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The role of microbiome in pancreatic cancer

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Abstract

Recent studies of the human microbiome have offered new insights into how the microbiome can impact cancer development and treatment. Specifically, in pancreatic ductal adenocarcinoma (PDAC), the microbiota has been shown to modulate PDAC risk, contribute to tumorigenesis, impact the tumor microenvironment, and alter treatment response. These findings provide rationale for further investigations into leveraging the microbiome to develop new strategies to diagnose and treat PDAC patients. There is growing evidence that microbiome analyses have the potential to become easily performed, non-invasive diagnostic, prognostic, and predictive biomarkers in pancreatic cancer. More excitingly, there is now emerging interest in developing interventions based on the modulation of microbiota. Fecal microbiota transplantation, probiotics, dietary changes, and antibiotics are all potential strategies to augment the efficacy of current therapeutics and reduce toxicities. While there are still challenges to overcome, this is a rapidly growing field that holds promise for translation into clinical practice and provides a new approach to improving patient outcomes.

Keywords Pancreatic cancer · Microbiome · Biomarker · Microbiota modulation

1 Introduction

Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of all pancreatic cancers and is the third leading cause of cancer-related deaths in the USA [1]. An estimated 60,430 new cases of PDAC will be diagnosed in 2021 [1]. Surgical resection remains the only curative therapy but only around 20% of patients have resectable tumors at diagnosis [2]. Most patients with PDAC present with either locally advanced (40%) or metastatic (40%) disease, and standard-of-care treatments are limited to palliative systemic therapies. Despite continued efforts, the 5-year overall survival rate of PDAC patients remains around 10% for all stages combined and drops to only 3% for those with distant disease [3]. Multiple factors contribute to the poor outcomes including nonspecific symptoms, the lack of early diagnostic markers, aggressive tumor biology/early metastasis, and resistance to chemotherapy. Clearly, there is a critical need for novel approaches to screening, prevention, and treatment.

The microbiota (bacteria, archaea, viruses, fungi, protozoa) inhabiting the human body are estimated to be in the range of 10–100 trillion [4]. The combined genetic material harbored by these microorganisms, known as the microbiome, far exceeds that of the human genome [5]. While the majority of microorganisms reside in the gastrointestinal tracts, microbiota can be found in other external and internal surfaces of the human body such as the skin, oral cavity, conjunctiva, and genitourinary tracts [6]. They play an essential role in maintaining homeostasis in the human body, and an imbalance of the microbiota, a state known as dysbiosis, can contribute to the pathogenesis of many diseases [7]. Increasingly, the microbiota is recognized to contribute to carcinogenesis and treatment outcomes [8]. Microbes such as *Helicobacter pylori*, human papillomaviruses, and hepatitis viruses have been linked to human malignancies, and infectious agents are thought to underlie the development of 10–20% of the global cancer cases each year [9]. Microbial dysbiosis can also positively and negatively impact tumor responses to therapies [10]. The microbiota has the ability to promote inflammatory responses, change the tumor immune microenvironment, alter tumor metabolism, and modulate tumor sensitivity to drugs [11–15]. Thus, the microbiome holds significant promise when developing PDAC management strategies.

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In this review, we will summarize the current understanding on the role of bacteria and fungi in PDAC tumorigenesis and explore the potential translational implications of targeting the microbiome in the management of PDAC.

2 The microbiome and pancreatic cancer

While researchers first noted the presence of bacteria in human tumors more than 100 years ago, these microbes were difficult to characterize due to their low biomass and the possibility of tumor sample contamination [16]. The development of newer analysis techniques has allowed for more in-depth examination and characterization of these microbes in recent years. The most common technique used to identify bacterial populations is the sequencing of amplified 16S ribosomal RNA (rRNA), and a similar technique that relies on the sequencing of amplified internal transcriber spacer (ITS) regions between various rRNA subunit genes can be used to identify the fungal composition in samples [17, 18]. Using these techniques, researchers have been able to interrogate the microbiomes of both healthy individuals and PDAC patients, with interesting patterns emerging (Figure 1).

2.1 Microbiota in pancreas

Historically, the pancreas was thought to be a sterile organ, but recent studies have found the existence of bacteria populations in both normal pancreatic tissue and PDAC tumor samples. Nejman et al. examined the bacterial composition of 1010 tumor samples across 7 different tumor types and 516 normal samples [16]. They found that pancreatic cancer had one of the highest proportions of tumor positive for bacterial DNA, and bacteria belonging to the Proteobacteria phylum were most abundant [16]. Geller et al. conducted real-time quantitative polymerase chain reaction (qPCR) to detect bacterial 16S ribosomal DNA in 113 human PDAC samples and 20 normal pancreas samples [15]. They were able to detect bacterial DNA in 15% of the normal pancreas samples and 76% of the PDAC samples, and the presence of intratumoral bacteria was confirmed by fluorescence *in situ* hybridization (FISH) targeted to 16S rRNA and immunohistochemistry. The authors further examined the specific bacterial species present in the tumors by sequencing amplified 16S rRNA genes and found that the most common species belonged to the Enterobacteriaceae and Pseudomonadaceae families [15]. Pushalkar et al. also compared the bacteria composition of 12 PDAC samples and 5 normal pancreas samples [19]. By using FISH, the authors found that the human PDAC samples harbored higher abundance of bacteria when compared to the normal pancreas samples. Sequencing of 16S rRNA revealed that the most prevalent intratumoral bacteria phyla included Proteobacteria, Bacteroidetes, and Firmicutes. Assessment of clade abundances confirmed that the bacterial composition of PDAC was distinct from normal human pancreas [19]. Additional studies of the microbiota in PDAC tissue also confirmed the presence of a distinct bacterial intratumoral profile [16, 20, 21].

Similar to the bacterial findings, Aykut et al. recently showed that the fungal flora (mycobiome) of PDAC samples are distinct from healthy samples as well [22]. They analyzed the intrapancreatic mycobiome of 13 PDAC patients and 5 healthy individuals, and they discovered that the fungal communities of PDAC samples clustered separately from the healthy samples. Specifically, PDAC samples were enriched for Malassezia species [22].

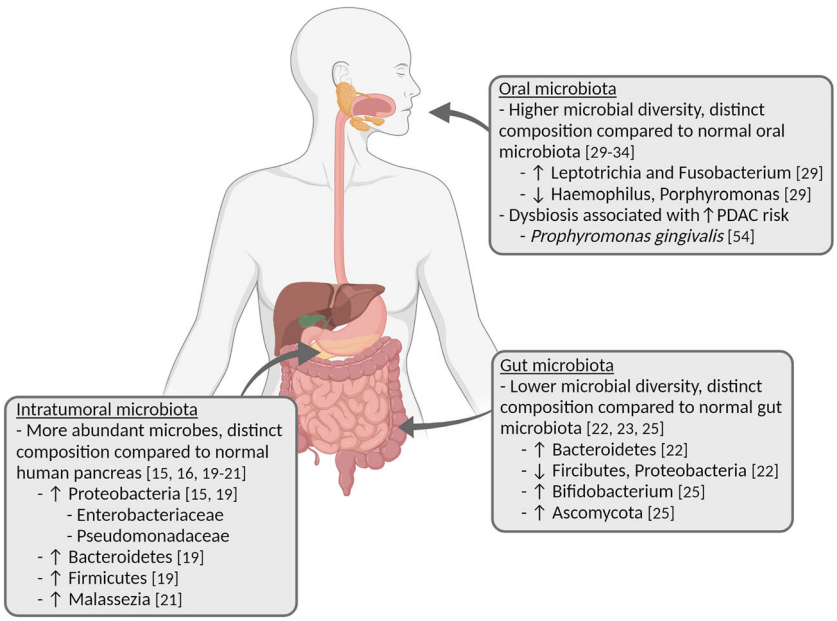

Fig. 1 Microbiota in pancreatic cancer. Created with BioRender.com

2.2 Gastrointestinal microbiota

In addition to intratumoral dysbiosis, several studies have shown a difference in the gut microbiota between PDAC patients and healthy individuals. In a study by Ren et al., fecal samples from 85 PDAC patients and 57 matched healthy controls were collected prospectively and analyzed for their microbial characteristics [23]. The gut microbial diversity was found to be significantly lower in PDAC patients. The composition of the gut microbiota was also unique in PDAC patients and contained significantly higher Bacteroidetes and lower Firmicutes and Proteobacteria when compared to healthy controls [23]. Rogers et al. characterized the bacterial composition of fecal samples, pancreatic fluid, bile, and jejunal fluid from 50 patients undergoing pancreaticoduodenectomy [21]. The microbial diversity of the fecal samples was significantly lower than samples from healthy volunteers, and they were enriched with Klebsiella and Bacteroides [21]. A separate study conducted by Half and colleagues examined the fecal microbiota of 30 PDAC patients, 13 healthy individuals, and 16 patients with non-alcoholic fatty liver disease [24]. While this study did not find any differences in the microbial diversity between groups, results did show that PDAC patients had a distinct microbial profile when compared to controls and had a higher Bacteroidetes to Firmicutes ratio, which is consistent with the findings of Ren et al. [23, 24].

In a small case-control study, analysis of the duodenal mucosal microbiota in 14 PDAC patients and 14 healthy individuals showed no significant difference between the two groups [25]. However, a later study of bacterial and fungal profiles of duodenal fluid from 74 PDAC patients, 98 patients with pancreatic cysts, and 134 normal controls revealed that patients with PDAC had significantly decreased bacterial and fungal diversity when compared to patients with pancreatic cysts and healthy controls [26]. PDAC patients also had an enrichment of Bifidobacterium and Ascomycota compared to healthy individuals. The duodenal fluid microbiota profiles were not significantly different between patients with pancreatic cysts and healthy controls [26].

The differences in study sizes, designs, sampling methods, and primers used for 16S rRNA amplification make interpretation and generalization difficult. Moreover, the decrease in gut microbial diversity compared to healthy individuals is observed in other chronic diseases as well [27–29].

2.3 Oral microbiota

Finally, the oral microbiota is different between PDAC patients and healthy controls as well. Lu and colleagues examined the tongue coating microbiota in 30 PDAC patients and 25 healthy controls [30]. They found that the microbiota diversity of the tongue coat in PDAC patients was significantly increased and the bacterial composition of the tongue coating is markedly different between PDAC patients and controls. A few bacterial genera (Haemophilus, Porphyromonas, Leptotrichia, and Fusobacterium) could distinguish PDAC patients from healthy individuals [30].

Additionally, multiple studies have shown a difference between the saliva microbiota profiles of PDAC patients compared to healthy controls, though different bacteria species were identified as the distinguishing factor in the different studies [31–35]. Similar to the gut microbiota studies, the inconsistency may be related to differences in study size, study design, and geographic location.

2.4 Microbial dysbiosis and PDAC risk

Prior epidemiologic studies have identified multiple risk factors for the development of PDAC including age, family history, cigarette smoking, obesity, chronic pancreatitis, and type 2 diabetes [36–41]. Microbial dysbiosis is associated with many of these environmental risk factors such obesity, chronic pancreatitis, and type 2 diabetes, which can potentially impact the risk of PDAC [42–48].

Interestingly, periodontal disease has been linked to increased PDAC risk. Hujoel et al. analyzed data from 11,328 patients from the National Health and Nutrition Examination Survey (NHANES) I Epidemiologic Follow-up Study and found a positive but nonsignificant association between periodontitis and PDAC risk (odds ratio [OR], 1.77; 95% confidence interval [CI], 0.85–3.67) [49]. In another prospective cohort study that included 48,375 US male health professionals, Michaud et al. found that after adjusting for known risk factors such as smoking history, body mass index, and history of diabetes, patients with a history of periodontal disease had significantly increased risk for PDAC (hazard ratio [HR], 1.54; 95% CI, 1.16–2.04) [50]. In a third cohort study, Chang et al. examined data from 139,805 patients and reported a significantly positive association between periodontal disease and PDAC risk (HR, 1.55; 95% CI, 1.02–2.33) [51]. This association is independent of age, sex, and medical comorbidities including diabetes, hyperlipidemia, allergies, viral hepatitis, peptic ulcer, pancreatitis, chronic obstructive pulmonary disease (as a proxy for cigarette smoking), and alcoholic-related conditions (as a proxy for alcohol drinking) [51]. Finally, a meta-analysis of 8 studies by Maisonneuve and colleagues confirmed a significantly positive association between periodontitis and PDAC risk (relative risk [RR], 1.74; 95% CI, 1.41–2.15) [52].

The increased risk for PDAC from periodontal disease may be related to oral dysbiosis. Oral microbes such as *Porphyromonas gingivalis* are important contributors to periodontal disease and may cause systemic inflammation leading to carcinogenesis [53, 54]. Michaud et al. conducted a nested case-control study of 405 PDAC patients and 416 healthy

controls to examine the relationship between antibodies to oral microbes and risk of PDAC [55]. Results show that higher levels of antibodies against a pathogenic strain of *P. gingivalis* were associated with a twofold increase in the risk of PDAC (OR, 2.14; 95% CI, 1.05–4.36; >200 ng/ml vs ≤200 ng/ml). Additionally, higher levels of antibodies against commensal (nonpathogenic) oral bacteria were associated with 45% lower risk of PDAC when compared to those with lower levels of antibodies (OR, 0.55; 95% CI, 0.36–0.83), suggesting that certain commensal oral bacteria may counter dysbiosis from pathogenic bacteria growth, thus decreasing the risk for PDAC [55].

The above results suggest that oral dysbiosis may play a role in PDAC development, though they need to be interpreted with caution given that it is difficult to know whether the change in the microbiota led to cancer development or whether the cancer caused the change in the microbiota. This has been explored by Fan et al. in a prospective nested case-control study, albeit not definitively, to assess the relationship of oral microbiota with risk of PDAC [56]. The study included 361 PDAC patients and 371 matched controls, and their pre-diagnostic oral wash samples were evaluated to determine the bacterial composition. The presence of *P. gingivalis* and *Aggregatibacter actinomycetemcomitans*, both considered oral pathogens, was associated with higher risk of PDAC (OR, 1.60; 95% CI, 1.15–2.22 for *P. gingivalis*; OR, 2.20; 95% CI, 1.16–4.18 for *A. actinomycetemcomitans*), while Fusobacteria was associated with decreased risk of PDAC (OR, 0.94, 95% CI, 0.89–0.99) [56]. These risks remained even after excluding patients who developed pancreatic cancer within 2 years of sample collection, so the likelihood of reverse causation is reduced. Further prospective cohort studies in high-risk patients evaluating the change in the microbiota over time will be needed to clarify this association.

2.5 Potential role of microbiota in pancreas carcinogenesis

While the aforementioned cohort studies linked microbial dysbiosis and PDAC risk, preclinical models have further clarified the potential mechanisms by which microbiota can contribute to tumorigenesis and establish a more causative role. Gnansekaran et al. evaluated the direct effects of *P. gingivalis* on PDAC development and proliferation using cell lines and a xenograft model [57]. They found that *P. gingivalis* infection enhanced proliferation of PDAC cells, and the enhanced tumor cell proliferation correlates with *P. gingivalis* intracellular survival. Hypoxia increased *P. gingivalis* intracellular survival. Consistent with the *in vitro* results, the authors found that *P. gingivalis* infection also led to enhanced tumor growth *in vivo*. A prior study in an oral squamous cell model indicated that of the effects of *P. gingivalis* on cancer cell growth was mediated through a Toll-like receptor 2 (TLR2)-dependent mechanism [58]. In contrast, Gnansekaran et al. found that *P. gingivalis*-induced PDAC cell proliferation was independent of TLR2 signaling and is associated with augmentation of the Akt signaling pathway [57].

Others showed that intestinal microbiota may affect the tumor immune microenvironment and impact PDAC tumor growth and therapy response. By using genetically engineered PDAC mouse models (KC and KPC), Pushalkar et al. showed that intestinal bacteria can migrate into the pancreas, and PDAC samples contained a distinct microbial profile when compared to normal samples [19]. Additionally, they demonstrated that tumor initiation and progression was delayed in both germ-free mice and mice treated with an oral antibiotic regimen to ablate the gut microbiota, and repopulation of the gut microbiota by fecal transplant from PDAC-bearing mice or treatment with *Bifidobacterium pseudolongum* accelerated disease progression. Microbial ablation altered the tumor microenvironment and resulted in increased M1 macrophage differentiation and tumor infiltration of T cells as well as decreased myeloid-derived suppressor cells. Microbial ablation also improved the antitumor effects of PD-1 blockade by upregulating PD-1 expression, suggesting that the microbiota may be a potential therapeutic target in PDAC [19]. A separate study by Thomas et al. in a different mouse model ($Kras^{G12D}$/$PTEN^{lox/+}$) showed that antibiotic treatment decreased the incidence of cancer [59]. Microbial depletion of Nod-SCID mice harboring PDAC xenografts resulted in increased time to xenograft formation, smaller tumors, and attenuated growth, and these tumors had higher immune cell infiltration [59]. Sethi et al. demonstrated that antibiotic therapy significantly reduced tumor growth and metastatic burden in a PDAC mouse model, and this was associated with an increase in effector T cells in the tumor microenvironment [60]. Antibiotic treatment did not decrease tumor size in Rag1-KO mice, which lack mature T and B cells, suggesting that the antitumor effect of antibiotics was not a direct cytotoxic effect and required active participation of adaptive immunity [60].

The role of mycobiome in PDAC tumorigenesis was examined by Aykut et al [22]. Amphotericin B-mediated mycobiome ablation resulted in delayed tumor development and growth in PDAC mouse models, and repopulation with Malassezia increased the growth of PDAC tumors. The tumorigenic effect of fungal pathogens was found to be mediated by the complement cascade, which was triggered by the binding of pathogens to mannose-binding lectin (MBL) [22].

Taken together, these animal studies provide evidence that microbial dysbiosis alters the tumor immune microenvironment and can influence PDAC tumor development/progression. These results suggest that the microbiota has the potential to be a therapeutic target, but significant challenges

remain. Detailed examination of the microbiota in large cohorts of real-world patients with pancreatic cancer is needed to investigate the specific communities that may contribute positively or negatively to disease development and reconcile contradictory findings between animal and human studies. For example, long-term antibiotic use may actually increase the risk of cancer occurrence in human [61], while microbial depletion delayed tumor growth in mice [19, 59, 60]. Another important observation is that prior antibiotic exposure, but not concurrent antibiotic use, can negatively impact the clinical efficacy of immunotherapy in some non-PDAC tumors [62], though there are conflicting data and this continues to be an area of active debate [63, 64]. Further studies are needed to better understand the dynamic functions of the microbiome and its interactions with our immune system so we can harness the microbiome to optimize therapies.

3 Translational implications

Biomarkers for early detection and diagnosis are urgently needed in PDAC, particularly due to the poor prognosis and potential for early metastasis. Recent studies have demonstrated that microbiome analyses have the potential to become non-invasive diagnostic, prognostic, and predictive biomarkers in the management of PDAC.

3.1 Microbiome for early detection/diagnosis of PDAC

Currently, there is no recommended screening program for pancreatic cancer. Early diagnosis allows prompt (and potentially curative) intervention that may lead to better patient outcomes. Serum tests for carbohydrate antigen 19-9 (CA 19-9) are not optimal for screening due to low positive predictive value in asymptomatic patients [65].

The distinct microbiota found in PDAC patients offers novel opportunities to develop diagnostic/screening biomarkers and the tests may be easily performed in the clinic since salivary and fecal samples will be less invasive and easier to obtain than tissue biopsy from metastatic tumors. Farrell and colleagues first explored the possibility of using salivary microbial profile as a diagnostic biomarker by using the Human Oral Microbe Identification Microarray (HOMIM) to compare the salivary microbiota between 10 PDAC patients and 10 matched controls to identify bacterial candidates [34]. This was then validated in a cohort of 28 PDAC samples, 28 matched controls, and 27 chronic pancreatitis samples. The authors observed a significant difference in the salivary microbiota between PDAC patients and controls. Specifically, levels of *Neisseria elongata* and *Streptococcus mitis* were significantly lower in patients with pancreatic cancer, and the combination of these two microbial biomarkers showed a sensitivity of 96.4% and specificity of 82.1% in differentiating PDAC patients from healthy subjects. Furthermore, they compared these biomarkers against a HOMIM profiling study in lung cancer and found that none of the bacterial candidates from the PDAC study was significantly altered in lung cancer [34]. Torres et al. also examined the salivary microbiota of PDAC patients, patients with other diseases, and healthy individuals (8 vs 78 vs 22 patients, respectively) [35]. By using high-throughput sequencing, the authors identified a significantly higher ratio of Leptotrichia to Porphyromonas (LP ratio) in the saliva of PDAC patients when compared to the other 2 groups. They thus proposed that the LP ratio may be a potential PDAC diagnostic biomarker. Interestingly, the relative abundances of Neisseria and Streptococcus were not significantly different between the patient groups in this study, and the authors attributed this to a difference in study methodology when compared to the study conducted by Farrell et al [35]. Mendez et al. approached early detection of PDAC by assessing the gut microbiota and its metabolic products in both KPC mice and PDAC patients [66]. They found that the bacterial phyla Proteobacteria and Firmicutes were enriched in the gut microbiota in early PDAC development. This was associated with increased serum polyamine levels as a product of active metabolic pathways. Thus, analysis of metabolites of microbial dysbiosis may be another non-invasive biomarker for early detection of PDAC [66].

Although these studies have identified potential strategies for developing diagnostic biomarkers based on the microbiota, many challenges still exist. One of the major barriers is the variability in the bacterial populations identified across these studies. While some bacterial phyla such as Proteobacteria, Bacteroidetes, and Firmicutes have been found to be enriched in PDAC patients in multiple studies, few studies have identified common genera or species of bacteria that are consistently altered in PDAC. Differences in host ethnicity, geographic location, lifestyle, and dietary intake can lead to divergent baseline microbiome composition, which likely contributes to the variability seen across studies [67–71]. This makes selecting a generalizable biomarker difficult. Thus, further studies will be needed to validate these analytic strategies and identify universal biomarkers for the clinic.

3.2 Microbiome as prognostic biomarker for PDAC

Accurate prognostic biomarkers are important for stratifying treatment strategies according to the biology of PDAC in patients. Several groups have demonstrated the potential of microbiota profile in prognosticating outcomes of PDAC patients.

In a study by Mitsuhashi et al., 283 PDAC tumor samples were tested for Fusobacterium species [72]. The samples were also tested for specific mutations, microRNA expression, and epigenetic alterations. Fusobacterium species were detected in 8.8% of the tumor samples, and Fusobacterium species

positivity was associated with significantly shorter cancer-specific survival independent of other clinical and molecular features, suggesting that intratumoral Fusobacterium status may be a potential prognostic biomarker [72]. Kohi et al. examined the bacterial composition of duodenal fluid collections from 74 PDAC patients and compared the microbial profiles of short-term vs long-term survivors [26]. Samples from short-term survivors were enriched with Fusobacterium, Rothia, and Neisseria [26]. In a retrospective study of intraoperative bile cultures from 211 locally advanced or borderline resectable PDAC patients, higher numbers of microbial species were associated with shorter progression free survival (PFS) [73].

However, not all intratumoral bacterial colonization is associated with worse outcomes. Riquelme et al. examined the intratumoral microbiome composition of 68 resected PDAC tumors; 36 of these patients were considered long-term survivors (> 5 years) and 32 were considered short-term survivors (<5 years) [74]. They found that the intratumoral microbiome diversity was significantly higher in the long-term survivors compared with the short-term survivors. A specific microbiome signature (*Pseudoxanthomonas*, *Streptomyces*, *Saccharopolyspora*, *Bacillus clausii*) was highly predictive of long-term survival [74].

Together, these results suggest that the microbiota diversity and composition of PDAC patients can potentially provide prognostic information to predict the survival of these patients. Once again, further studies are needed to clarify discrepant results and better define the biomarkers.

3.3 Microbiome as a predictive biomarker for treatment response in PDAC

Finally, biomarkers that predict patient response to therapies will be crucial to improve the outcomes of PDAC patients. Cytotoxics remain the main class of anticancer drugs used in treating PDAC patients, and recent studies show that the microbiota can significantly alter their efficacy. *In vitro* and *in vivo* studies evaluating gemcitabine in multiple cancer cell lines showed that gemcitabine efficacy was attenuated in the presence of *Escherichia coli* [75]. A separate study by Geller et al. showed that PDAC tumor samples harbored the bacteria class Gammaproteobacteria, which is capable of metabolizing gemcitabine to the inactive 2′,2′-difluorodeoxyuridine by a long isoform of the enzyme cytidine deaminase (CDD_L) [15]. This bacteria-induced gemcitabine resistance was abrogated by ciprofloxacin co-treatment in a colon cancer mouse model. Furthermore, bacteria derived from PDAC tumor samples induced gemcitabine resistance in 2 human colorectal cancer cell lines [15]. A retrospective study by Weniger et al. of PDAC patients receiving adjuvant gemcitabine found better PFS in PDAC patients without *Klebsiella pneumoniae* (which belongs to the class Gammaproteobacteria) in their bile culture than those with *K. pneumoniae* [73]. Antibiotic treatment with quinolones was associated with improved overall survival in patients who were positive for *K. pneumoniae* [73]. Overall, these results suggest that microbial dysbiosis can induce gemcitabine resistance, and appropriate antibiotic therapy may reverse the resistance and improve clinical outcomes. The successful identification of deleterious microbial profiles can potentially help triage PDAC patients for antibiotic therapy either prior to starting or during chemotherapy treatment.

Immune checkpoint inhibitors (ICIs) have so far been a failure in PDAC therapy despite successes in other cancer types [76]. The failure is partly attributed to the 'immunosuppressive' tumor microenvironment in PDAC [77–79]. As mentioned above, the microbiota may alter tumor microenvironment and immune cell infiltration in PDAC, which can potentially impact the efficacy of ICI therapy [19, 59, 60, 80, 81]. Research on the impact of microbiota on ICI in PDAC is sparse except for the study by Pushalkar et al., which showed that PD-1 was upregulated during antibiotic-mediated microbial ablation, and this was linked to improved antitumor effects of PD-1 blockade in a PDAC mouse model [19]. However, the microbial composition in melanoma patients was found to be different between immunotherapy responders and non-responders, and bacteria such as Bifidobacterium were associated with better response to ICIs [16, 82].

Not only can the microbiota impact drug efficacy, it can also predict and mediate chemotherapy-related toxicity [83]. For instance, the metabolism and clearance of irinotecan, a chemotherapeutic agent commonly used in PDAC that is known to induce dose-limiting diarrhea, is affected by gut microbiota [84]. Irinotecan is a prodrug of SN-38 and is converted to SN-38 by hepatic carboxylesterase; SN-38 is then metabolized by UGT1A1 in the liver to an inactive product, SN-38G [85, 86]. Patients with homozygous UGT1A1*28 allele, associated with decreased UGT1A1 activity, are at increased risk for irinotecan-related neutropenia due to decreased SN-38 clearance. The concentration of SN-38 is found unexpectedly high in feces as SN-38G can be converted back to SN-38 in the intestine by β-glucuronidase of naturally present, nonpathogenic gut bacteria such as *E. coli*, *Bacteroides* spp., and *Clostridium perfringens*, leading to the development of mucosal damage and severe diarrhea [86]. A preclinical study in rats by Stringer et al. showed that following irinotecan administration, there was an increase in the β-glucuronidase producing gut microbiota, which may be the primary cause of excessive gastrointestinal toxicity experienced by some patients [87]. Another study by Bhatt et al. demonstrated that inhibition of β-glucuronidase significantly decreased the incidence of diarrhea when co-administered with irinotecan in a mouse model [88]. A pilot study in cancer patients receiving irinotecan-containing regimens is currently underway at the Mayo Clinic that is designed to assess the activity of β-glucuronidase in stool samples with a specialized

Table 1 Ongoing and completed observational studies assessing the microbiome in pancreatic ductal adenocarcinoma and other GI cancers (source: ClinicalTrials.gov)

Study title	Study design	Approach	Conditions	Samples collected	NCT identifier	Status
Oral Microbiome and Pancreatic Cancer	Observational	$^{16S\ rRNA\ gene}$ sequencing	Pancreatic cancer	Oral	NCT03302637	Completed
Microbial Diversity of Pancreatic Diseases	Observational	16S rRNA sequencing and metagenomics	Pancreatic cancer Pancreatic diseases	Stool, blood, other digestive secretions	NCT03809247	Not yet recruiting
The Microbiome of Pancreatic Cancer: "PANDEMIC" Study (PANDEMIC)	Observational	Microbiome evaluation (unspecified)	Pancreatic cancer	Oral, stool, PDAC tissue, intestinal mucosal tissue, bile	NCT04274972	Recruiting
A Prospective Translational Tissue Collection Study in Early and Advanced Pancreatic Ductal Adenocarcinoma and Pancreatic Neuroendocrine Tumours to Enable Further Disease Characterisation and the Development of Potential Predictive and Prognostic Biomarkers (PaC-MAn)	Observational	miRNA analysis, DNA and RNA sequencing nanostring, RT-PCR, immunohistochemistry	Pancreatic cancer	Blood, urine, stool, oral, bile, tissue	NCT03840460	Recruiting
Microbiome Analysis in esoPhageal, PancreatIc and Colorectal CaNcer Patients Undergoing Gastrointestinal Surgery (MA-PPING)	Observational	$^{16S\ rRNA\ gene}$ sequencing	Pancreatic cancer Colorectal cancer Esophageal cancer	Oral, stool	NCT03840460	Not yet recruiting
ARGONAUT: Development and Analysis of a Blood and Stool Sample Bank for Cancer Patients, Enabling the Systematic Study of the Effect of Gut Microbiomes on Response to Treatment	Observational	Whole genome sequencing and metabolomics	Pancreatic cancer Colorectal cancer Non-small cell lung cancer Triple negative breast cancer	Blood, stool	NCT04638751	Recruiting
The Mechanism of Enhancing the Anti- tumor Effects of CAR-T on PC by Gut Microbiota Regulation	Observational	16S rRNA sequencing	Pancreatic cancer	Blood, stool	NCT04203459	Recruiting
Interaction Between Host, Microenvironment and Immunity on Gastrointestinal Neoplasms(HoMING)	Observational	miRNA analysis, cytokine analysis, metabolomics, ctDNA, microbiota analysis	Gastrointestinal neoplasms	Blood, stool, liver	NCT04363983	Not yet recruiting
Role of gut microbiome in irinotecan pharmacology	Observational	16S rRNA sequencing, β-glucuronidase activity, PK profiles	Any cancer being treated with irinotecan	Blood, stool	--- (Institutional)	Recruiting

probe [89] and evaluate the impact of gut microbiota on the efficacy and toxicity of irinotecan. The results of these studies will provide rationale for further development of the fecal microbiota as a predictive biomarker for treatment-related toxicities in gastrointestinal cancer (including PDAC) patients receiving irinotecan.

4 Current clinical trials

There is nascent but growing interest in leveraging the microbiome for PDAC detection and treatment. Several studies are underway to comprehensively evaluate the impact of dysbiosis in pancreas and other gastrointestinal malignancies. These range from understanding the impact of the microbiota on carcinogenesis and tumor microenvironment to developing microbiome signatures for diagnosis and predicting prognosis and treatment response (Table 1). There are also interventional trials to evaluate how to leverage our understanding of the microbiome to develop novel therapies to improve patient outcomes (Table 2).

MRx0518 is a live biotherapeutic consisting of a lyophilized formulation of a proprietary bacterium and is currently undergoing clinical investigation in several phase I trials. The preliminary results from two of these trials were presented at the Society for Immunotherapy of Cancer (SITC) Annual Meeting in 2020. In one of the trials, MRx0518 was used as monotherapy in the neoadjuvant setting for multiple solid tumors (NCT03934827). Results from part A of the study showed safety and tolerability of MRx0518, and exploratory analyses of post-treatment samples demonstrated increased infiltration of immune cells [90]. In another trial, MRx0518 was used in combination with pembrolizumab in patients with advanced solid tumors refractory to ICIs (NCT03637803). Part A of the study enrolled patients with

Table 2 Interventional clinical trials that target the microbiome in pancreatic ductal adenocarcinoma and other GI cancers (source: ClinicalTrials.gov)

Study title	Study design	Intervention	Conditions	NCT identifier	Status
Gut Microbiome Modulation to Enable Efficacy of Checkpoint-based Immunotherapy in Pancreatic Adenocarcinoma	Interventional	Drug: Pembrolizumab Drug: Ciprofloxacin Drug: Metronidazole	Pancreatic Cancer	NCT03891979	Withdrawn
A Study of Live Biotherapeutic Product MRx0518 With Hypofractionated Radiation Therapy in Resectable Pancreatic Cancer	Interventional phase I	Drug: MRx0518 Radiation: hypofractionated preoperative radiation	Pancreatic Cancer	NCT04193904	Recruiting
MS-20 on Gut Microbiota and Risk/Severity of Cachexia in Pancreatic Cancer Patients	Interventional	Drug: MS-20 Other: Placebo	Pancreatic Cancer	NCT04600154	Recruiting
Phase II Study of Nivolumab (Anti-PD1), Tadalafil and Oral Vancomycin in Patients With Refractory Primary Hepatocellular Carcinoma or Liver Dominant Metastatic Cancer From Colorectal or Pancreatic Cancers	Interventional phase II	Drug: Nivolumab Drug: Tadalafil Drug: oral vancomycin	Hepatocellular carcinoma Metastatic pancreatic cancer Metastatic colorectal cancer	NCT03785210	Recruiting
Pilot Trial of Fecal Microbiota Transplantation and Re- Introduction of Anti-PD-1 Therapy in dMMR Colorectal Adenocarcinoma Anti-PD 1 Non-Responders	Interventional phase I	Drug: fecal microbiota transplantation capsule Drug: Nivolumab Drug: Pembrolizumab	Colorectal cancer	NCT04729322	Recruiting
Investigator-initiated Trial of Fecal Microbiota Transplant (FMT) Capsule for Improving the Efficacy of Anti-PD-1 in Patients With PD-1 Resistant Digestive System Cancers	Interventional phase I	Drug: fecal microbiota transplantation capsule Drug: anti-PD-1 therapy	Gastrointestinal cancers	NCT04130763	Recruiting
A Phase I/II Open-label Study of EDP1503 Alone and in Combination With Pembrolizumab in Patients With Advanced Metastatic Colorectal Carcinoma, Triple- negative Breast Cancer, and Checkpoint Inhibitor Relapsed Tumors	Interventional phase I/II	Drug: EDP1503 Drug: Pembrolizumab	Colorectal cancer Gastroesophageal cancer Triple negative breast cancer Non-small cell lung cancer Renal cell carcinoma	NCT03775850	Recruiting
Fusobacterium Nucleatum Eradication in Postoperative Stage II/III Colorectal Cancer (FINER-PACE) by Oral Metronidazole: A Multi-left, Randomized, Double-Blind, Placebo-Controlled Clinical Trial.	Interventional phase II/III	Drug: Metronidazole Drug: Placebo	Colorectal cancer	NCT04264676	Recruiting

metastatic non-small cell lung cancer and renal cell carcinoma. The study demonstrated safety and tolerability of the combination, and 5 of 12 (42%) patients achieved clinical benefit [91]. There is now an active phase I trial in pancreatic cancer of using MRx0518 with hypofractionated radiation therapy (NCT04193904).

Several other interventional trials are ongoing, but no results have been reported yet. MS-20 is a biotherapeutic agent consisting of a mixture of soybean fermentation metabolites and microorganisms that mimic the human intestinal environment. There is an ongoing trial to examine the effects of MS-20 on gut microbiota and risk/severity of cachexia in pancreatic cancer patients undergoing chemotherapy treatment (NCT04600154). Another phase II trial is evaluating the hypothesis that oral vancomycin can impact gut commensal bacteria and increase cytokine expression, which results in liver-selective NKT-mediated antisssstumor effects, by assessing the combination of nivolumab, tadalafil, and oral vancomycin in patients with refractory hepatocellular carcinoma or liver dominant metastatic colorectal or pancreatic cancers (NCT03785210) [92].

While not many interventional trials involving the microbiome exist for pancreatic cancer, various microbiome-based strategies are under clinical investigation for other malignancies and may provide future study directions for pancreatic cancer. The most well-studied intervention involves fecal microbiota transplantation (FMT), where fecal materials from donors are transferred to recipients via endoscopy, colonoscopy, or oral capsules. Several phase I trials have demonstrated that FMT can improve response to ICI in patients with ICI-resistant metastatic melanoma and induce favorable changes in the tumor microenvironment [93, 94]. Similar studies that combine FMT and immunotherapy are underway in gastrointestinal cancers, prostate cancer, non-small cell lung cancer, and mesothelioma (NCT04130763, NCT04729322, NCT04521075, NCT04116775, NCT04056026). FMT is also being evaluated for its impact on ICI toxicities in various malignancies (NCT03819296, NCT04163289). In addition to FMT, administration of probiotics in combination with immunotherapy is under evaluation in renal cell carcinoma, breast cancer, non-small cell lung cancer, and colorectal cancer (NCT03829111, NCT03775850, NCT04909034). There is growing evidence that specific dietary changes can alter the intestinal microbiota [95], which can be associated with altered response to immunotherapy. Despite these studies, immune checkpoint inhibitor therapies in the current forms have yet to demonstrate activity in pancreatic cancer and will likely limit the applicability of FMT to enhance ICI in this disease.

Several trials are underway to evaluate the clinical outcomes of melanoma patients receiving high fiber diets and immunotherapy (NCT04645680, NCT04866810). Finally, as mentioned previously, antibiotic use may alter the gut microbiome and impact sensitivity to chemotherapy. There is an active phase II trial exploring the impact of metronidazole on the efficacy of adjuvant chemotherapy in patients with colorectal cancer (NCT04264676).

5 Conclusions

Despite decades of research, pancreatic cancer remains a deadly disease with limited treatment options and poor patient outcomes. Our growing understanding of the role of microbiome in pancreatic cancer will provide new insights and potentially lead to opportunities for the development of novel biomarkers and interventional strategies. Current studies have revealed challenges in the evaluation of microbiome in pancreatic cancer such as conflicting results on the specific microbial signatures associated with tumor development, progression, and treatment response. We need to improve the quality of future studies by controlling for patient comorbidities and standardizing the study design, methods of sampling, and primers used for sequencing and analysis. There is also a paucity of data regarding the role of other microorganisms such as viruses and protozoa, and better techniques are needed to advance our understanding in this area. Microbiome-based interventional studies in pancreatic cancer are an emerging field and are currently limited to evaluating the impact of a few biotherapeutic agents on patient response to radiation therapy and treatment toxicities. However, the modulation of microbiota has the potential to augment drug efficacy and reduce toxicity, and future studies should integrate microbiome-based biomarkers as well as evaluate the role of FMT, probiotics, dietary changes, and antibiotics in altering treatment response and patient outcomes. Well-designed, geographically diverse, prospective clinical trials will be needed to validate the results. Overall, the study of microbiome in pancreatic cancer holds great promise as a new frontier for precision medicine in the management of pancreatic cancer and deserves further investigation.

Data and materials availability Not applicable

Code availability Not applicable

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Declarations

Conflict of interest The authors declare no competing interests.

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