

REVIEW ARTICLE

Microbiome as a modulator of immunotherapy response in pancreatic cancer

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Abstract

Pancreatic adenocarcinoma is widely regarded as one of the most lethal malignancies due to its rapid progression and the limited success of early detection methods and therapeutic interventions. While immunotherapy has emerged as an effective treatment option for various solid tumors, it has not demonstrated comparable efficacy in pancreatic cancer. Further research is required to evaluate the safety and efficacy of different immunotherapy modalities, including immune checkpoint inhibitors, T-cell transfer therapy, chimeric antigen receptor T-cell therapy, neoantigen vaccines, and epigenome-targeting treatments, specifically in the context of pancreatic cancer. Emerging evidence highlights the crucial role of the microbiome in modulating cancer cells' responses to immunotherapy. Studies have increasingly implicated the gut microbiota composition as a direct influencer of tumorigenesis in pancreatic cancer. Certain microbial species have been shown to exert immunostimulatory or immunosuppressive effects on pancreatic cancer cells, thereby directly enhancing or suppressing their response to immunotherapeutic regimens. Despite these findings, there remains a paucity of comprehensive reviews on microbiome studies specific to individual immunotherapy modalities in pancreatic cancer. This review highlights the exciting potential of the microbiome in modulating pancreatic cancer responses across various immunotherapy subtypes and emphasizes the clinical need for further research in the field.

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1. Introduction

Pancreatic adenocarcinoma (PAC) is one of the most lethal malignancies worldwide, with a 5-year survival rate of approximately 10%.¹ It is the third leading cause of cancer-related deaths in the United States and the sixth leading cause globally.² PAC can be classified into endocrine or exocrine tumors, with the majority of exocrine tumors

being malignant. Pancreatic ductal adenocarcinoma (PDAC) comprises 90% of all exocrine tumors.³ Globally, the incidence of PDAC is rising, with over 500,000 cases reported, making it the 14th most common malignancy and the 7th leading cause of cancer-related deaths.⁴ Other classes of pancreatic cancers include cystic neoplasms (i.e., serous cystadenomas, mucinous neoplasms, intraductal papillary mucinous neoplasms, and cystic papillary and epithelial neoplasms) and endocrine tumors, such as insulinomas. Prognosis in PAC mainly relies on clinical staging and histopathological assessment. Molecular profiling further subclassifies PAC into classical and basal-like subtypes, with the basal-like subtype being more aggressive and associated with worse prognoses.⁵

The poor prognosis of pancreatic cancer is attributed to late diagnosis, rapid progression, and resistance to conventional treatment.^{6,7} The majority of pancreatic cancer cases (approximately 85%) are diagnosed at an advanced stage, and only 10 – 15% of patients are eligible for surgical resection, the best effective curative option.⁸ While immunotherapy has revolutionized the treatment of various cancers by harnessing the immune system to target cancer cells,⁹ pancreatic cancers have demonstrated limited responsiveness to such therapies. However, emerging evidence from clinical trials challenges this perspective by suggesting that combination therapies, such as chemoimmunotherapy, may enhance treatment efficacy in PAC.¹⁰⁻¹²

Pancreatic cancer cells exhibit unique characteristics that enable them to evade immune detection and proliferate uncontrollably, disrupting normal tissues and cellular functions. While immunotherapy has shown robust efficacy in other solid tumors, such as melanoma, lung cancer, and renal cell carcinoma,^{13,14} these distinctive characteristics hinder similar success in treating PDAC.¹⁵⁻¹⁷ The failure of immunotherapy in PDAC has been attributed to the unique nature of the tumor microenvironment (TME), which is often described as immunologically “cold.” This phenomenon can be broadly classified into two distinct mechanisms: (i) Evasion of recognition by the host immune system and (ii) induction of an immunosuppressive environment surrounding the TME.¹⁸⁻²⁰

In roughly 40% of pancreatic cancers, the loss of human leukocyte antigens (HLA) reduces the presentation of antigens on the tumor cell surface, thereby facilitating evasion of immunosurveillance.²¹ Similarly, studies on breast and lung cancers have demonstrated that a decrease in antigen-presenting molecules results in the downregulation of natural killer (NK) activator molecules on the cell surface. This downregulation ultimately allows

cancer cells to escape detection by the innate immune system's NK T cells.²² The second mechanism involves active immunosuppression with the TME. Pancreatic cancer cells achieve this immunosuppression by engaging specific target molecules, such as interleukin (IL)-10, transforming growth factor-beta (TGF- β), prostaglandin E2, programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), V-domain immunoglobulin suppressor of T cell activation protein, and vascular endothelial growth factor.²³⁻²⁶

Immunotherapy strategies generally aim to either enhance immune system activity or prevent immunosuppression. However, the various subtypes of immunotherapy operate through distinct mechanisms. One prominent approach involves immune checkpoint inhibitors, which target checkpoint proteins, such as CTLA-4 and programmed cell death protein 1 (PD-1), to restore T-cell activity by preventing excessive immune suppression.²⁷⁻²⁹ Another strategy employs monoclonal antibodies (mAbs), which are artificially produced antibodies designed to target specific tumor antigens.³⁰ In addition, T-cell transfer therapies, including chimeric antigen receptor T-cell (CAR-T) therapy, modify a patient's T cells to improve their ability to identify and destroy cancer cells.³¹

Epigenetic alterations, such as DNA methylation, histone acetylation, and microRNA (miRNA) suppression, also play crucial roles in aberrant gene expression associated with cancer progression.³² Clinical trials targeting epigenetic regulators – such as histone deacetylase (HDAC) inhibitors, DNA methyltransferase (DNMT) inhibitors, and enhancers of zeste homolog 2 (EZH2) inhibitors – show promise in this context.³³ Furthermore, therapeutic vaccines stimulate the immune system by presenting tumor antigens to elicit a robust immune response. Whole-cell vaccines and antigen-specific vaccines have demonstrated potential in converting non-immunogenic tumors into immunogenic ones, thereby enhancing the body's ability to fight cancer.³⁴

Several clinical studies suggest that the gut microbiota plays a vital role in modulating the immune system,^{35,36} tumorigenesis,^{37,38} and tumor response to treatment.³⁹⁻⁴³ Research on the impact of microbiome on pancreatic cancer has primarily focused on its influence on tumor development^{44,45} and response to immune checkpoint inhibitors.^{46,47} Specific findings indicate that certain microbes, such as *Bacteroides fragilis* and *Bifidobacterium* species, enhance therapeutic responses to CTLA-4 and PD-L1 inhibitors, highlighting the potential for microbiome-based therapies in cancer treatment. Certain gut bacterial species, such as *Akkermansia muciniphila*

and *Enterococcus hirae*, have been found to enhance T-cell function by increasing recruitment and secretion of IL-12. These effects potentially aid antitumor activity in various cancer types, including melanoma and PAC. Conversely, species such as *Fusobacterium* and *Escherichia coli* exhibit adverse effects by downregulating T-cell responses and increasing resistance to chemotherapy in pancreatic cancer.

Studies linking specific microbes to pancreatic cancer, similar to established correlations such as *Helicobacter pylori* with gastric cancer or *Schistosoma hematobium* with bladder cancer, suggest the potential for developing customized vaccines targeting tumorigenic microbial species. For instance, Proteobacteria and *Porphyromonas gingivalis* have been implicated in PDAC. Findings of altered microbiota profiles in PDAC patients, coupled with evidence of microbial migration across organs, underscore the clinical and scientific significance of investigating such vaccine strategies.

Furthermore, the impact of the microbiome on epigenetic modifications in pancreatic cancer remains underexplored.^{48,49} Preliminary findings suggest that microbial species such as *Fusobacterium*, *Porphyromonas*, and *Gemella* with S100P methylation patterns, indicating potential immunosuppressive roles. These findings necessitate further investigation into the underlying mechanisms and the potential of these microbes as immunostimulatory agents in epigenetic modification therapies.

This review will focus on the potential use of various immunotherapies for pancreatic cancer and the adjunctive role of the microbiome in enhancing or inhibiting the efficacy of these immunotherapies.⁵⁰ The first part of the review discusses the efficacy and potential of various types of immunotherapy treatments specifically for pancreatic cancer. The second part of the review explores the role of the microbiome in modulating the efficacy of these therapies.

2. Concepts of immune-based therapies in pancreatic cancer

2.1. Immune checkpoint inhibitors

Immune checkpoint proteins are integral components of the innate immune system, functioning to prevent immune cells from attacking the body's own cells.⁵¹ Prominent examples include CTLA-4, PD-1, and PD-L1, which play essential roles in regulating T-cell proliferation (Figure 1). CTLA-4 and PD-1 act collectively as negative co-stimulators to restrict T-cell expansion⁵² and suppress the activation of autoreactive T-cells.⁵³ The interaction between PD-1 and its partner protein PD-L1 inhibits

T-cell-mediated cytotoxic activity, contributing to tumor immunosuppression.⁵⁴ Immune checkpoint inhibitor therapies (such as anti-CTLA-4, anti-PD-1, and anti-PD-L1) block these checkpoint proteins, thereby allowing T-cells to attack cancerous cells directly.

Monoclonal antibodies can be engineered to target a wide range of molecules, including, but not limited to, tumor growth factors and immune cells. Pre-clinical studies on mAbs targeting large-cell lymphoma antigens have demonstrated that radioisotope-labeled mAbs are most effective in hematological malignancies due to their inherent radiosensitivity compared to solid tumors.⁵⁵ Combination therapies using external beam radiation and radioimmunotherapy have yielded promising preliminary results in brain malignancies. These mAbs exhibit low uptake in healthy tissues but can localize to regions with an altered endothelial barrier caused by inflammation or angiogenesis, making them particularly relevant for brain tumors.⁶² However, despite their success in other cancers, mAb therapy has not yet been clinically approved for pancreatic cancer and remains an area requiring further exploration.

Blocking the PD-1/PD-L1 pathway with immune checkpoint inhibitors has shown significant clinical responses in several cancers.^{56,57} For example, in the treatment of 15 types of microsatellite instability-high solid tumors, the KEYNOTE-158 trial reported a complete response rate of 7%, an overall response rate (ORR) of 39.6%, and a duration of response of 6 months or longer in 78% of patients.⁵⁸ However, the efficacy of these therapies in pancreatic cancer is markedly lower. In the phase II arm of the KEYNOTE-158 trial, while the ORR across tumor types was 30.8%, the ORR for pancreatic cancer was significantly lower at 18.2%, with a duration of response varying between 8.1 and 24.3 months.⁵⁸ Furthermore, patients in the pancreatic cancer cohort had the lowest median progression-free survival following pembrolizumab therapy (2.1 months) compared with ovarian (2.2 months), gastric (3.2 months), and biliary tract carcinoma (4.2 months).⁵⁸

In pancreatic cancer, the limited clinical efficacy of monotherapy with immune checkpoint inhibitors has raised questions about the role of immunosuppression in the TME.^{57,59} Studies exploring combinations of immune checkpoint inhibitors have yielded mixed results. For instance, a pre-clinical study in murine models demonstrated that administering a TGF- β pathway inhibitor (galunisertib) before treatment with anti-PD-L1 and anti-CTLA-4 inhibitors improved efficacy in colon cancer with liver metastases.⁶⁰ However, other studies found that combination therapy with anti-PD-L1 (durvalumab)

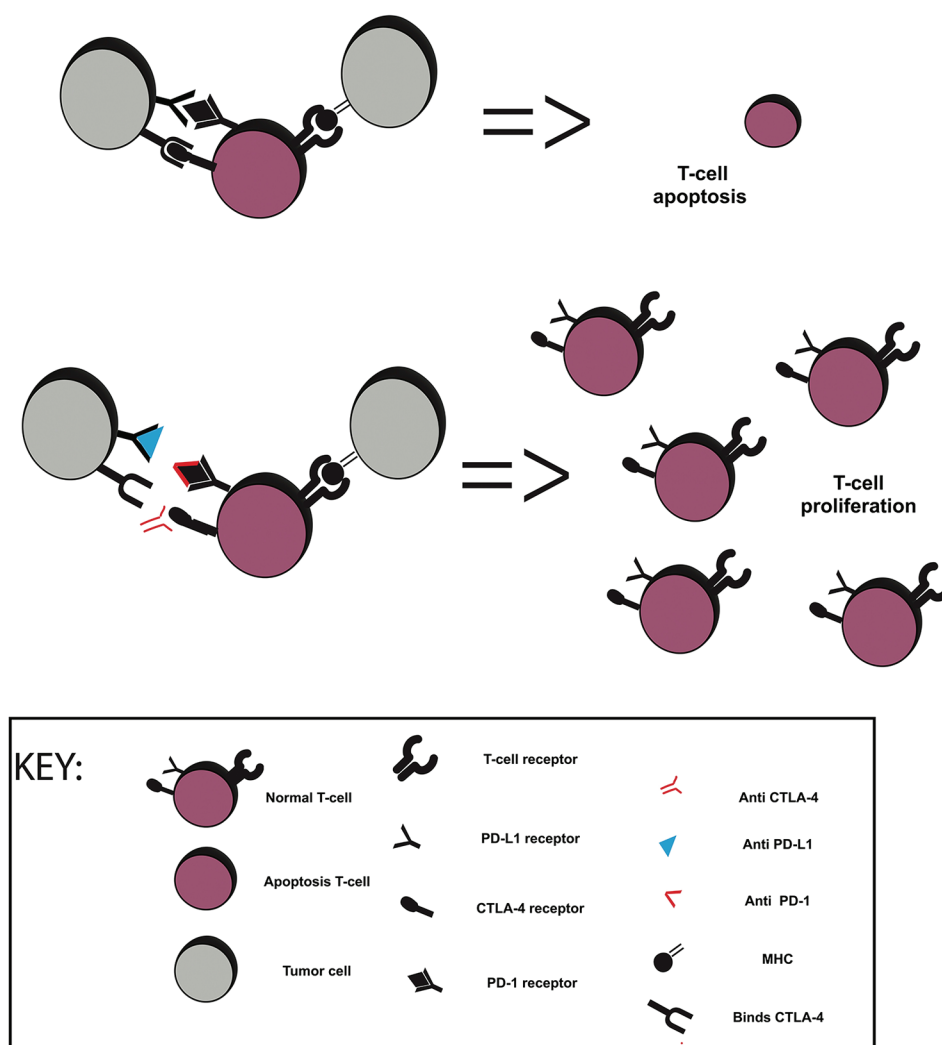


Figure 1. PD-L1 immune checkpoint inhibitor pathway. (A) Checkpoint proteins PD-L1 and PD-1 bind together to act as negative co-stimulators, preventing T-cell proliferation in untreated tumor cells. (B) Immune checkpoint inhibitors, including anti-PD-L1 (e.g., atezolizumab, avelumab, and durvalumab), block the binding of PD-L1 and PD-1, allowing T-cell proliferation and targeted tumor cell destruction in cancer patients undergoing immunotherapy. Similarly, anti-PD-1 drugs (e.g., nivolumab and pembrolizumab) inhibit PD-1 activity. In contrast, anti-CTLA-4 drugs (e.g., ipilimumab and tremelimumab) inhibit the CTLA-4 receptor.

and anti-CTLA-4 did not significantly improve outcomes in patients with PDAC.⁶¹ Similarly, a phase II clinical trial in patients with metastatic pancreatic cancer reported disease control in 76% of patients treated with a combination of anti-epidermal growth factor receptor (EGFR) mAbs and gemcitabine, a nucleoside analog.⁵⁵ While the study demonstrated limited cytotoxic effects of anti-EGFR mAb monotherapy in pancreatic cancer,^{10,62} the mechanisms driving the success of combination therapy remain unclear. Only a small subset of patients with PDAC possess a mismatch repair-deficient (MMR-D) genotype, for which immune checkpoint inhibitors have shown clinical efficacy. In a phase II study of MMR-D

solid tumors, six patients with pancreatic cancer exhibited an ORR of 62%.⁶³ However, for the majority of patients with advanced PDAC, immune checkpoint inhibitors lack an established clinical role. Further research is needed to identify the barriers preventing effective tumor responses and to improve therapeutic outcomes in pancreatic cancer.

2.2. T-cell transfer and chimeric antigen therapy

T-cell therapy involves genetically recombining patients' T cells with tumor antigen-targeting receptors, enabling them to specifically attack cancer cells.⁶⁴ Prominent receptors used in this approach include T-cell receptors (TCRs) and chimeric antigen receptors (CARs). TCRs recognize HLA

peptides and bind directly to macromolecules on the surface of cancer cells.⁶⁵ However, this therapy is HLA-specific, which limits its applicability to patients with compatible TCR-HLA profiles.⁶⁶ In contrast, CAR-T therapy is a multi-step process that bypasses HLA dependency. T-cells are obtained through leukapheresis, isolated from the blood, genetically reprogrammed with CARs, expanded to generate millions of copies, and subsequently re-infused into the patient. While CAR-T therapy is not restricted by HLA compatibility, it is limited to targeting surface antigens.⁶⁶

Research on non-CAR TCRs for pancreatic cancer remains sparse, and clinical trials for CAR-T therapy in pancreatic cancer are still in their early stages. Initial findings suggest that CAR-T treatment may have limited effectiveness in PDAC. For example, preliminary results from the CARsgen trial in 2018 demonstrated that patients with gastrointestinal cancers showed an ORR of 50% to anti-Claudin-18.2 CAR treatment (a target antigen receptor specifically expressed in pancreatic cancer)⁶⁷ after failing anti-PD-1/PD-L1 immune checkpoint inhibitor therapy. However, follow-up results published in 2022 indicated that Claudin18.2-specific CAR-T treatment resulted in progressive disease in all five pancreatic cancer patients included in the study.^{67,68}

The shortcomings of CAR-T therapy in pancreatic cancer appear to stem from off-target toxicity caused by the high sensitivity of CAR-T cells to low-level antigen expression (Figure 2).⁶⁸ In the CARsgen trial, for example, one patient exhibited signs of healthy tissue destruction. Similarly, a separate clinical study assessing CAR-T therapy safety in four patients with mesothelioma reported anaphylaxis in one patient,⁶⁹ although the results were not statistically significant. Unfortunately, limited evidence exists on off-target toxicities, specifically in PDAC treated with CAR-T therapy. Further evidence from phase II/III clinical trials is required to evaluate the efficacy and safety of CAR-T therapy in pancreatic cancer and to establish guidelines for its clinical application.

2.3. Vaccines in therapeutics

Therapeutic vaccines aim to stimulate the immune systems to recognize and attack tumor cells by exploiting antigenic differences between tumor cells and healthy host cells.^{70,71} These vaccines include whole-cell vaccines, which induce delayed-type hypersensitivity responses to tumor cells,⁷² and antigen-specific, vector-based vaccines, which are thought to provide strong safety profiles and antitumor activity.⁷¹ For instance, whole-cell vaccines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) gene-transduced tumor vaccine (GVAX),

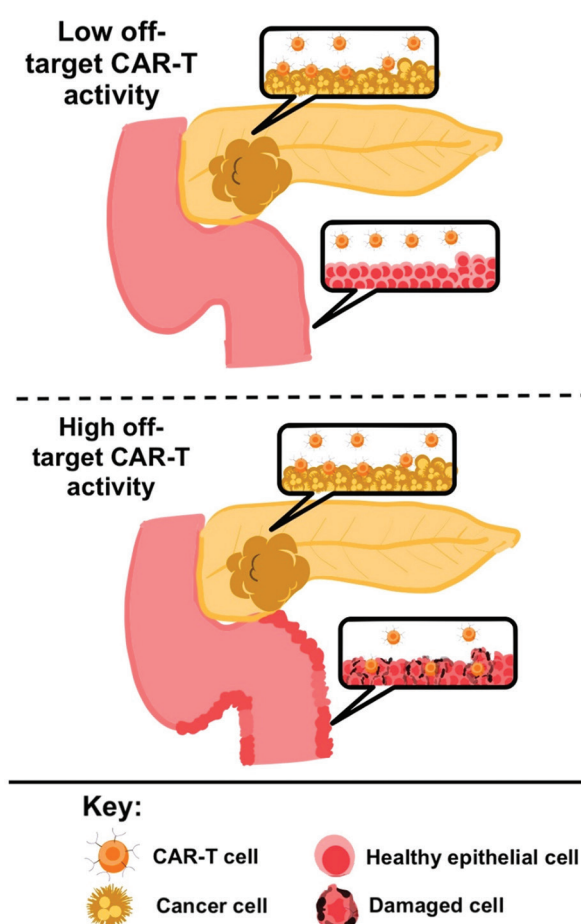


Figure 2. Precision targeting of CAR-T therapies is crucial to maximizing efficacy and minimizing harm. (A) Low off-target activity depicting CAR-T cells (orange) primarily targeting and eliminating cancer cells (yellow) in the pancreas, with minimal impact on healthy epithelial cells in nearby tissues. (B) High off-target activity depicting CAR-T cells causing significant damage to both cancer cells and epithelial cells, potentially resulting in detrimental side effects – a key limitation of this therapy.

have shown evidence of converting non-immunogenic tumors into immunogenic ones. When combined with cyclophosphamide, GVAX may improve outcomes in pancreatic cancer patients by depleting regulatory T cells and enhancing tumor infiltration.⁷⁰ This effect has been demonstrated in preclinical studies. For example, a murine model with early-stage, non-metastatic solid tumors showed that a single, personalized PD-L1 blockade cancer vaccine administered post-surgery inhibited tumor recurrence and distant metastases.⁷¹ Similarly, a clinical study of 48 patients with inoperable PAC reported statistically significant survival improvements when treated with a combination of mutant Ras peptides and GM-CSF.⁷² Furthermore, a phase I clinical trial using mRNA vaccines derived from neoantigens in resected

pancreatic cancer cells demonstrated promising activity and delayed recurrence. Although no vaccines for PDAC have been approved by the United States Food and Drug Administration, experimental treatments, albeit limited, have shown promise. Further research is crucial to advance these findings into clinical applications.

2.4. Epigenetic modifications

Several well-characterized epigenetic modifications – namely, DNA methylation, histone acetylation, and miRNA suppression – are linked to aberrant gene expression patterns that play critical roles in the pathophysiology of pancreatic cancer.⁵⁶ Evidence shows that aberrant DNA methylation is the most common alteration in cancer cells, surpassing cytogenetic abnormalities or gene mutations.^{73,74} Recent studies have mapped specific histone modifications and their suspected underlying correlations with particular cancer types (e.g., mono acetylation of histone-4 has been associated with breast and liver tumorigenesis).⁷³

In pancreatic cancer, a notable epigenetic modification is the hypomethylation of the *S100P* gene, which leads to the suppression of the expression of B-cell leukemia/lymphoma 2 protein (BCL2)/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3). This suppression inhibits apoptosis and induces chemoresistance.⁷⁵⁻⁷⁷ Similarly, miRNA silencing via aberrant epigenetic modifications has been significantly associated with carcinogenesis and the progression of metastasis in a wide array of human malignancies.⁷⁸⁻⁸⁰ Specific miRNA sequences, such as miR-93, miR-106b, miR-142, and miR195-5p, have all been shown to downregulate PD-L1 expression and modulate T-cell functions in pancreatic cancer.⁸¹⁻⁸⁴ Beyond their role in pathogenesis, epigenetic modifications hold promise for identifying disease biomarkers and developing therapeutic interventions. For instance, cell-free nucleosomes containing DNA and histone modifications have been identified as important markers of drug sensitivity in pancreatic cancer patients.⁸⁰

Epigenetic modifications, such as HDAC inhibitors, DNMT inhibitors, and EZH2 inhibitors, have emerged as promising targets for PDAC in clinical trials. While preclinical studies have shown some degree of efficacy, clinical trials targeting epigenetic regulators are still in their early stages. HDAC inhibitors have been evaluated in single-arm dose-finding studies, but the results have been inconsistent, showing minimal effects even at higher toxic doses.⁷⁸ Phase I trials involving DNMT inhibitors have shown promise in inducing antitumor responses in solid organ cancers, including pancreatic cancer.⁸¹ However, these therapies are limited by poor toxicity profiles and limited efficacy, achieving, at best, stable disease. Similarly,

EZH2 inhibitors tested in Phase I trials on two pancreatic cancer patients demonstrated safety but showed no partial or complete responses.³³

Although the results are mixed, epigenetic modifications remain a promising area for improving therapeutic outcomes in pancreatic cancer. Further research is needed to validate their efficacy.

Table 1 summarizes the various types of immunotherapies used in pancreatic cancer and their mechanisms of action.

2.5. Challenges in pancreatic cancer immunotherapy

Immunotherapy has demonstrated robust and significant efficacy in recent years as a treatment regime for solid tumors (such as melanoma, lung cancer, and renal cell carcinoma).^{83,84} However, phase I and II clinical trials have unfortunately not shown similar results in the realm of PAC.^{85,86} This disparity is hypothesized to stem from the unique TME of PAC tumors⁸⁷ and the lack of comprehensive mechanistic validation. The prevailing hypothesis for the immunogenic or “cold” nature of the PAC TME can be broadly classified into two major factors: (i) A marked deficiency in high-quality, effective immune effector cells, particularly anti-tumor T cells and NK cells, and (ii) an overabundance of immunosuppressive molecules, including epigenetic targets, that actively release suppressive chemokines into the surrounding environment.

While pre-clinical trials using various PAC mouse models demonstrated improved chemotherapy delivery upon depletion of the desmoplastic stroma in the TME,⁵⁰ these results did not translate effectively to clinical settings. For instance, a large-scale phase II clinical trial employing gemcitabine and a hedgehog pathway antagonist to target tumor stroma showed no significant improvements in overall survival or progression-free survival.⁷⁹ Interestingly, a more recent pre-clinical trial investigating fibroblast depletion in the TME stroma of PAC-bearing mice revealed that such depletion induced immunosuppression and was associated with reduced survival.³³ Despite these varying results, a consistent theme across studies highlights the pivotal role of the TME in PAC and its critical impact on the lack of response to immunotherapy.

3. The adjunct role of the microbiome in immunotherapy for pancreatic cancer

3.1. The microbiome and immune checkpoint inhibitor therapy

While numerous pre-clinical and clinical studies have investigated the microbiome's role in the initiation and progression of pancreatic cancer, relatively little research

Table 1. Examples of immune-targeting therapies

Therapy category	Example	Mechanism of action
Immune checkpoint inhibitors	Pembrolizumab (anti-PD-1), nivolumab (anti-PD-1)	Blocks interaction between checkpoint inhibitor proteins, enhancing T-cell response against cancer cells ⁵³⁻⁵⁵
T-cell therapy	TILs, T-cell receptor-engineered T-cells	Isolates and expands TILs from the tumor, then re-infusing them to target tumor cells ⁵⁷
CAR-T therapy	Mesothelin-specific CAR-T cells, mucin 1-specific CAR-T cells	Genetically engineered T cells targeting antigens overexpressed in pancreatic ductal adenocarcinoma ⁶⁹
Vaccines	GVAX, CRS-207 (<i>Listeria</i> -based vaccine)	Stimulates antitumor immune responses through inoculation with whole cells or bacteria ⁷⁴⁻⁷⁶
Epigenetic modifications	Azacitidine (DNA methyltransferase inhibitor), entinostat (histone deacetylase inhibitor)	Alters DNA expression patterns without modifying the underlying DNA structure ⁵⁹

Abbreviations: CAR-T: Chimeric antigen receptor T cell; GVAX: Granulocyte-macrophage colony-stimulating factor gene-transfected tumor cell vaccine; PD-1: Programmed cell death protein 1; TIL: Tumor-infiltrating lymphocyte.

has focused on the effect of specific microbes in modulating response to immune checkpoint inhibitor therapies. A landmark study by Vetizou *et al.*⁸⁴ investigated the effects of the microbiome on responses to CTLA-4 blockade therapy in both animal models and human subjects. The 2016 study demonstrated that tumors in melanoma-bearing mice responded to CTLA-4 blockade therapy only when the mice were also treated with *B. fragilis*, suggesting an immunostimulatory role for certain *Bacteroides* species in immune checkpoint inhibitor therapy. Furthermore, the study revealed that fecal microbiota transplant from melanoma patients treated with CTLA-4 antibodies induced an overgrowth of *B. fragilis* in recipient mice, implying a similar immunostimulatory effect may occur in humans.

Further evidence was provided by Sivan *et al.*,⁸⁵ who, in 2015, demonstrated the antitumor effects of *Bifidobacterium* species in mice with melanoma. Oral administration of *Bifidobacterium* was shown to be as effective as PD-L1 antibody immunotherapy in controlling tumor growth, while a combination of the two therapies nearly eliminated tumor outgrowth.

In the context of pancreatic cancer, Pushalkar *et al.*,⁸⁶ in 2018, identified Proteobacteria, *Bacteroidetes*, and *Firmicutes* as the predominant phyla in PAC patients, with relative abundances of 45%, 31%, and 22%, respectively, compared to healthy controls. Ablation of these bacterial species led to the upregulation of PD-1 expression in tumor cells. Importantly, in macrophages deficient in toll-like receptor (TLR) signaling, the immunosuppressive effects of these bacterial species were not observed. Further analysis revealed that Proteobacteria, in particular, were associated with advanced pancreatic disease, providing evidence that certain pathogenic Gram-negative bacteria promote an immunosuppressive TME through mechanisms involving lipopolysaccharides and flagella-mediated TLR activation.⁸⁶

Most recently, in 2019, a defined commensal consortium of 11 rare, healthy human-associated bacterial strains (7 *Bacteroidales* sp. and 4 non-*Bacteroidales* sp.) was shown to enhance the therapeutic efficacy of both PD-L1 and CTLA-4 checkpoint inhibitors.⁸⁷ This finding suggests that the microbiome not only modulates immune checkpoint inhibitor therapy responses in pancreatic cancers but also offers potential therapeutic applications across various cancer types.

3.2. The microbiome in T-cell transfer and chimeric antigen therapies

While specific bacterial species in the gut microbiome have not yet been directly linked to the modulation of TCR or CAR-T therapies, some evidence suggests certain species can enhance or suppress T-cell function, thereby influencing antitumor activity. For example, *A. muciniphila* was shown in a 2018 study by Routy *et al.*⁸⁸ to actively increase recruitment of CD4⁺ T-lymphocytes to epithelial tumor beds. The same study also demonstrated that *A. muciniphila* promotes IL-12 secretion by dendritic cells, enhancing T-helper (Th)1 cell differentiation and antitumor efficacy.

Similarly, a 2016 study reported that *E. hirae* exerts comparable effects on epithelial tumor cells by inducing IL-12 secretion, which subsequently upregulates Th1 cell differentiation into active T-cells.⁸⁹ In melanoma patients, certain species from the *Ruminococcaceae* family, in addition to their immunostimulatory effect on PD-L1 therapy, were associated with increased levels of peripheral and tumor-infiltrating T-cells in the tumor stroma.⁹⁰

In the context of PAC, species such as *Bifidobacterium* and *Bacteroidales* have been shown to enhance CD8⁺ T-cell infiltration and activation in murine models.⁹¹ This finding suggests these microbes may increase the efficacy of T-cell-targeting immunotherapies, such as CAR-T or TCR

therapies. Conversely, species such as *Fusobacterium* or *E. coli* have been linked to worse prognoses in pancreatic cancer due to their role in T-cell downregulation.⁸⁹ In addition, these bacteria have been associated with increased resistance to chemotherapy agents, such as gemcitabine, fludarabine, and cladribine, in PDAC.^{92,93}

3.3. The microbiome and therapeutic vaccines

Decades of research have established strong correlations between specific microbial pathogens, such as *H. pylori* and gastric cancer or *S. hematobium* and bladder cancer. Emerging evidence now links certain microbes to pancreatic cancer, highlighting their potential as targets for vaccine development. A randomized, double-blind, placebo-controlled clinical trial conducted in China involving over 4,400 patients demonstrated that an oral recombinant vaccine against *H. pylori* not only significantly reduced infection incidence but also decreased the long-term risk of gastric cancer.⁹⁴ While specific studies examining the microbiome's role in vaccine-based immunotherapy for PDAC are limited (likely due to the nascent stage of this technology), there is considerable evidence linking tumorigenic microbial species to PDAC pathogenesis. This connection underscores the potential clinical relevance of developing customized vaccines targeting these microbes.

For example, Proteobacteria comprises approximately 50% of the gut microbiome in PDAC patients, compared to only about 8% in healthy controls.⁸⁶ In addition, *P. gingivalis*, a periodontal pathogen, has been associated with an almost two-fold increased risk of pancreatic cancer, as demonstrated in a 2013 European study involving over 800 subjects.⁹⁵ Mechanistic studies suggest that *P. gingivalis* may release peptidyl-arginine deaminase, resulting in point mutations in *P53* and *RAS* genes, thereby promoting tumorigenesis in PDAC.⁹⁵

Multiple studies have examined salivary flora for its potential use in screening for the early detection of PDAC. A 2015 study involving 20 subjects demonstrated that the reduction of a combination of *Neisseria elongate* and *Streptococcus mitis* served as a reliable screening test for PDAC, yielding 96% sensitivity and 82% specificity.⁹⁶ Similarly, sequencing of the tongue microbiome in a 2019 study revealed that an increased prevalence of *Haemophilus*, *Porphyromonas*, *Leptotrichia*, and *Fusobacterium* effectively distinguished PDAC patients from healthy controls.⁹⁷ While significant overlap in microbial profiles may exist between PDAC patients and those with colorectal cancer or hepatocellular carcinoma, a separate 2019 study found that *Lactobacillus* levels were significantly higher in healthy controls compared to PDAC patients.⁹⁸ This finding not only highlights the presence of

tumorigenic microbial species, as observed in most studies but also suggests that dysbiosis can result in a decrease in immunostimulatory microbes, thereby facilitating PDAC development. Furthermore, the study identified microbial similarities between the pancreas and adjacent organs, such as the colon and liver, suggesting the potential migration of bacterial species across organs, which could directly influence dysplasia development. These findings underline the need for further research into customized vaccines targeting microbial species strongly correlated with PDAC pathogenesis, which could have tremendous clinical and scientific implications.

3.4. The microbiome and epigenetic modifications

Among immunotherapy types, the role of the microbiome in epigenetic modifications within pancreatic cancer remains the least studied. Although the mechanisms underlying miRNA and DNA methylation changes in PDAC oncogenesis are still unclear, their downstream effects on tumor response remain an active area of research.

In 2004, Sato *et al.*⁷⁵ identified significant patterns of *S100P* hypomethylation in pancreatic cancer cells compared with healthy controls. Building on this, Riquelme *et al.*⁹⁹ in 2019 linked changes in *S100P* methylation patterns with increased presence of *Fusobacterium*, *Porphyromonas*, and *Gemella*, suggesting a potential immunosuppressive role for these species in the context of epigenetic modification therapy. Similarly, Geller *et al.*¹⁰⁰ in 2017 associated certain intratumoral bacterial species, particularly *Porphyromonas* and *Streptococcus*, with elevated *S100P* expression in pancreatic cancer cells. These findings collectively suggest that certain microbial species may enhance immunostimulatory responses in epigenome modification therapies, although the underlying mechanisms remain unclear, and potential confounding factors cannot be excluded. This represents a significant opportunity to establish correlations between the microbiome and its immunomodulatory roles in epigenetic modification therapies.

Table 2 summarizes the roles of specific microbial species in modulating tumor responses to various immunotherapy types.

4. Future directions

The studies discussed in this article highlight the fundamental role of microbiome composition in modulating tumor responses to immunotherapy in cancer patients. Multiple ongoing clinical trials are evaluating the efficacy of combination immunotherapies in human PAC tissues compared to standard-of-care chemotherapy or radiotherapy. These studies further emphasize the potential of immunotherapy in pancreatic cancer management.

Table 2. Examples of specific microbial species that are associated with immunotherapy modulation

Immunotherapy type	Microbiota species with immunomodulatory potential
Immune checkpoint inhibitors	<i>Bacteriodes fragilis</i> (+) ⁸⁴ <i>Bifidobacterium</i> (+) ⁸⁵ Proteobacteria (-) ⁸⁶ <i>Bacterioidetes</i> (-) ⁸⁶ <i>Firmicutes</i> (-) ⁸⁹
T-cell therapy and CAR-T therapy	<i>Akkermansia muciniphila</i> (+) ⁸⁸ <i>Enterococcus hirae</i> (+) ⁹¹ <i>Bifidobacterium</i> (+) ⁸⁵ <i>Bacterioidales</i> (+) ⁸⁵ <i>Fusobacterium</i> (-) ^{89,92} <i>Escherichia coli</i> (-) ^{89,92}
Vaccines	<i>Neisseria elongate</i> (+) ⁹⁶ <i>Streptococcus mitis</i> (+) ⁹⁶ <i>Lactobacillus</i> (+) ⁹⁸ Proteobacteria (-) ⁸⁶ <i>Porphyromonas gingivalis</i> (-) ^{94,97} <i>Haemophilus</i> (-) ⁹⁷ <i>Leptotrichia</i> (-) ⁹⁷ <i>Fusobacterium</i> (-) ⁹⁷
Epigenetic modifications	<i>Fusobacterium</i> (-) ^{85,99} <i>Porphyromonas</i> (-) ^{85,99} <i>Gemella</i> (-) ^{85,99} <i>Streptococcus</i> (-) ¹⁰⁰

Notes: (+) Refers to associations with an immunostimulatory effect; (-) Refers to associations with an immunosuppressive effect.
Abbreviation: CAR-T: Chimeric antigen receptor T cell.

Evidence suggesting a correlation between increased microbiota diversity and altered responses to immunotherapy indicates that the gut microbiome warrants further exploration in the context of pancreatic cancer. Specifically, leveraging advanced technologies to evaluate the effects of specific microbes on the TME could significantly reduce the reliance on animal experiments for studying the role of gut flora in drug resistance.

As modern oncology increasingly adopts personalized therapies enabled by genomic profiling and genetic sequencing, microbiome composition analysis could emerge as a valuable predictive tool for prognosis and treatment efficacy. Individualized microbiome profiling may also provide valuable insights into potential correlations between microbiome diversity, epigenetic modifications, and treatment efficacy in modalities such as radiation therapy or surgical tumor resection.

5. Conclusion

Despite a wide array of technological and biological advancements in the realm of immunotherapy since its initial introduction as a potential treatment for pancreatic

cancer, several questions remain. Cytotoxic chemotherapy continues to be the cornerstone treatment for localized and advanced PDAC. While multiple preclinical and early-phase clinical studies support promising approaches such as immune checkpoint inhibitor therapies, T-cell transfer, CAR-T therapies, neoantigen vaccines, and epigenetic modification therapies, large-scale, practice-changing trials are still lacking.

The limited research into these modalities leaves gaps in understanding their efficacy and safety profiles. For instance, immune checkpoint inhibitors are effective in a very small subset of pancreatic cancer patients, while CAR-T therapy is hindered by off-target toxicities. Similarly, vaccine-based and epigenetic therapies show potential but are constrained by a lack of robust clinical evidence in pancreatic cancer.

Emerging evidence underscores the critical role of the gut microbiome in immunomodulation and its influence on tumor response to treatment. Species such as *Firmicutes* and *P. gingivalis* have been associated with immunosuppressive effects, whereas *B. fragilis* and *A. muciniphila* are linked to immunostimulatory responses. Conversely, Proteobacteria has been implicated in the progression to advanced stages of pancreatic cancer.

Despite the critical clinical and therapeutic potential of microbiome modulation, research in this area remains sparse, and translation from bench to bedside has proven challenging. Both pre-clinical and clinical studies are urgently needed to elucidate the microbiome's role in immunomodulation and metabolome interactions, potentially paving the way for a new class of therapeutics in pancreatic cancer.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

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References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin.* 2022;72(1):7-33.
doi: 10.3322/caac.21708
2. Lippi G, Mattiuzzi C. The global burden of pancreatic cancer. *Arch Med Sci.* 2020;16(4):820-824.
doi: 10.5114/aoms.2020.94845
3. Barreto SG, Shukla PJ, Shrikhande SV. Tumors of the pancreatic body and tail. *World J Oncol.* 2010;1(2):52-65.
doi: 10.4021/wjon2010.04.200w
4. Ushio J, Kanno A, Ikeda E, *et al.* Pancreatic ductal adenocarcinoma: Epidemiology and risk factors. *Diagnostics (Basel).* 2021;11(3):562.
doi: 10.3390/diagnostics11030562
5. Suurmeijer JA, Soer EC, Dings MPG, *et al.* Impact of classical and basal-like molecular subtypes on overall survival in resected pancreatic cancer in the SPACIOUS-2 multicentre study. *Br J Surg.* 2022;109(11):1150-1155.
doi: 10.1093/bjs/znac272
6. Rogers S, Charles A, Thomas RM. The prospect of harnessing the microbiome to improve immunotherapeutic response in pancreatic cancer. *Cancers.* 2023;15(24):5708.
doi: 10.3390/cancers15245708
7. Bangolo AI, Trivedi C, Jani I, *et al.* Impact of gut microbiome in the development and treatment of pancreatic cancer: Newer insights. *World J Gastroenterol.* 2023;29(25):3984-3998.
doi: 10.3748/wjg.v29.i25.3984
8. Kamisawa T, Wood LD, Itoi T, Takaori K. Pancreatic cancer. *Lancet.* 2016;388(10039):73-85.
doi: 10.1016/S0140-6736(16)00141-0
9. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science.* 2018;359(6382):1350-1355.
doi: 10.1126/science.aar4060
10. O'Reilly EM, Oh DY, Dhani N, *et al.* Durvalumab with or without tremelimumab for patients with metastatic pancreatic ductal adenocarcinoma: A phase 2 randomized clinical trial. *JAMA Oncol.* 2019;5(10):1431-1438.
doi: 10.1001/jamaoncol.2019.1588
11. Kamath SD, Kalyan A, Kircher S, *et al.* Ipilimumab and gemcitabine for advanced pancreatic cancer: A phase Ib study. *Oncologist.* 2020;25(5):e808-e815.
doi: 10.1634/theoncologist.2019-0473
12. Mahalingam D, Wilkinson GA, Eng KH, *et al.* Pembrolizumab in combination with the oncolytic virus Pelareorep and chemotherapy in patients with advanced pancreatic adenocarcinoma: A phase Ib study. *Clin Cancer Res.* 2020;26(1):71-81.
doi: 10.1158/1078-0432.CCR-19-2078
13. Krishnamurthy A, Jimeno A. Atezolizumab: A novel PD-L1 inhibitor in cancer therapy with a focus in bladder and non-small cell lung cancers. *Drugs Today (Barc).* 2017;53(4):217-237.
doi: 10.1358/dot.2017.53.4.2589163
14. Necchi A, Joseph RW, Loriot Y, *et al.* Atezolizumab in platinum-treated locally advanced or metastatic urothelial carcinoma: Post-progression outcomes from the phase II IMvigor210 study. *Ann Oncol.* 2017;28(12):3044-3050.
doi: 10.1093/annonc/mdx518
15. Fukunaga A, Miyamoto M, Cho Y, *et al.* CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. *Pancreas.* 2004;28(1):e26-e31.
doi: 10.1097/00006676-200401000-00023
16. Schmitz-Winnenthal FH, Volk C, Z'Graggen K, *et al.* High frequencies of functional tumor-reactive T cells in bone marrow and blood of pancreatic cancer patients. *Cancer Res.* 2005;65(21):10079-10087.
doi: 10.1158/0008-5472.CAN-05-1098
17. Neesse A, Algul H, Tuveson DA, Gress TM. Stromal biology and therapy in pancreatic cancer: A changing paradigm. *Gut.* 2015;64(9):1476-1484.
doi: 10.1136/gutjnl-2015-309304
18. Zhao P, Wang Y, Kang X, *et al.* Dual-targeting biomimetic delivery for anti-glioma activity via remodeling the tumor microenvironment and directing macrophage-mediated immunotherapy. *Chem Sci.* 2018;9(10):2674-2689.
doi: 10.1039/c7sc04853j
19. Kaplanov I, Carmi Y, Kornetsky R, Shemesh A, Shaked Y, Yefenof E. Blocking IL-1 β reverses the immunosuppression in mouse breast cancer and synergizes with anti-PD-1 for tumor abrogation. *Proc Natl Acad Sci U S A.* 2019;116(4):1361-1369.
doi: 10.1073/pnas.1812266115
20. Tsunedomi R, Shindo Y, Nakajima M, Yoshimura K, Nagano H. The tumor immune microenvironment in pancreatic cancer and its potential in the identification of immunotherapy biomarkers. *Expert Rev Mol Diagn.* 2023;23(12):1121-1134.

- doi: 10.1080/14737159.2023.2281482
21. Ryschich E, Notzel T, Hinz U, Autschbach F, Kaufmann M, Klar E. Control of T-cell-mediated immune response by HLA class I in human pancreatic carcinoma. *Clin Cancer Res.* 2005;11(2 Pt 1):498-504.
 22. Malladi S, Macalinao DG, Jin X, *et al.* Metastatic latency and immune evasion through autocrine inhibition of WNT. *Cell.* 2016;165(1):45-60.
doi: 10.1016/j.cell.2016.02.025
 23. Böttcher JP, Bonavita E, Chakravarty P, *et al.* NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell.* 2018;172(5):1022.e14-1037.e14.
doi: 10.1016/j.cell.2018.01.004
 24. Skertich NJ, Chu F, Tarhoni IA, Szajek S, Borgia JA, Madonna MB. Expression of immunomodulatory checkpoint molecules in drug-resistant neuroblastoma: An exploratory study. *Cancers.* 2022;14(3):751.
doi: 10.3390/cancers14030751
 25. Böger C, Behrens HM, Krüger S, Röcken C. The novel negative checkpoint regulator VISTA is expressed in gastric carcinoma and associated with PD-L1/PD-1: A future perspective for a combined gastric cancer therapy? *Oncoimmunology.* 2017;6(4):e1293215.
doi: 10.1080/2162402x.2017.1293215
 26. Faget J, Biota C, Bachelot T, *et al.* Early detection of tumor cells by innate immune cells leads to T(reg) recruitment through CCL22 production by tumor cells. *Cancer Res.* 2011;71(19):6143-6152.
doi: 10.1158/0008-5472.Can-11-0573
 27. Padoan A, Plebani M, Basso D. Inflammation and pancreatic cancer: Focus on metabolism, cytokines, and immunity. *Int J Mol Sci.* 2019;20(3):676.
doi: 10.3390/ijms20030676
 28. Wang D, Dubois RN. Eicosanoids and cancer. *Nat Rev Cancer.* 2010;10(3):181-193.
doi: 10.1038/nrc2809
 29. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer.* 2012;12(4):252-264.
doi: 10.1038/nrc3239
 30. ElTanbouly MA, Croteau W, Noelle RJ, Lines JL. VISTA: A novel immunotherapy target for normalizing innate and adaptive immunity. *Semin Immunol.* 2019;42:101308.
doi: 10.1016/j.smim.2019.101308
 31. Thomas AA, Fisher JL, Hampton TH, Galbraith K, Gilbert MR, Lathia JD. Immune modulation associated with vascular endothelial growth factor (VEGF) blockade in patients with glioblastoma. *Cancer Immunol Immunother.* 2017;66(3):379-389.
doi: 10.1007/s00262-016-1941-3
 32. Bagchi S, Yuan R, Engleman EG. Immune checkpoint inhibitors for the treatment of cancer: Clinical impact and mechanisms of response and resistance. *Annu Rev Pathol.* 2021;16(1):223-249.
doi: 10.1146/annurev-pathol-042020-042741
 33. Waterhouse P, Penninger JM, Timms E, *et al.* Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science.* 1995;270(5238):985-988.
doi: 10.1126/science.270.5238.985
 34. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity.* 1999;11(2):141-151.
doi: 10.1016/s1074-7613(00)80089-8
 35. León-Letelier RA, Dou R, Vykoukal J, *et al.* Contributions of the microbiome-derived metabolome for risk assessment and prognostication of pancreatic cancer. *Clin Chem.* 2024;70(1):102-115.
doi: 10.1093/clinchem/hvad186
 36. Cruz MS, Tintelnot J, Gagliani N. Roles of microbiota in pancreatic cancer development and treatment. *Gut Microbes.* 2024;16(1):2320280.
doi: 10.1080/19490976.2024.2320280
 37. Attebury H, Daley D. The gut microbiome and pancreatic cancer development and treatment. *Cancer J.* 2023;29(2):49-56.
doi: 10.1097/PPO.0000000000000647
 38. Zhao F, Chen A, Wu X, Sun J, Wang X, Zhang J. Heterogeneous changes in gut and tumor microbiota in patients with pancreatic cancer: Insights from clinical evidence. *BMC Cancer.* 2024;24:478.
doi: 10.1186/s12885-024-12202-z
 39. Pourali G, Kazemi D, Chadeganipour AS, Rezvanfar M. Microbiome as a biomarker and therapeutic target in pancreatic cancer. *BMC Microbiol.* 2024;24:16.
doi: 10.1186/s12866-023-03166-4
 40. Guo X, Zhang L, Li Q, Chen Z. Microbiomes in pancreatic cancer can be an accomplice or a weapon. *Crit Rev Oncol Hematol.* 2024;194:104262.
doi: 10.1016/j.critrevonc.2024.104262
 41. Bastos AR, Pereira-Marques J, Ferreira RM, Figueiredo C. Harnessing the microbiome to reduce pancreatic cancer burden. *Cancers (Basel).* 2023;15(9):2629.
doi: 10.3390/cancers15092629
 42. Ansari D, Ibrahim H, Andersson R. Microbiome alterations in pancreatic cancer: New insights and potential clinical

- implications. *Scand J Gastroenterol*. 2023;59(2):202-203.
doi: 10.1080/00365521.2023.2261062
43. Kang X, Lau HC, Yu J. Modulating gut microbiome in cancer immunotherapy: Harnessing microbes to enhance treatment efficacy. *Cell Rep Med*. 2024;5(4):101478.
doi: 10.1016/j.xcrm.2024.100598
44. Papa V, Schepis T, Coppola G, et al. The role of microbiota in pancreatic cancer. *Cancers (Basel)*. 2023;15(12):3143.
doi: 10.3390/cancers15123143
45. Chai Y, Huang Z, Shen X, et al. Microbiota regulates pancreatic cancer carcinogenesis through altered immune response. *Microorganisms*. 2023;11(5):1240.
doi: 10.3390/microorganisms11051240
46. Halle-Smith JM, Hall LA, Powell-Brett SF, et al. Pancreatic exocrine insufficiency and the gut microbiome in pancreatic cancer: A target for future diagnostic tests and therapies? *Cancers (Basel)*. 2023;15(21):5140.
doi: 10.3390/cancers15215140
47. Zhang B, Liu J, Li H, et al. Integrated multi-omics identified the novel intratumor microbiome-derived subtypes and signature to predict the outcome, tumor microenvironment heterogeneity, and immunotherapy response for pancreatic cancer patients. *Front Pharmacol*. 2023;14:1244752.
doi: 10.3389/fphar.2023.1244752
48. Villemain C, Six A, Neville BA, Lawley TD, Robinson MJ, Bakdash G. The heightened importance of the microbiome in cancer immunotherapy. *Trends Immunol*. 2023;44(1):44-59.
doi: 10.1016/j.it.2023.10.004
49. Nista EC, Del Gaudio A, Del Vecchio LE, et al. Pancreatic cancer resistance to treatment: The role of microbiota. *Biomedicines*. 2023;11(1):157.
doi: 10.3390/biomedicines11010157
50. Halle-Smith JM, Pearce H, Nicol S, et al. Involvement of the gut microbiome in the local and systemic immune response to pancreatic ductal adenocarcinoma. *Cancers (Basel)*. 2024;16(5):996.
doi: 10.3390/cancers16050996
51. Freeman GJ, Long AJ, Iwai Y, Dahan R, Fitz LJ, Byrne MC. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*. 2000;192(7):1027-1034.
doi: 10.1084/jem.192.7.1027
52. Nishimura, H., Nose, M., Hiai, H., Minato, N., & Honjo, T. (1999). Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity*, 11(2), 141-151.
doi: 10.1016/s1074-7613(00)80089-8
53. Rataj F, Kraus FBT, Chaloupka M, Rajasekaran D, Hecht C, Maier B. PD1-CD28 fusion protein enables CD4+ T cell help for adoptive T cell therapy in models of pancreatic cancer and non-Hodgkin lymphoma. *Front Immunol*. 2018;9:955.
doi: 10.3389/fimmu.2018.01955
54. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. *Cancer Discov*. 2013;3(4):388-398.
doi: 10.1158/2159-8290.CD-12-0548
55. Xiong HQ, Rosenberg A, LoBuglio A, Bennett M, McCarthy R. Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: A multicenter phase II trial. *J Clin Oncol*. 2004;22(13):2610-2616.
doi: 10.1200/JCO.2004.12.040
56. Thompson JK, Bednar F. Clinical utility of epigenetic changes in pancreatic adenocarcinoma. *Epigenomes*. 2021;5(4):20.
doi: 10.3390/epigenomes5040020
57. Lutz ER, Wu AA, Bigelow E, Svoronos N, Fisher R. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. *Cancer Immunol Res*. 2014;2(7):616-631.
doi: 10.1158/2326-6066.CIR-14-0027
58. Marcus L, Lemery SJ, Keegan P, Pazdur R. FDA approval summary: Pembrolizumab for the treatment of microsatellite instability-high solid tumors. *Clin Cancer Res*. 2019;25(13):3753-3758.
doi: 10.1158/1078-0432.CCR-18-4070
59. Buchsbaum DJ, Bonner JA, Grizzle WE, Allred DC, Gehan EA. Treatment of pancreatic cancer xenografts with Erbitux (IMC-C225) anti-EGFR antibody, gemcitabine, and radiation. *Int J Radiat Oncol Biol Phys*. 2002;54(4):1180-1193.
doi: 10.1016/S0360-3016(02)03788-4
60. Tauriello DVE, Palomo-Ponce S, Stork D, Sancho E. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature*. 2018;554(7693):538-543.
doi: 10.1038/nature25492
61. Brahmer JR, Tykodi SS, Chow LQM, Hwu WJ, Topalian SL, Smith DC. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366(26):2455-2465.
doi: 10.1056/NEJMoa1200694
62. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. *Oncogene*. 2000;19(56):6550-6565.
doi: 10.1038/sj.onc.1204082
63. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh R. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409-413.

- doi: 10.1126/science.aan6733
64. Ahmed N, Salsman VS, Yvon E, McIntyre R, Nishida Y. Immunotherapy for osteosarcoma: Genetic modification of T cells overcomes low levels of tumor antigen expression. *Mol Ther*. 2009;17(10):1779-1787.
doi: 10.1038/mt.2009.133
65. Morello A, Sadelain M, Adusumilli PS. Mesothelin-targeted CARs: Driving T cells to solid tumors. *Cancer Discov*. 2016;6(2):133-146.
doi: 10.1158/2159-8290.CD-15-0583
66. Wachsmann TLA, Wouters AK, Remst DFG, Santeagoets SJAM. Comparing CAR and TCR engineered T cell performance as a function of tumor cell exposure. *Oncoimmunology*. 2022;11(1):2033528.
doi: 10.1080/2162402X.2022.2033528
67. Jiang H, Shi Z, Wang P, Liu X, Zhang Y. Claudin18.2-specific chimeric antigen receptor engineered T cells for the treatment of gastric cancer. *JNCI J Natl Cancer Inst*. 2018;111(4):409-418.
doi: 10.1093/jnci/djy134
68. Foley K, Kim V, Jaffee E, Zheng L. Current progress in immunotherapy for pancreatic cancer. *Cancer Lett*. 2016;381(1):244-251.
doi: 10.1016/j.canlet.2015.12.020
69. Maus MV, Haas AR, Beatty GL, Albelda SM. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. *Cancer Immunol Res*. 2013;1(1):26-31.
doi: 10.1158/2326-6066.CIR-13-0006
70. Jaffee EM, Hruban RH, Biedrzycki B, Lutz ER. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: A phase I trial of safety and immune activation. *J Clin Oncol*. 2001;19(1):145-156.
doi: 10.1200/JCO.2001.19.1.145
71. Wang T, Wang D, Yu H, et al. A cancer vaccine-mediated postoperative immunotherapy for recurrent and metastatic tumors. *Nat Commun*. 2018;9(1):1532.
doi: 10.1038/s41467-018-03915-4
72. Gjertsen MK, Breivik J, Saeterdal I, Kvalheim G. Vaccination with mutant ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding RAS mutation. *Lancet*. 1995;346(8987):1399-1400.
doi: 10.1016/S0140-6736(95)92408-6
73. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet*. 2007;8(4):286-298.
doi: 10.1038/nrg2005
74. Esteller M. Aberrant DNA methylation as a cancer-inducing mechanism. *Annu Rev Pharmacol Toxicol*. 2005;45:629-656.
doi: 10.1146/annurev.pharmtox.45.120403.095832
75. Sato N, Fukushima N, Matsubayashi H, Goggins M. Identification of maspin and S100P as novel hypomethylation targets in pancreatic cancer using global gene expression profiling. *Oncogene*. 2004;23(8):1531-1538.
doi: 10.1038/sj.onc.1207269
76. Mahon PC, Baril P, Bhakta V, Stevens M. S100A4 contributes to the suppression of BNIP3 expression, chemoresistance, and inhibition of apoptosis in pancreatic cancer. *Cancer Res*. 2007;14(6):6786-6795.
doi: 10.1158/0008-5472.CAN-07-0440
77. Zhou WY, Zhang MM, Liu C, Kang Y, Wang JO, Yang XH. Long noncoding RNA LINC00473 drives the progression of pancreatic cancer via upregulating programmed death-ligand 1 by sponging microRNA-195-5p. *J Cell Physiol*. 2019;234(12):23176-23189.
doi: 10.1002/jcp.28884
78. Bauden M, Pamart D, Ansari D, Andersson R. Circulating nucleosomes as epigenetic biomarkers in pancreatic cancer. *Clin Epigenet*. 2015;7:106.
doi: 10.1186/s13148-015-0139-4
79. Von Hoff DD, Rasco DW, Heath EI, Wang X, Wei X, Zhang Y. Phase I study of CC-486 alone and in combination with carboplatin or nab-paclitaxel in patients with relapsed or refractory solid tumors. *Clin Cancer Res*. 2018;24(17):4072-4080.
doi: 10.1158/1078-0432.CCR-17-3716
80. Kanwal R, Gupta S. Epigenetic modifications in cancer. *Clin Genet*. 2012;81(4):303-311.
doi: 10.1111/j.1399-0004.2011.01809.x
81. Fabbri M, Calore F, Paone A, Galli R, Calin GA. Epigenetic regulation of miRNAs in cancer. *Adv Exp Med Biol*. 2013;754:137-148.
doi: 10.1007/978-1-4419-9967-2_6
82. Cioffi M, Trabulo SM, Vallespinos M, Dusetti NJ, De Giorgio A. The miR-25-93-106b cluster regulates tumor metastasis and immune evasion via modulation of CXCL12 and PD-L1. *Oncotarget*. 2017;8(13):21609-21625.
doi: 10.18632/oncotarget.15450
83. Jia L, Xi Q, Wang H, Zhang Q, Liu T. miR-142-5p regulates tumor cell PD-L1 expression and enhances anti-tumor immunity. *Biochem Biophys Res Commun*. 2017;488(2):425-431.
doi: 10.1016/j.bbrc.2017.05.074
84. Vetizou M, Pitt JM, Daillere R, Messaoudi I. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*. 2015;350(6264):1079-1084.
doi: 10.1126/science.aad1329
85. Sivan A, Corrales L, Hubert N, Williams JA, Aquino-Michaels K, Gajewski TF. Commensal *Bifidobacterium*

- promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science*. 2015;350(6264):1084-1089.
doi: 10.1126/science.aac4255
86. Pushalkar S, Hundeyin M, Daley D, Mantri C. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. *Cancer Discov*. 2018;8(4):403-416.
doi: 10.1158/2159-8290.CD-17-1134
87. Tanoue T, Morita S, Plichta DR, Shinohara M. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature*. 2019;565(7741):600-605.
doi: 10.1038/s41586-019-0878-z
88. Routy B, Le Chatelier E, Derosa L, Alou MT. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*. 2018;359(6371):91-97.
doi: 10.1126/science.aan3706
89. Komai-Koma M, Wang E, Kurowska-Stolarska M, Li D, McSharry C, Xu D. Interleukin-33 promoting Th1 lymphocyte differentiation depends on IL-12. *Immunobiology*. 2016;221(3):412-417.
doi: 10.1016/j.imbio.2015.11.013
90. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science*. 2018;359(6371):97-103.
doi: 10.1126/science.aan4236
91. Mitsuhashi K, Noshio K, Sukawa Y, Ogawa S. Association of Fusobacterium species in pancreatic cancer tissues with molecular features and prognosis. *Oncotarget*. 2015;6(9):7209-7210.
doi: 10.18632/oncotarget.3109
92. Lehouritis P, Cummins J, Stanton M, Smith T. Local bacteria affect the efficacy of chemotherapeutic drugs. *Sci Rep*. 2015;5:14554.
doi: 10.1038/srep14554
93. Vande Voorde J, Sabuncuoglu S, Noppen S, Vandembroucke R. Nucleoside-catabolizing enzymes in mycoplasma-infected tumor cell cultures compromise the cytostatic activity of the anticancer drug gemcitabine. *J Biol Chem*. 2014;289(19):13054-13065.
doi: 10.1074/jbc.M114.558924
94. Zeng M, Mao XH, Li JX, Liu CM. Efficacy, safety, and immunogenicity of an oral recombinant Helicobacter pylori vaccine in children in China: A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2015;386(10002):1457-1464.
doi: 10.1016/S0140-6736(15)60310-5
95. Ogrendik M. Periodontal pathogens in the etiology of pancreatic cancer. *Gastrointest Tumors*. 2017;3(3-4):125-127.
doi: 10.1159/000452708
96. Farrell JJ, Zhang L, Zhou H, Liu Y. Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. *Gut*. 2012;61(4):582-588.
doi: 10.1136/gutjnl-2011-300784
97. Lu H, Ren Z, Li A, Ma L. Tongue coating microbiome data distinguish patients with pancreatic head cancer from healthy controls. *J Oral Microbiol*. 2019;11(1):1563409.
doi: 10.1080/20002297.2018.1563409
98. Del Castillo E, Meier R, Chung M, Rausch P. The microbiomes of pancreatic and duodenum tissue overlap and are highly subject-specific but differ between pancreatic cancer and noncancer subjects. *Cancer Epidemiol Biomark Prev*. 2019;28(2):370-383.
doi: 10.1158/1055-9965.EPI-18-0542
99. Riquelme E, Zhang Y, Zhang L, Yoon S. Tumor microbiome diversity and composition influence pancreatic cancer outcomes. *Cell*. 2019;178(4):795.e12-806.e12.
doi: 10.1016/j.cell.2019.07.008
100. Geller LT, Barzily-Rokni M, Danino T, Jamin Y. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science*. 2017;357(6356):1156-1160.
doi: 10.1126/science.aah5043