beck, K. Bornemann-Kolatzki, H. Urnovitz, E. Schütz, et al., Phase Ib/II study of gemcitabine, nab-paclitaxel, and pembrolizumab in metastatic pancreatic adenocarcinoma, Invest. New Drugs 36 (1) (2018 Feb 1) 96–102.
[78] C. Morizane, M. Ueno, M. Ikeda, K. Sudo, Y. Hirashima, M. Kuroda, et al., A phase 2 study of nivolumab in combination with modified FOLFIRINOX for metastatic pancreatic cancer, BJC Rep. 2 (1) (2024 Jan 23) 1–7.
[79] D.J. Renouf, J.M. Loree, J.J. Knox, J.T. Topham, P. Kavan, D. Jonker, et al., The CCTG PA.7 phase II trial of gemcitabine and nab-paclitaxel with or without durvalumab and tremelimumab as initial therapy in metastatic pancreatic ductal adenocarcinoma, Nat. Commun. 26 (13) (2022 Aug) 5020.
[80] Gang J, Guo S, Zhang Y, Ma Y, Guo X, Zhou X, et al. A phase II study of KN046 monotherapy as 2nd line and above treatment for unresectable locally advanced or metastatic pancreatic ductal adenocarcinoma (PDAC). J Clin Oncol. 2022 Jun; 40(16_suppl):e16305–e16305.
[81] Jin G, Guo S, Zhang Y, Ma Y, Guo X, Zhou X, et al. Efficacy and safety of KN046 plus nab-paclitaxel/gemcitabine as first-line treatment for unresectable locally advanced or metastatic pancreatic ductal adenocarcinoma (PDAC). J Clin Oncol. 2021 May 20;39(15_suppl):4138–4138.
[82] Jin G, Guo S, Xu J, Liu R, Liang Q, Yang Y, et al. A multicenter, randomized, double-blind phase III clinical study to evaluate the efficacy and safety of KN046 combined with nab-paclitaxel and gemcitabine versus placebo combined with nab-paclitaxel and gemcitabine in patients with advanced pancreatic cancer (ENREACH-PDAC-01). J Clin Oncol. 2022 Jun 1;40(16_suppl): TPS4189–TPS4189.
[83] M. Piechutta, A.S. Berghoff, New emerging targets in cancer immunotherapy: the role of Cluster of Differentiation 40 (CD40/TNFR5), ESMO Open 4 (Suppl 3) (2019 Jun 12) e000510.
[84] L.J. Padrón, D.M. Maurer, M.H. O'Hara, E.M. O'Reilly, R.A. Wolff, Z.A. Wainberg, et al., Sotigalimab and/or nivolumab with chemotherapy in first-line metastatic pancreatic cancer: clinical and immunologic analyses from the randomized phase 2 PRINCE trial, Nat. Med. 28 (6) (2022) 1167–1177.
[85] A. Ene-Obong, A.J. Clear, J. Watt, J. Wang, R. Fatah, J.C. Riches, et al., Activated Pancreatic Stellate Cells Sequester CD8+ T-Cells to Reduce their Infiltration of the Juxtatumoral Compartment of Pancreatic Ductal Adenocarcinoma, Gastroenterology 145 (5) (2013 Nov) 1121–1132.
[86] Fan J qiao, Wang MF, Chen HL, Shang D, Das JK, Song J. Current advances and outlooks in immunotherapy for pancreatic ductal adenocarcinoma. Mol Cancer. 2020 Feb 15;19:32.
[87] Y.D. Seo, X. Jiang, K.M. Sullivan, F. Jalikis, K.S. Smythe, A. Abbasi, et al., Mobilization of CD8+ T cells via CXCR4 blockade facilitates PD-1 checkpoint therapy in human pancreatic cancer, Clin Cancer Res off J Am Assoc Cancer Res. 25 (13) (2019 Jul 1) 3934–3945.
[88] B. Bockorny, V. Semenisty, T. Macarulla, E. Borazanci, B.M. Wolpin, S. M. Stemmer, et al., BL-8040, a CXCR4 antagonist, in combination with pembrolizumab and chemotherapy for pancreatic cancer: the COMBAT trial, Nat. Med. 26 (6) (2020 Jun) 878–885.
[89] A. Wang-Gillam, C.P. Li, G. Bodoky, A. Dean, Y.S. Shan, G. Jameson, et al., Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial, Lancet Lond Engl. 387 (10018) (2016 Feb 6) 545–557.
[90] J.C. Dawson, A. Serrels, D.G. Stupack, D.D. Schlaepfer, M.C. Frame, Targeting FAK in anticancer combination therapies, Nat. Rev. Cancer 21 (5) (2021 May) 313–324.
[91] A. Wang-Gillam, K.H. Lim, R. McWilliams, R. Suresh, A.C. Lockhart, A. Brown, et al., Defactinib, Pembrolizumab, and Gemcitabine in patients with Advanced Treatment Refractory Pancreatic Cancer: a phase I Dose Escalation and expansion Study, Clin Cancer Res off J Am Assoc Cancer Res. 28 (24) (2022 Dec 15) 5254–5262.
[92] Lim KH, Spencer KR, Safyan RA, Picozzi VJ, Varghese AM, Siolas D, et al. Avutometinib/defactinib and gemcitabine/nab-paclitaxel combination in first-line metastatic pancreatic ductal adenocarcinoma: Initial safety and efficacy of phase 1b/2 study (RAMP 205). J Clin Oncol. 2024 Jun;42(16_suppl):4140–4140.
[93] A.J. Gunderson, M.M. Kaneda, T. Tsujikawa, A.V. Nguyen, N.I. Affara, B. Ruffell, et al., Bruton Tyrosine Kinase-Dependent Immune Cell Cross-talk Drives Pancreas Cancer, Cancer Discov. 6 (3) (2016 Mar) 270–285.
[94] M. Overman, M. Javle, R.E. Davis, P. Vats, C. Kumar-Sinha, L. Xiao, et al., Randomized phase II study of the Bruton tyrosine kinase inhibitor acalabrutinib, alone or with pembrolizumab in patients with advanced pancreatic cancer, J. Immunother. Cancer 8 (1) (2020 Feb) e000587.
[95] D. Hong, D. Rasco, M. Veeder, J.J. Luke, J. Chandler, A. Balmanoukian, et al., A phase 1b/2 Study of the Bruton Tyrosine Kinase Inhibitor Ibrutinib and the PD-L1 Inhibitor Durvalumab in patients with Pretreated Solid Tumors, Oncol Switz. 97 (2) (2019 Jul 1) 102–111.
[96] A. Naing, H. Mamdani, M.A. Barve, M.L. Johnson, D. Morgensztern, A. J. Olszanski, et al., Efficacy and safety of NT-I7, long-acting interleukin-7, plus pembrolizumab in patients with advanced solid tumors: results from the phase 2a study, J. Clin. Oncol. 1 (2022 Jun).
[97] G. Lamberti, E. Andrini, M. Sisi, A.D. Federico, B. Ricciuti, Targeting DNA damage response and repair genes to enhance anticancer immunotherapy: rationale and clinical implication, Future Oncol Lond Engl. 16 (23) (2020 Aug) 1751–1766.
[98] K.A. Reiss, R. Mick, U. Teitelbaum, M. O'Hara, C. Schneider, R. Massa, et al., A Randomized phase Ib/II Study of Niraparib plus Nivolumab or Ipilimumab in

patients with Platinum-Sensitive Advanced Pancreatic Cancer, Lancet Oncol. 23 (8) (2022 Aug) 1009–1020.
[99] Zhen DB, Whittle M, Ritch PS, Hochster HS, Coveler AL, George B, et al. Phase II study of PEGPH20 plus pembrolizumab for patients (pts) with hyaluronan (HA)-high refractory metastatic pancreatic adenocarcinoma (mPC): PCRT16-001. J Clin Oncol. 2022 Jan 5;40(4_suppl):576–576.
[100] Algaze S, Hanna DL, Azad NS, Thomas JS, Iqbal S, Habib D, et al. A phase Ib study of guadecitabine and durvalumab in patients with advanced hepatocellular carcinoma, pancreatic adenocarcinoma, and biliary cancers. J Clin Oncol. 2022 Feb 1;40(4_suppl):574–574.
[101] G. Biffi, T.E. Oni, B. Spielman, Y. Hao, E. Elyada, Y. Park, et al., IL1-Induced JAK/STAT Signaling is Antagonized by TGFβ to Shape CAF Heterogeneity in Pancreatic Ductal Adenocarcinoma, Cancer Discov. 9 (2) (2019 Feb 8) 282–301.
[102] A.R. Parikh, A. Szabolcs, J.N. Allen, J.W. Clark, J.Y. Wo, M. Raabe, et al., Radiation Therapy Enhances Immunotherapy Response in Microsatellite-stable Colorectal and Pancreatic Adenocarcinoma in a phase II Trial, Nat Cancer. 2 (11) (2021 Nov) 1124–1135.
[103] I.M. Chen, J.S. Johansen, S. Theile, J.X. Hjaltelin, S.I. Novitski, S. Brunak, et al., Randomized phase II Study of Nivolumab with or without Ipilimumab combined with Stereotactic Body Radiotherapy for Refractory Metastatic Pancreatic Cancer (CheckPAC), J. Clin. Oncol. 20 (2022 Sep).
[104] C. Xie, A.G. Duffy, G. Brar, S. Fioravanti, D. Mabry-Hrones, M. Walker, et al., Immune Checkpoint Blockade in Combination with Stereotactic Body Radiotherapy in patients with Metastatic Pancreatic Ductal Adenocarcinoma, Clin Cancer Res off J Am Assoc Cancer Res. 26 (10) (2020 May 15) 2318–2326.
[105] I.M. Chen, M. Donia, C.A. Chamberlain, A.W.P. Jensen, A. Draghi, S. Theile, et al., Phase 2 study of ipilimumab, nivolumab, and tocilizumab combined with stereotactic body radiotherapy in patients with refractory pancreatic cancer (TRIPLE-R), Eur J Cancer Oxf Engl 1990 (180) (2023 Feb) 125–133.
[106] C. Lynch, M.C. Korpics, R.R. Katipally, C.M. Bestvina, S.P. Pitroda, J.D. Patel, et al., Safety of combined ablative radiotherapy and immune checkpoint inhibitors in three phase I trials, Eur. J. Cancer 1 (209) (2024 Sep) 114264.
[107] L. Dercle, J. McGale, S. Sun, A. Marabelle, R. Yeh, E. Deutsch, et al., Artificial intelligence and radiomics: fundamentals, applications, and challenges in immunotherapy, J. Immunother. Cancer 10 (9) (2022 Sep 1) e005292.
[108] D.U. Ozsahin, N. Usanase, I. Ozsahin, Advancing pancreatic cancer management: the role of artificial intelligence in diagnosis and therapy, Beni-Suef Univ J Basic Appl Sci. 14 (1) (2025 Apr 7) 32.
[109] Y. Bian, C. Liu, Q. Li, Y. Meng, F. Liu, H. Zhang, et al., Preoperative Radiomics Approach to evaluating Tumor-Infiltrating CD8+ T Cells in patients with Pancreatic Ductal Adenocarcinoma using Noncontrast magnetic Resonance Imaging, J. Magn. Reson. Imaging 55 (3) (2022) 803–814.
[110] C. Saillard, R. Dubois, O. Tchita, N. Loiseau, T. Garcia, A. Adriansen, et al., Validation of MSIntuit as an AI-based pre-screening tool for MSI detection from colorectal cancer histology slides, Nat. Commun. 14 (1) (2023 Nov 6) 6695.
[111] Z.A. Wainberg, D. Melisi, T. Macarulla, R. Pazo Cid, S.R. Chandana, C. De La Fouchardière, et al., NALIRIFOX versus nab-paclitaxel and gemcitabine in treatment-naive patients with metastatic pancreatic ductal adenocarcinoma (NAPOLI 3): a randomised, open-label, phase 3 trial, Lancet Lond Engl. 402 (10409) (2023 Oct 7) 1272–1281.
[112] G. Milano, F. Innocenti, H. Minami, Liposomal irinotecan (Onivyde): Exemplifying the benefits of nanotherapeutic drugs, Cancer Sci. 113 (7) (2022 Jul) 2224–2231.
[113] S.H. Kiaie, H. Salehi-Shadkami, M.J. Sanaei, M. Azizi, M. Shokrollahi Barough, M. S. Nasr, et al., Nano-immunotherapy: overcoming delivery challenge of immune checkpoint therapy, J Nanobiotechnology. 21 (1) (2023 Sep 21) 339.
[114] Y. Zong, Y. Lin, T. Wei, Q. Cheng, Lipid Nanoparticle (LNP) Enables mRNA delivery for Cancer Therapy, Adv. Mater. 35 (51) (2023) 2303261.
[115] S. Wilhelm, A.J. Tavares, Q. Dai, S. Ohta, J. Audet, H.F. Dvorak, et al., Analysis of nanoparticle delivery to tumours, Nat. Rev. Mater. 1 (5) (2016 Apr 26) 16014.
[116] S. Camorani, S. Tortorella, L. Agnello, C. Spanu, A. d'Argenio, R. Nilo, et al., Aptamer-Functionalized Nanoparticles Mediate PD-L1 siRNA delivery for Effective Gene Silencing in Triple-negative Breast Cancer Cells, Pharmaceutics 14 (10) (2022 Oct) 2225.
[117] Y. Wu, W. Gu, J. Li, C. Chen, Z.P. Xu, Silencing PD-1 and PD-L1 with nanoparticle-delivered small interfering RNA increases cytotoxicity of tumor-infiltrating lymphocytes, Nanomed 14 (8) (2019 Apr) 955–967.
[118] J. Tang, L. Li, C.B. Howard, S.M. Mahler, L. Huang, Z.P. Xu, Preparation of optimized lipid-coated calcium phosphate nanoparticles for enhanced in vitro gene delivery to breast cancer cells, J. Mater. Chem. B Mater. Biol. Med. 3 (33) (2015 Sep 7) 6805–6812.
[119] A.K. Kosmides, J.W. Sidhom, A. Fraser, C.A. Bessell, J.P. Schneck, Dual Targeting Nanoparticle Stimulates the Immune System to Inhibit Tumor Growth, ACS Nano 11 (6) (2017 Jun 27) 5417–5429.
[120] Z. Su, Z. Xiao, Y. Wang, J. Huang, Y. An, X. Wang, et al., Codelivery of Anti-PD-1 Antibody and Paclitaxel with Matrix Metalloproteinase and pH Dual-Sensitive Micelles for Enhanced Tumor Chemoimmunotherapy, Small Weinh Bergstr Ger. 16 (7) (2020 Feb) e1906832.
[121] Wan W jun, Qu C xi, Zhou Y juan, Zhang L, Chen M tian, Liu Y, et al. Doxorubicin and siRNA-PD-L1 co-delivery with T7 modified ROS-sensitive nanoparticles for tumor chemoimmunotherapy. Int J Pharm. 2019 Jul 20;566:731–44.
[122] L. Miao, J. Li, Q. Liu, R. Feng, M. Das, C.M. Lin, et al., Transient and Local Expression of Chemokine and Immune Checkpoint Traps to Treat Pancreatic Cancer, ACS Nano 11 (9) (2017 Sep 26) 8690–8706.
[123] H.E. Daldrup-Link, Ten things you Might not know about Iron Oxide Nanoparticles, Radiology 284 (3) (2017 Sep) 616–629.
[124] S. Zhao, X. Yu, Y. Qian, W. Chen, J. Shen, Multifunctional magnetic iron oxide nanoparticles: an advanced platform for cancer theranostics, Theranostics 10 (14) (2020) 6278–6309.
[125] S.M. Dadfar, K. Roemhild, N.I. Drude, S. von Stillfried, R. Knüchel, F. Kiessling, et al., Iron oxide nanoparticles: Diagnostic, therapeutic and theranostic applications, Adv. Drug Deliv. Rev. 1 (138) (2019 Jan) 302–325.
[126] T. Anani, S. Rahmati, N. Sultana, A.E. David, MRI-traceable theranostic nanoparticles for targeted cancer treatment, Theranostics 11 (2) (2021) 579–601.
[127] B. Yu, B. Choi, W. Li, D.H. Kim, Magnetic field boosted ferroptosis-like cell death and responsive MRI using hybrid vesicles for cancer immunotherapy, Nat. Commun. 11 (1) (2020 Jul 20) 3637.
[128] S. Zanganeh, G. Hutter, R. Spitler, O. Lenkov, M. Mahmoudi, A. Shaw, et al., Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues, Nat. Nanotechnol. 11 (11) (2016 Nov) 986–994.
[129] Ruiz-de-Angulo A, Bilbao-Asensio M, Cronin J, Evans SJ, Clift MJD, Llop J, et al. Chemically Programmed Vaccines: Iron Catalysis in Nanoparticles Enhances Combination Immunotherapy and Immunotherapy-Promoted Tumor Ferroptosis. iScience. 2020 Sep 25;23(9):101499.
[130] H.W. Cheng, H.Y. Tsao, C.S. Chiang, S.Y. Chen, Advances in magnetic Nanoparticle-Mediated Cancer Immune-Theranostics, Adv. Healthc. Mater. 10 (1) (2021 Jan) e2001451.
[131] S. Liu, J. He, R. Song, M. Zhang, L. Huang, J. Chen, et al., Fe2P nanorods based photothermal therapy combined with immune checkpoint inhibitors for pancreatic cancer, Nanophotonics 10 (12) (2021 Sep 2) 3267–3278.
[132] J. Pan, P. Hu, Y. Guo, J. Hao, D. Ni, Y. Xu, et al., Combined magnetic Hyperthermia and Immune Therapy for Primary and Metastatic Tumor Treatments, ACS Nano 14 (1) (2020 Jan 28) 1033–1044.
[133] B. Xu, Y. Chen, M. Peng, J.H. Zheng, C. Zuo, Exploring the potential of exosomes in diagnosis and drug delivery for pancreatic ductal adenocarcinoma, Int. J. Cancer 152 (2) (2023 Jan 15) 110–122.
[134] S. Kamerkar, V.S. LeBleu, H. Sugimoto, S. Yang, C.F. Ruivo, S.A. Melo, et al., Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer, Nature 546 (7659) (2017 Jun) 498–503.
[135] M. Zhuang, X. Chen, D. Du, J. Shi, M. Deng, Q. Long, et al., SPION decorated exosome delivery of TNF-α to cancer cell membranes through magnetism, Nanoscale 12 (1) (2019 Dec 19) 173–188.
[136] S.K. Libutti, G.F. Paciotti, A.A. Byrnes, H.R. Alexander Jr, W.E. Gannon, M. Walker, et al., Phase I and Pharmacokinetic Studies of CYT-6091, a Novel PEGylated Colloidal gold-rhTNF Nanomedicine, Clin. Cancer Res. 16 (24) (2010 Dec 16) 6139–6149.
[137] Sandin LC, Eriksson ,Fredrik, Ellmark ,Peter, Loskog ,Angelica SI, Tötterman , Thomas H, and Mangsbo SM. Local CTLA4 blockade effectively restrains experimental pancreatic adenocarcinoma growth in vivo. OncoImmunology. 2014 Jan 1;3(1):e27614.
[138] E. Minaei, M. Ranson, M. Aghmesheh, R. Sluyter, K.L. Vine, Enhancing pancreatic cancer immunotherapy: Leveraging localized delivery strategies through the use of implantable devices and scaffolds, J. Controlled Release 1 (373) (2024 Sep) 145–160.
[139] Y. Liu, F. Liu, Y. Zeng, L. Lin, H. Yu, S. Zhang, et al., Hydrogel systems for spatiotemporal controlled delivery of immunomodulators: engineering the tumor immune microenvironment for enhanced cancer immunotherapy, Front. Cell Dev. Biol. 13 (2024 Dec) 12.
[140] J. Kim, W.A. Li, Y. Choi, S.A. Lewin, C.S. Verbeke, G. Dranoff, et al., Injectable, spontaneously assembling, inorganic scaffolds modulate immune cells in vivo and increase vaccine efficacy, Nat. Biotechnol. 33 (1) (2015 Jan) 64–72.
[141] H. Zhou, W. Wang, Z. Cai, Z.Y. Jia, Y.Y. Li, W. He, et al., Injectable hybrid hydrogels enable enhanced combination chemotherapy and roused anti-tumor immunity in the synergistic treatment of pancreatic ductal adenocarcinoma, J Nanobiotechnology. 22 (1) (2024 Jun 20) 353.
[142] H. Liu, D. Davila Gonzalez, D.I. Viswanath, R.S. Vander Pol, S.Z. Saunders, N. Di Trani, et al., Sustained Intratumoral Administration of Agonist CD40 Antibody Overcomes Immunosuppressive Tumor Microenvironment in Pancreatic Cancer, Adv. Sci. 10 (9) (2023 Jan 19) 2206873.
[143] Wang C, Wang J, Zhang X, Yu S, Wen D, Hu Q, et al. In situ formed reactive oxygen species-responsive scaffold with gemcitabine and checkpoint inhibitor for combination therapy. Sci Transl Med. 2018 Feb 21;10(429):eaan3682.
[144] M. Wang, Q. Hu, J. Huang, F. Zhang, Z. Yao, S. Shao, et al., In Situ formed ROS-Responsive Hydrogel with STING Agonist and Gemcitabine to Intensify Immunotherapy against Pancreatic Ductal Adenocarcinoma, Adv. Healthc. Mater. 12 (20) (2023) 2203264.
[145] R. Zhao, Q. Xiao, Y. Wu, W. Zhang, J. Liu, Y. Zeng, et al., Dual-crosslinking immunostimulatory hydrogel synchronously suppresses pancreatic fistula and pancreatic cancer relapse post-resection, Biomaterials 305 (2024 Mar) 122453.
[146] B.L. Musher, E.K. Rowinsky, B.G. Smaglo, W. Abidi, M. Othman, K. Patel, et al., LOAd703, an oncolytic virus-based immunostimulatory gene therapy, combined with chemotherapy for unresectable or metastatic pancreatic cancer (LOKON001): results from arm 1 of a non-randomised, single-centre, phase 1/2 study, Lancet Oncol. 25 (4) (2024 Apr) 488–500.
[147] K. Kirtane, H. Elmariah, C.H. Chung, D. Abate-Daga, Adoptive cellular therapy in solid tumor malignancies: review of the literature and challenges ahead, J. Immunother. Cancer 9 (7) (2021 Jul 23) e002723.
[148] C. Delm, Z.E. Tano, A.M. Varghese, P.S. Adusumilli, CAR T-cell Therapy for Pancreatic Cancer, J. Surg. Oncol. 116 (1) (2017 Jul) 63–74.
[149] M. Sadelain, R. Brentjens, I. Riviere, The basic principles of chimeric antigen receptor (CAR) design, Cancer Discov. 3 (4) (2013 Apr) 388–398.

[150] R. Leidner, N.S. Silva, H. Huang, D. Sprott, C. Zheng, Y.P. Shih, et al., Neoantigen T-Cell Receptor Gene Therapy in Pancreatic Cancer, N. Engl. J. Med. 386 (22) (2022 Jun 2) 2112–2119.
[151] N. Tokarew, J. Ogonek, S. Endres, M. von Bergwelt-Baildon, S. Kobold, Teaching an old dog new tricks: next-generation CAR T cells, Br. J. Cancer 120 (1) (2019 Jan) 26–37.
[152] M. Sadelain, CAR therapy: the CD19 paradigm, J. Clin. Invest. 125 (9) (2015 Sep 1) 3392–3400.
[153] A. Morello, M. Sadelain, P.S. Adusumilli, Mesothelin-Targeted CARs: Driving T cells to Solid Tumors, Cancer Discov. 6 (2) (2016 Feb) 133–146.
[154] G.L. Beatty, M.H. O'Hara, S.F. Lacey, D.A. Torigian, F. Nazimuddin, F. Chen, et al., Activity of Mesothelin-specific Chimeric Antigen Receptor T cells against Pancreatic Carcinoma Metastases in a phase 1 Trial, Gastroenterology 155 (1) (2018 Jul) 29–32.
[155] A.R. Haas, J.L. Tanyi, M.H. O'Hara, W.L. Gladney, S.F. Lacey, D.A. Torigian, et al., Phase I Study of Lentiviral-Transduced Chimeric Antigen Receptor-Modified T Cells Recognizing Mesothelin in Advanced Solid Cancers, Mol. Ther. 27 (11) (2019 Nov) 1919–1929.
[156] A.H. Ko, A.C. Jordan, E. Tooker, S.F. Lacey, R.B. Chang, Y. Li, et al., Dual Targeting of Mesothelin and CD19 with Chimeric Antigen Receptor-Modified T Cells in patients with Metastatic Pancreatic Cancer, Mol. Ther. 28 (11) (2020 Nov 4) 2367–2378.
[157] University of Pennsylvania. Phase I Study of Human Chimeric Antigen Receptor Modified T Cells (CAR T Cells) in Patients With Pancreatic Cancer. clinicaltrials.gov; 2024 Oct. Report No.: NCT03323944.
[158] X. Wang, C.S. Zhang, X.Y. Dong, Y. Hu, B.J. Duan, J. Bai, et al., Claudin 18.2 is a potential therapeutic target for zolbetuximab in pancreatic ductal adenocarcinoma. World J Gastrointest, Oncol 14 (7) (2022 Jul 15) 1252–1264.
[159] C. Wang, N. Wu, B. Pei, X. Ma, W. Yang, Claudin and pancreatic cancer. Front, Oncol 7 (2023 Mar) 13.
[160] C. Deiana, M. Agostini, G. Brandi, E. Giovannetti, The trend toward more target therapy in pancreatic ductal adenocarcinoma, Expert Rev. Anticancer Ther. 24 (7) (2024 Jul 2) 525–565.
[161] W. Park, E.M. O'Reilly, J. Furuse, C.P. Li, D.Y. Oh, R. Garcia-Carbonero, et al., Zolbetuximab plus gemcitabine and nab-paclitaxel (GN) in first-line treatment of claudin 18.2–positive metastatic pancreatic cancer (mPC): phase 2, open-label, randomized study, J. Clin. Oncol. 41(4_suppl):TPS782–TPS782 (2023 Feb).
[162] C. Qi, T. Xie, J. Zhou, X. Wang, J. Gong, X. Zhang, et al., CT041 CAR T cell therapy for Claudin18.2-positive metastatic pancreatic cancer, J Hematol OncolJ Hematol Oncol. 16 (1) (2023 Sep 9) 102.
[163] C. Li, D.G. Heidt, P. Dalerba, C.F. Burant, L. Zhang, V. Adsay, et al., Identification of Pancreatic Cancer Stem Cells, Cancer Res. 67 (3) (2007 Feb 1) 1030–1037.
[164] A. Maliar, C. Servais, T. Waks, M. Chmielewski, R. Lavy, P. Altevogt, et al., Redirected T cells that target pancreatic adenocarcinoma antigens eliminate tumors and metastases in mice, Gastroenterology 143 (5) (2012 Nov) 1375–1384.e5.
[165] K. Feng, Y. Liu, Y. Guo, J. Qiu, Z. Wu, H. Dai, et al., Phase I study of chimeric antigen receptor modified T cells in treating HER2-positive advanced biliary tract cancers and pancreatic cancers, Protein Cell 9 (10) (2018 Oct) 838–847.
[166] Y. Wang, M. Chen, Z. Wu, C. Tong, H. Dai, Y. Guo, et al., CD133-directed CAR T cells for advanced metastasis malignancies: a phase I trial, Oncoimmunology 7 (7) (2018 May 7) e1440169.
[167] S. Ueda, S. Ogata, H. Tsuda, N. Kawarabayashi, M. Kimura, Y. Sugiura, et al., The Correlation between Cytoplasmic Overexpression of Epidermal Growth factor Receptor and Tumor Aggressiveness: Poor Prognosis in patients with Pancreatic Ductal Adenocarcinoma, Pancreas 29 (1) (2004 Jul) e1.
[168] Y. Liu, Y. Guo, Z. Wu, K. Feng, C. Tong, Y. Wang, et al., Anti-EGFR chimeric antigen receptor-modified T cells in metastatic pancreatic carcinoma: a phase I clinical trial, Cytotherapy 22 (10) (2020 Oct 1) 573–580.
[169] G.L. Beatty, M. O'Hara, Chimeric antigen receptor-modified T cells for the treatment of solid tumors: defining the challenges and next steps, Pharmacol. Ther. 166 (2016 Oct) 30–39.
[170] C.Y. Yang, M.H. Fan, C.H. Miao, Y.J. Liao, R.H. Yuan, C.L. Liu, Engineering Chimeric Antigen Receptor T Cells against Immune Checkpoint Inhibitors PD-1/PD-L1 for Treating Pancreatic Cancer, Mol. Ther. Oncolytics 26 (17) (2020 May) 571–585.
[171] T.T. Smith, H.F. Moffett, S.B. Stephan, C.F. Opel, A.G. Dumigan, X. Jiang, et al., Biopolymers codelivering engineered T cells and STING agonists can eliminate heterogeneous tumors, J. Clin. Invest. 127 (6) (2017 Jun 1) 2176–2191.
[172] Y. Zhao, Y. Dong, S. Yang, Y. Tu, C. Wang, J. Li, et al., Bioorthogonal Equipping CAR-T Cells with Hyaluronidase and Checkpoint Blocking Antibody for Enhanced Solid Tumor Immunotherapy, ACS Cent. Sci. 8 (5) (2022 May 25) 603–614.
[173] Q. Zhang, W. Wei, P. Wang, L. Zuo, F. Li, J. Xu, et al., Biomimetic Magnetosomes as Versatile Artificial Antigen-Presenting Cells to Potentiate T-Cell-Based Anticancer Therapy, ACS Nano 11 (11) (2017 Nov 28) 10724–10732.
[174] M. Ma, J. Liu, S. Jin, L. Wang, Development of tumour peptide vaccines: from universalization to personalization, Scand. J. Immunol. 91 (6) (2020) e12875.
[175] E.R. Lutz, A.A. Wu, E. Bigelow, R. Sharma, G. Mo, K. Soares, et al., Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation, Cancer Immunol. Res. 2 (7) (2014 Jul) 616–631.
[176] T. Heumann, C. Judkins, K. Li, S.J. Lim, J. Hoare, R. Parkinson, et al., A platform trial of neoadjuvant and adjuvant antitumor vaccination alone or in combination with PD-1 antagonist and CD137 agonist antibodies in patients with resectable pancreatic adenocarcinoma, Nat. Commun. 20 (14) (2023 Jun) 3650.
[177] K. Li, J.A. Tandurella, J. Gai, Q. Zhu, S.J. Lim, D.L. Thomas, et al., Multi-omic analyses of changes in the tumor microenvironment of pancreatic adenocarcinoma following neoadjuvant treatment with anti-PD-1 therapy, Cancer Cell 40 (11) (2022 Nov 14) 1374–1391.e7.
[178] S.T. Muth, M.T. Saung, A.B. Blair, M.G. Henderson, D.L. Thomas, L. Zheng, CD137 Agonist-based Combination Immunotherapy Enhances Activated, Effector memory T cells and Prolongs Survival in Pancreatic Adenocarcinoma, Cancer Lett. 28 (499) (2021 Feb) 99–108.
[179] T. Conroy, P. Hammel, M. Hebbar, M. Ben Abdelghani, A.C. Wei, J.L. Raoul, et al., FOLFIRINOX or Gemcitabine as Adjuvant Therapy for Pancreatic Cancer, N. Engl. J. Med. 379 (25) (2018 Dec 20) 2395–2406.
[180] M. Morishita, Y. Takahashi, A. Matsumoto, M. Nishikawa, Y. Takakura, Exosome-based tumor antigens-adjuvant co-delivery utilizing genetically engineered tumor cell-derived exosomes with immunostimulatory CpG DNA, Biomaterials 111 (2016 Dec) 55–65.
[181] Xiao L, Erb ,Ulrike, Zhao ,Kun, Hackert ,Thilo, and Zöller M. Efficacy of vaccination with tumor-exosome loaded dendritic cells combined with cytotoxic drug treatment in pancreatic cancer. OncoImmunology. 2017 Jun 3;6(6): e1319044.
[182] L.A. Rojas, Z. Sethna, K.C. Soares, C. Olcese, N. Pang, E. Patterson, et al., Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer, Nature 618 (7963) (2023 Jun) 144–150.
[183] Z. Sethna, P. Guasp, C. Reiche, M. Milighetti, N. Ceglia, E. Patterson, et al., RNA neoantigen vaccines prime long-lived CD8+ T cells in pancreatic cancer, Nature 19 (2025 Feb) 1–10.
[184] S. Escalera-Anzola, M. Rosado, Y. Yang, D. Parra-Sanchez, C.S. Pedro-Liberal, P. Acedo, Breakthroughs in nanoparticle-based strategies for pancreatic cancer therapy, Biochem. Pharmacol. 1 (232) (2025 Feb) 116685.
[185] C.J. Bishop, S.Y. Tzeng, J.J. Green, Degradable polymer-coated gold nanoparticles for co-delivery of DNA and siRNA, Acta Biomater. 1 (11) (2015 Jan) 393–403.
[186] J.E. Won, Y. Byeon, T.I. Wi, C.M. Lee, J.H. Lee, T.H. Kang, et al., Immune checkpoint silencing using RNAi-incorporated nanoparticles enhances antitumor immunity and therapeutic efficacy compared with antibody-based approaches, J. Immunother. Cancer 10 (2) (2022 Feb) e003928.
[187] R. Endo, T. Nakamura, K. Kawakami, Y. Sato, H. Harashima, The silencing of indoleamine 2,3-dioxygenase 1 (IDO1) in dendritic cells by siRNA-loaded lipid nanoparticles enhances cell-based cancer immunotherapy, Sci. Rep. 9 (1) (2019 Aug 5) 11335.
[188] Y. Liu, L. Xiao, K.I. Joo, B. Hu, J. Fang, P. Wang, In Situ Modulation of Dendritic Cells by Injectable Thermosensitive Hydrogels for Cancer Vaccines in mice, Biomacromolecules 15 (10) (2014 Oct 13) 3836–3845.
[189] S. Chattopadhyay, Y.H. Liu, Z.S. Fang, C.L. Lin, J.C. Lin, B.Y. Yao, et al., Synthetic Immunogenic Cell Death Mediated by Intracellular delivery of STING Agonist Nanoshells Enhances Anticancer Chemo-immunotherapy, Nano Lett. 20 (4) (2020 Apr 8) 2246–2256.
[190] Y. Guo, Y. Ran, Z. Wang, J. Cheng, Y. Cao, C. Yang, et al., Magnetic-responsive and targeted cancer nanotheranostics by PA/MR bimodal imaging-guided photothermally triggered immunotherapy, Biomaterials 219 (2019 Oct) 119370.
[191] B. Yoo, V.C. Jordan, P. Sheedy, A.M. Billig, A. Ross, P. Pantazopoulos, et al., RNAi-Mediated PD-L1 Inhibition for Pancreatic Cancer Immunotherapy, Sci. Rep. 9 (1) (2019 Mar 18) 4712.
[192] A.I. Bangolo, C. Trivedi, I. Jani, S. Pender, H. Khalid, B. Alqinai, et al., Impact of gut microbiome in the development and treatment of pancreatic cancer: Newer insights, World J. Gastroenterol. 29 (25) (2023 Jul 7) 3984–3998.
[193] C.M. Doocey, K. Finn, C. Murphy, C.M. Guinane, The impact of the human microbiome in tumorigenesis, cancer progression, and biotherapeutic development, BMC Microbiol. 12 (22) (2022 Feb) 53.
[194] J. Durack, S.V. Lynch, The gut microbiome: Relationships with disease and opportunities for therapy, J. Exp. Med. 216 (1) (2019 Jan 7) 20–40.
[195] S. Viaud, F. Saccheri, G. Mignot, T. Yamazaki, R. Daillère, D. Hannani, et al., The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide, Science 342 (6161) (2013 Nov 22) 971–976.
[196] M. Vétizou, J.M. Pitt, R. Daillère, P. Lepage, N. Waldschmitt, C. Flament, et al., Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota, Science 350 (6264) (2015 Nov 27) 1079–1084.
[197] A. Sivan, L. Corrales, N. Hubert, J.B. Williams, K. Aquino-Michaels, Z.M. Earley, et al., Commensal Bifidobacterium promotes antitumor immunity and facilitates anti–PD-L1 efficacy, Science 350 (6264) (2015 Nov 27) 1084–1089.
[198] V. Matson, J. Fessler, R. Bao, T. Chongsuwat, Y. Zha, M.L. Alegre, et al., The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients, Science 359 (6371) (2018 Jan 5) 104–108.
[199] B. Routy, E. Le Chatelier, L. Derosa, C.P.M. Duong, M.T. Alou, R. Daillère, et al., Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors, Science 359 (6371) (2018 Jan 5) 91–97.
[200] J. Ahn, R. Sinha, Z. Pei, C. Dominianni, J. Wu, J. Shi, et al., Human Gut Microbiome and risk for Colorectal Cancer, JNCI J Natl Cancer Inst. 105 (24) (2013 Dec 18) 1907–1911.
[201] Goedert JJ, Jones G, Hua X, Xu X, Yu G, Flores R, et al. Investigation of the Association Between the Fecal Microbiota and Breast Cancer in Postmenopausal Women: a Population-Based Case-Control Pilot Study. JNCI J Natl Cancer Inst. 2015 Jun 1;107(8):djv147.
[202] Z. Ren, J. Jiang, H. Xie, A. Li, H. Lu, S. Xu, et al., Gut microbial profile analysis by MiSeq sequencing of pancreatic carcinoma patients in China, Oncotarget 8 (56) (2017 Jun 29) 95176–95191.
[203] V. Gopalakrishnan, C.N. Spencer, L. Nezi, A. Reuben, M.C. Andrews, T. V. Karpinets, et al., Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients, Science 359 (6371) (2018 Jan 5) 97–103.

[204] D. Davar, A.K. Dzutsev, J.A. McCulloch, R.R. Rodrigues, J.M. Chauvin, R. M. Morrison, et al., Fecal microbiota transplant overcomes resistance to anti–PD-1 therapy in melanoma patients, Science 371 (6529) (2021 Feb 5) 595–602.
[205] E.N. Baruch, I. Youngster, G. Ben-Betzalel, R. Ortenberg, A. Lahat, L. Katz, et al., Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients, Science 371 (6529) (2021 Feb 5) 602–609.
[206] Lin Y, Xie M, Lau HCH, Zeng R, Zhang R, Wang L, et al. Effects of gut microbiota on immune checkpoint inhibitors in multi-cancer and as microbial biomarkers for predicting therapeutic response. Med. 2024 Nov;100530.
[207] A. Elkrief, L. El Raichani, C. Richard, M. Messaoudene, W. Belkaid, J. Malo, et al., Antibiotics are associated with decreased progression-free survival of advanced melanoma patients treated with immune checkpoint inhibitors, Oncoimmunology 8 (4) (2019 Feb 18) e1568812.
[208] F. Huemer, G. Rinnerthaler, T. Westphal, H. Hackl, G. Hutarew, S. P. Gampenrieder, et al., Impact of antibiotic treatment on immune-checkpoint blockade efficacy in advanced non-squamous non-small cell lung cancer, Oncotarget 9 (23) (2018 Mar 27) 16512–16520.
[209] X.Z. Huang, P. Gao, Y.X. Song, Y. Xu, J.X. Sun, X.W. Chen, et al., Antibiotic use and the efficacy of immune checkpoint inhibitors in cancer patients: a pooled analysis of 2740 cancer patients, Oncoimmunology 8 (12) (2019 Sep 23) e1665973.
[210] D.J. Pinato, S. Howlett, D. Ottaviani, H. Urus, A. Patel, T. Mineo, et al., Association of Prior Antibiotic Treatment with Survival and Response to Immune Checkpoint Inhibitor Therapy in patients with Cancer, JAMA Oncol. 5 (12) (2019 Dec) 1774–1778.
[211] Y. Yang, W. Weng, J. Peng, L. Hong, L. Yang, Y. Toiyama, et al., *Fusobacterium nucleatum* increases Proliferation of Colorectal Cancer Cells and Tumor Development in mice by Activating Toll-like Receptor 4 Signaling to Nuclear Factor−κB, and Up-regulating Expression of MicroRNA-21, Gastroenterology 152 (4) (2017 Mar 1) 851–866.e24.
[212] T. Yu, F. Guo, Y. Yu, T. Sun, D. Ma, J. Han, et al., Fusobacterium nucleatum Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy, Cell 170 (3) (2017 Jul) 548–563.e16.
[213] Z.L. Chen, M. Li, Y. Zhang, M. Sun, L. Wang, et al., Fusobacterium nucleatum reduces METTL3-mediated m6A modification and contributes to colorectal cancer metastasis, Nat. Commun. (2022). Mar 10;13(1):1248.
[214] X. Wang, Y. Fang, W. Liang, C.C. Wong, H. Qin, Y. Gao, et al., Fusobacterium nucleatum facilitates anti-PD-1 therapy in microsatellite stable colorectal cancer, Cancer Cell 42 (10) (2024 Oct) 1729–1746.e8.
[215] Michaud DS, Izard J, Wilhelm-Benartzi CS, You DH, Grote VA, Tjønneland A, et al. Plasma antibodies to oral bacteria and risk of pancreatic cancer in a large European prospective cohort study. Gut. 2013 Dec;62(12):10.1136/gutjnl-2012–303006.
[216] S. Pushalkar, M. Hundeyin, D. Daley, C.P. Zambirinis, E. Kurz, A. Mishra, et al., The Pancreatic Cancer Microbiome Promotes Oncogenesis by Induction of Innate and Adaptive Immune suppression, Cancer Discov. 8 (4) (2018 Apr) 403–416.
[217] V. Sethi, S. Kurtom, M. Tarique, S. Lavania, Z. Malchiodi, L. Hellmund, et al., Gut Microbiota Promotes Tumor Growth in mice by Modulating Immune Response, Gastroenterology 155 (1) (2018 Jul) 33–37.e6.
[218] F. McAllister, J.M. Bailey, J. Alsina, C.J. Nirschl, R. Sharma, H. Fan, et al., Oncogenic Kras activates a hematopoietic-to-epithelial IL-17 signaling axis in preinvasive pancreatic neoplasia, Cancer Cell 25 (5) (2014 May 12) 621–637.
[219] C. Loncle, L. Bonjoch, E. Folch-Puy, M.B. Lopez-Millan, S. Lac, M.I. Molejon, et al., IL-17 functions through the novel REG3β-JAK2-STAT3 inflammatory pathway to promote the transition from chronic pancreatitis to pancreatic cancer, Cancer Res. 75 (22) (2015 Nov 15) 4852–4862.
[220] Y. Zhang, M. Zoltan, E. Riquelme, H. Xu, I. Sahin, S. Castro-Pando, et al., Immune Cell production of Interleukin 17 Induces Stem Cell Features of Pancreatic Intraepithelial Neoplasia Cells, Gastroenterology 155 (1) (2018 Jul) 210–223.
[221] Y. Zhang, V. Chandra, E. Riquelme Sanchez, P. Dutta, P.R. Quesada, A. Rakoski, et al., Interleukin-17-induced neutrophil extracellular traps mediate resistance to checkpoint blockade in pancreatic cancer, J. Exp. Med. 217 (12) (2020 Dec 7) e20190354.
[222] P. Kumar, L. Monin, P. Castillo, W. Elsegeiny, W. Horne, T. Eddens, et al., Intestinal interleukin-17 receptor signaling mediates reciprocal control of the gut microbiota and autoimmune inflammation, Immunity 44 (3) (2016 Mar 15) 659–671.
[223] V. Chandra, L. Li, O. Le Roux, Y. Zhang, R.M. Howell, D.N. Rupani, et al., Gut epithelial Interleukin-17 receptor a signaling can modulate distant tumors growth through microbial regulation, Cancer Cell 42 (1) (2024 Jan 8) 85–100.e6.
[224] J. Ahn, S. Segers, R.B. Hayes, Periodontal disease, Porphyromonas gingivalis serum antibody levels and orodigestive cancer mortality, Carcinogenesis 33 (5) (2012 May) 1055–1058.
[225] D.L. D'Antonio, A. Zenoniani, S. Umme, A. Piattelli, M.C. Curia, Intratumoral Fusobacterium nucleatum in Pancreatic Cancer: current and Future Perspectives, Pathogens 14 (1) (2025 Jan) 2.
[226] M. Ögrendik, Periodontal Pathogens in the Etiology of Pancreatic Cancer, Gastrointest Tumors. 3 (3–4) (2017 Mar) 125–127.
[227] R.A. Gaiser, A. Halimi, H. Alkharaan, L. Lu, H. Davanian, K. Healy, et al., Enrichment of oral microbiota in early cystic precursors to invasive pancreatic cancer, Gut 68 (12) (2019 Dec) 2186–2194.
[228] K. Mitsuhashi, K. Nosho, Y. Sukawa, Y. Matsunaga, M. Ito, H. Kurihara, et al., Association of Fusobacterium species in pancreatic cancer tissues with molecular features and prognosis, Oncotarget 6 (9) (2015 Mar 13) 7209–7220.
[229] E. Half, N. Keren, L. Reshef, T. Dorfman, I. Lachter, Y. Kluger, et al., Fecal microbiome signatures of pancreatic cancer patients, Sci. Rep. 14 (9) (2019 Nov) 16801.
[230] Y. Zhu, X. Liang, M. Zhi, L. Li, G. Zhang, C. Chen, et al., Succession of the multi-site microbiome along pancreatic ductal adenocarcinoma tumorigenesis, Front. Immunol. 15 (2024) 1487242.
[231] E. Nistal, A. Caminero, A.R. Herrán, L. Arias, S. Vivas, J.M.R. de Morales, et al., Differences of small intestinal bacteria populations in adults and children with/without celiac disease: effect of age, gluten diet, and disease, Inflamm. Bowel Dis. 18 (4) (2012 Apr) 649–656.
[232] L.T. Geller, M. Barzily-Rokni, T. Danino, O.H. Jonas, N. Shental, D. Nejman, et al., Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine, Science 357 (6356) (2017 Sep 15) 1156–1160.
[233] B. Aykut, S. Pushalkar, R. Chen, Q. Li, R. Abengozar, J.I. Kim, et al., The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL, Nature 574 (7777) (2019 Oct) 264–267.
[234] S. Nakano, Y. Komatsu, Y. Kawamoto, R. Saito, K. Ito, H. Nakatsumi, et al., Association between the use of antibiotics and efficacy of gemcitabine plus nab-paclitaxel in advanced pancreatic cancer, Medicine (Baltimore) 99 (39) (2020 Sep 25) e22250.
[235] H. Imai, K. Saijo, K. Komine, Y. Otsuki, K. Ohuchi, Y. Sato, et al., Antibiotic therapy augments the efficacy of gemcitabine-containing regimens for advanced cancer: a retrospective study, Cancer Manag. Res. 11 (2019 Aug) 7953–7965.
[236] C. Mohindroo, M. Hasanov, J.E. Rogers, W. Dong, L.R. Prakash, S. Baydogan, et al., Antibiotic use influences outcomes in advanced pancreatic adenocarcinoma patients, Cancer Med. 10 (15) (2021) 5041–5050.
[237] S. Sayin, B. Rosener, C.G. Li, B. Ho, O. Ponomarova, D.V. Ward, et al., Evolved bacterial resistance to the chemotherapy gemcitabine modulates its efficacy in co-cultured cancer cells, Elife 3 (12) (2023 Feb) e83140.
[238] J. Tintelnot, Y. Xu, T.R. Lesker, M. Schönlein, L. Konczalla, A.D. Giannou, et al., Microbiota-derived 3-IAA influences chemotherapy efficacy in pancreatic cancer, Nature 615 (7950) (2023 Mar) 168–174.
[239] M.D. Molin, M. Zhang, R.F. de Wilde, N.A. Ottenhof, N. Rezaee, C.L. Wolfgang, et al., Very long-term survival following resection for pancreatic cancer is not explained by commonly mutated genes: results of whole-exome sequencing analysis, Clin Cancer Res off J Am Assoc Cancer Res. 21 (8) (2015 Apr 15) 1944–1950.
[240] E. Riquelme, Y. Zhang, L. Zhang, M. Montiel, M. Zoltan, W. Dong, et al., Tumor Microbiome Diversity and Composition Influence Pancreatic Cancer Outcomes, Cell 178 (4) (2019 Aug 8) 795–806.e12.
[241] A. Świdnicka-Siergiejko, J. Daniluk, K. Miniewska, U. Daniluk, K. Guzińska-Ustymowicz, A. Pryczynicz, et al., Inflammatory Stimuli and Fecal Microbiota Transplantation Accelerate Pancreatic Carcinogenesis in Transgenic mice, Accompanied by changes in the Microbiota Composition, Cells 14 (5) (2025 Feb 28) 361.
[242] Goedert JJ, Jones G, Hua X, Xu X, Yu G, Flores R, et al. Investigation of the Association Between the Fecal Microbiota and Breast Cancer in Postmenopausal Women: a Population-Based Case-Control Pilot Study. JNCI J Natl Cancer Inst. 2015 Jun 1;107(8):djv147.
[243] Decker-Farrell AR, Sastra SA, Harimoto T, Hasselluhn MC, Palermo CF, Ballister ER, et al. Tumor-selective treatment of metastatic pancreatic cancer with an engineered, probiotic living drug. BioRxiv Prepr Serv Biol. 2024 May 5; 2024.05.02.592216.
[244] W. Tan, M.T.Q. Duong, C. Zuo, Y. Qin, Y. Zhang, Y. Guo, et al., Targeting of pancreatic cancer cells and stromal cells using engineered oncolytic Salmonella typhimurium, Mol Ther J Am Soc Gene Ther. 30 (2) (2022 Feb 2) 662–671.
[245] D. Chandra, B.C. Selvanesan, Z. Yuan, S.K. Libutti, W. Koba, A. Beck, et al., 32-Phosphorus selectively delivered by listeria to pancreatic cancer demonstrates a strong therapeutic effect, Oncotarget 8 (13) (2017 Feb 6) 20729–20740.
[246] Y. Xiao, T. Pan, W. Da, Y. Liu, S. Chen, D. Chen, et al., Aptamer-drug conjugates-loaded bacteria for pancreatic cancer synergistic therapy, Signal Transduct. Target. Ther. 9 (1) (2024 Oct 14) 272.
[247] X. Xu, Y. Ding, Y. Dong, H. Yuan, P. Xia, C. Qu, et al., Nanobody-Engineered Biohybrid Bacteria Targeting Gastrointestinal Cancers Induce Robust STING-Mediated Anti-Tumor Immunity, Adv. Sci. 11 (31) (2024) 2401905.
[248] W.Q. Yao, W.F. Song, X.C. Deng, Y.T. Lin, R. Meng, J.W. Wang, et al., Harnessing the Engineered Probiotic-Nanosystem to Remodulate Tumor Extracellular Matrix and Regulate Tumor-Colonizing Bacteria for improving Pancreatic Cancer Chemo-Immunotherapy, Small Weinh Bergstr Ger. 21 (3) (2025 Jan) e2406837.
[249] Z.Y. Han, Z.J. Fu, Y.Z. Wang, C. Zhang, Q.W. Chen, J.X. An, et al., Probiotics functionalized with a gallium-polyphenol network modulate the intratumor microbiota and promote anti-tumor immune responses in pancreatic cancer, Nat. Commun. 15 (1) (2024 Aug 17) 7096.
[250] D. Lee, C. Lee, K. Han, T. Goo, B. Kim, Y. Han, et al., Machine learning models for pancreatic cancer diagnosis based on microbiome markers from serum extracellular vesicles, Sci. Rep. 15 (1) (2025 Mar 31) 10995.

[251] Y. Chen, F. Nian, J. Chen, Q. Jiang, T. Yuan, H. Feng, et al., Metagenomic Microbial Signatures for Noninvasive Detection of Pancreatic Cancer, Biomedicines 13 (4) (2025 Apr 21) 1000.
[252] Zhang Y, Zhang H, Liu B, Ning K. Highly accurate diagnosis of pancreatic cancer by integrative modeling using gut microbiome and exposome data. iScience. 2024 Feb 21;27(3):109294.
[253] Villani A, Fontana A, Panebianco C, Ferro C, Copetti M, Pavlovic R, et al. A powerful machine learning approach to identify interactions of differentially abundant gut microbial subsets in patients with metastatic and non-metastatic pancreatic cancer. Gut Microbes. 16(1):2375483.
[254] S. Li, M. Yang, L. Ji, H. Fan, A multi-omics machine learning framework in predicting the recurrence and metastasis of patients with pancreatic adenocarcinoma, Front. Microbiol. 3 (2022 Nov) 13.