Therapeutic potential of microbial modulation in pancreatic cancer

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INTRODUCTION

Pancreatic cancer, particularly pancreatic ductal adenocarcinoma (PDAC), is an aggressive disease with a poor prognosis.[1] Surgery is the only potential curative treatment, but it is only possible in patients with early-stage disease, and most patients present with non-resectable disease.[1] Even in patients who undergo resection, the recurrence rate is very high due to early systemic dissemination.[2] Combinatorial chemotherapy remains the standard of care for patients with advanced disease, but responses to it are heterogeneous, and its toxicity limits treatment duration.[3–5] Results from clinical trials of single-agent immunotherapy have not proven its efficacy in patients with pancreatic cancer, not even when used with chemotherapy or other immunotherapeutic agents.[6–8] A highly immunosuppressive tumour microenvironment (TME) has been postulated as one of the main reasons for the lack of efficacy of immunotherapy for pancreatic cancer.[9–11] Therefore, novel strategies that modulate the suppressive TME are urgently needed.

The GI tract is the largest reservoir of microbes, which play important roles in modulating metabolism and immunity through interaction with host cells.[12] The discovery of the association between infection with the bacterium *Helicobacter pylori* and the incidence of gastritis and peptic ulcer disease earned Barry Marshall and Robin Warren the Nobel Prize in Physiology or Medicine in 2005, underscoring the pivotal role of the microbiota in influencing inflammatory conditions which may predispose to cancer.[13] The microbial α-diversity is a metric that quantifies the number of different species within a defined sample.[14 15] In patients with many malignancies, including colorectal, breast and pancreatic cancer, the α-diversity of the gut microbiota is lower than that in healthy controls.[16–18] With respect to clinical outcomes, the gut microbial α-diversity is higher in patients with melanoma that responded to immunotherapy than in those unresponsive.[19] In this review, we highlight recent studies analysing microbes present in various compartments (oral cavity, gut, cysts and pancreatic tumours) in patients with pancreatic cancer, as well as murine studies. We also discuss various strategies for microbial modulation. A summary of the published data on enriched microbes at different sites in clinical studies and murine models of pancreatic cancer is included in table 1.

RELEVANCE OF THE ORAL AND GUT MICROBIOMES IN PANCREATIC CANCER

Several recent studies have analysed the oral and gut microbiomes associated with pancreatic cancer risk and progression.[18 20–25] Periodontal disease has been recognised as a risk factor for pancreatic cancer and may be an initiator of oral microbial dysbiosis.[25] A prospective population-based nested case–control study demonstrated that the presence of *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans* in the oral cavity was indicative of increasing the risk of pancreatic cancer.[20] Notably, high levels of plasma antibodies reactive against *P. gingivalis* corresponded with reduced risk of pancreatic cancer, potentially due to systemic immunity against cancer-associated oral pathogens.[24] Another study demonstrated that the oral microbial composition differs in healthy controls and patients with established pancreatic cancer, and the investigators proposed that detection of two microbes

Table 1 The enriched microbes in the oral cavity, gut and tumours in murine models and human samples

Host	Study group	Enriched bacteria	Reference
Oral microbiota			
Human	Prediagnostic samples, PDAC versus matched controls	*Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*	Fan *et al*[20]
Human	PDAC versus healthy controls	*Neisseria elongate*, *Streptococcus mitis*	Farrell *et al*[21]
Gut microbiota			
Human	PDAC versus healthy controls	*Prevotella*, *Veillonella*, *Klebsiella*, *Selenomonas*, *Hallella*, *Enterobacter*, *Cronobacter*	Ren *et al*[18]
Human	PDAC versus healthy controls	*Bacteroidetes*, *Veillonellaceae*, *Akkermansia*, *Odoribacter*	Half *et al*[22]
Human	PDAC versus healthy controls	*Proteobacteria*, *Synergistetes*, *Euryarchaeota*	Pushalkar *et al*[23]
Mouse	PDAC spontaneous model	*Actinobacteria*, *Deferribacteres*, *Bifidobacterium pseudolongum*	
Mouse	PDAC subcutaneous model	*Bacteroidetes*, *Firmicutes*	Sethi *et al*[48]
Mouse	PDAC spontaneous model	*Bacteroides*, *Alphaproteobacteria*	Mendez *et al*[26]
Tumour microbiota			
Human	PDAC versus healthy controls	*Gammaproteobacteria*	Geller *et al*[37]
Human	PDAC tumour versus gut	*Proteobacteria* (*Pseudomonas*, *Elizabethkingia*)	Pushalkar *et al*[23]
Human	PDAC	*Acinetobacter*, *Afipia*, *Corynebacterium*, *Escherichia*, *Propionibacterium*	Thomas *et al*[27]
Human	PDAC	*Gammaproteobacteria*, *Bacilli*, *Actinobacteria*	Riquelme *et al*[40]
Human	PDAC long-term survivors versus short-term survivors	*Pseudoxanthomonas*, *Streptomyces*, *Saccharopolyspora*, *Bacillus clausii*	
Cyst microbiota			
Human	IPMN, MCN, SCA, pseudocysts	*Bacteroides*, *Escherichia/Shigella*, *Fusobacterium*, *Acidaminococcus*, *Sphingomonas*, *Bifidobacterium*	Li *et al*[35]
Human	Cancerous versus non-cancerous PCNs	*Fusobacterium nucleatum*, *Granulicatella adiacens*	Gaiser *et al*[36]

IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; PCN, pancreatic cystic neoplasias; PDAC, pancreatic ductal adenocarcinoma; SCA, serous cystadenoma.

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in the oral compartment (*Neisseria elongata* and *Streptococcus mitis*) can differentiate patients with pancreatic cancer from healthy individuals.[21]

The gut microbiota associated with pancreatic cancer development has been assessed in a few clinical studies.[18 22] A study in China examined a population of patients with PDAC and matched healthy controls.[18] Besides a lower α-diversity in the gut microbiome of patients with PDAC versus healthy controls, a unique gut microbial profile was detected in patients with PDAC, including increased abundance of *Veillonella*, *Klebsiella* and *Selenomonas* species and lipopolysaccharide-producing bacteria (*Prevotella*, *Hallella* and *Enterobacter* species) but decreased abundance of *Bifidobacterium* species and some butyrate-producing bacteria (eg, *Coprococcus*, *Clostridium*, *Blautia*, *Flavonifractor* and *Anaerostipes* species). Of note, patients who presented with biliary obstruction had a unique microbiome, suggesting that biliary fluid stasis has a role in the microbial changes found in these patients.[18] Another study that compared gut microbes in patients with pancreatic cancer and healthy controls found an increase in *Bacteroidetes* and a reduction in *Firmicutes* abundance in patients with pancreatic cancer in two independent cohorts.[22] Analysis of the gut microbiotas of patients with PDAC in a third study revealed enrichment in *Proteobacteria*, *Synergistetes* and *Euryarchaeota* species than in matched healthy controls.[23]

The functional role of the gut microbiome in pancreatic cancer development has been examined in murine studies.[23 26 27] Transgenic mice engineered to develop the full spectrum of pancreatic premalignant to malignant lesions exhibited marked temporal changes in their gut microbiota composition as well as bacteria-related metabolites throughout tumourigenesis.[23 26] To examine progressive changes in the gut microbiota during pancreatic tumourigenesis, Pushalkar *et al*[23] longitudinally sampled faeces from the premalignant PDAC mouse model KC (Ptf1aCre, LSL-Kras^{G12D}). Actinobacteria and *Bifidobacterium pseudolongum* were abundant in KC mice with advanced stages of disease progression.[23] Moreover, a clear delay in tumourigenesis was seen in KC mice located in a germ-free environment but not in their littermates raised in a regular mouse housing facility, highlighting the functional importance of the gut microbiota in pancreatic cancer development.[23] The mechanisms implicated in tumourigenic induction by the dysbiotic microbiota included activation of Toll-like receptors (TLRs) on immunosuppressive monocytic cells. Antibiotics-based microbial ablation increased CD4$^+$ T-cell polarisation towards a Th1 phenotype and increased cytotoxic CD8$^+$ T cells based on upregulation of T-bet, tumour necrosis factor-α (TNF-α), interferon gamma (IFN-γ), and CD38. Both CD4$^+$ and CD8$^+$ T cells had overexpression of programmed cell death protein 1 (PD-1), and CD44 and CD4$^+$ T cells had overexpression of inducible costimulator (ICOS) and LFA-1 on microbial ablation. They also found that faecal microbial transplants (FMT) from the spontaneous PDAC mouse model KPC (LSL-Kras^{G12D}, LSL-Trp53^{R172H} and Pdx1Cre) into either germ-free mice or antibiotic-treated KC mice significantly accelerated tumour growth in both settings. Bacterial ablation on recipient mice prior to FMT improves colonisation with donor's stools. This study further showed that infiltration of immunosuppressive myeloid cells and macrophages into the TME decreased with antibiotic-mediated bacterial ablation, which was reversed with murine FMT from mice with PDAC in an orthotopic PDAC murine model.

A second mechanism postulated for the microbial effect in tumourigenesis implicates metabolites, which are well-known host modulators.[26] Mendez *et al*[26] profiled the gut microbial communities in mice throughout tumourigenesis and identified metabolic pathways associated with microbial changes via metagenomics. They used the genetically engineered spontaneous PDAC mouse model KPC to examine microbial and metabolic changes over time.[26] They found that the main metabolic pathways enriched in KPC mice were related to the biosynthesis of pyrimidines and polyamines, in particular, those involving putrescine, spermidine and spermine. Polyamine levels were measured in the serum of KPC mice and were found to be increased at 4 months, the time at which most of the KPC mice had advanced pancreatic intraepithelial neoplasia (PanIN).[26] To validate this finding, polyamine levels were analysed in serum samples and were found to be higher in the serum of patients with PDAC than in the serum of healthy controls.[26] Polyamines, which are mainly produced by intestinal microbiota, are known to induce cellular proliferation by contributing to purine/pyrimidine cellular biosynthesis.[28] One of the microbes associated with polyamine metabolism is *Lactobacillus*. Authors have reported that *Lactobacillus rhamnosus* is capable of affecting polyamine metabolism and tumour growth in gastric cancer cases.[29] The role of bacteria-derived polyamines in pancreatic cancer development has yet to be established. Also, because of their wide repertoire of microbial enzymes, microbes have great potential to influence host metabolism downstream of dietary influences, including metabolism of xenobiotics by altering their toxicity.[30]

In summary, the oral and gut microbiomes may play an important role in pancreatic cyst biology and cancer initiation and progression through modulation of immune and metabolic pathways. Functional mechanisms implicated in these processes must be further delineated. Nevertheless, sequential analysis of oral and/or gut bacteria, as well as their serum-associated metabolites, could emerge as an inexpensive, non-invasive strategy for early detection of pancreatic cancer. Furthermore, targeting microbial populations associated with increased risk of PDAC may also represent a novel cancer-preventive methodology.

RELEVANCE OF THE PANCREATIC INTRATUMORAL MICROBIOME

The presence of micro-organisms in tumours has been described multiple times over the past 130 years.[31–34] Early pancreatic cystic lesions have been described to possess bacterial DNA.[35 36] In examination of different types of pancreatic cystic lesions, such as intraductal papillary mucinous neoplasms (IPMNs), mucinous cystic neoplasms (MCN), serous cystadenomas (SCA) and pseudocysts, most samples had enrichment in *Bacteroides* spp, *Escherichia*/*Shigella* species, *Fusobacterium acidaminococcus*, *Sphingomonas* species and *Bifidobacterium* spp.[35] Another study demonstrated that the microbiotas of high-grade IPMNs were commonly found as part of oral microbiome, like *F. nucleatum* and *Granulicatella adiacens*; this potential translocation was attributed in part to invasive endoscopic procedures.[36]

In 2017, Geller *et al*[37] reported for the first time the presence of bacteria in human PDACs while demonstrating that intratumoral Gammaproteobacteria, among the most common bacteria detected in human pancreatic tumours, reduce the efficacy of chemotherapeutic drugs like gemcitabine. The mechanism for this involves the bacterial production of cytidine deaminase, which is capable of metabolising gemcitabine into its inactive form.[37] Other comprehensive studies have revealed the presence of microbiotas in several other tumour types, such as melanoma and breast, lung, ovarian, bone and brain tumours.[38] Tumour microbial

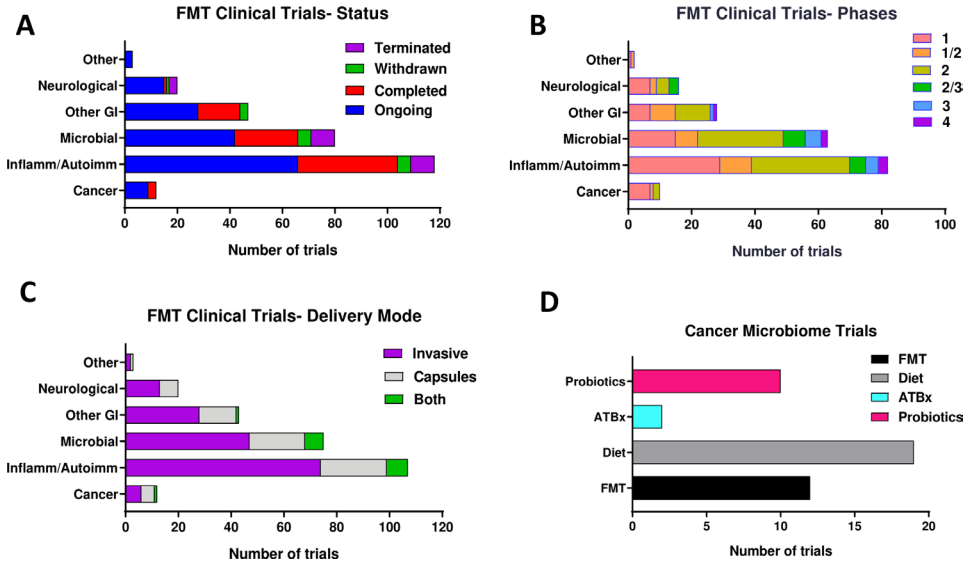

Figure 1 Overview of clinical FMT trials by status (A), clinical phase (B), delivery mode (C) and cancer (D) reported in ClinicalTrials.gov as of September 2020. autoimm, autoimmune; FMT, faecal microbial transplant; inflamm, inflammatory.

signatures can also be extracted from publicly available data sets in The Cancer Genome Atlas (TCGA) by filtering out non-human sequences from tumour and blood samples and can be used, through artificial intelligence driven predictive models, to distinguish between cancer and healthy signatures as well as different cancer types.[39]

Intratumoral bacteria have also been identified in murine PDAC models.[23 27] Of note, though, is that Thomas *et al*[27] reported that genetically engineered mice ($Kras^{G12D/+}$, $PTEN^{lox/+}$ and Pdx1-Cre) had tumourigenesis delay when raised in a germ-free environment. However, intratumoral microbiotas did not suffer major changes after they were transferred from germ-free to specific pathogen-free housing conditions, suggesting that the local tumour microbial communities in this model may not be as relevant as systemic/gut communities.

More recently, Riquelme *et al*[40] profiled intratumoral bacteria from patients with resected PDAC and compared short-term and long-term survivors in two geographically distant cohorts. Long-term survivors had greater intratumoral microbial α-diversity than did those who died of the disease within 5 years after resection.[40] Overall tumour microbial characterisation revealed a microbial composition similar to the one in human PDAC previously described by Geller *et al*,[37] but unique enrichment in the following microbes was found in tumours from long-term survivors in both independent cohorts studied by Riquelme and colleagues: *Pseudoxanthomonas*, *Streptomyces* and *Saccharopolyspora* and *Bacillus clausii*. In this study, tumour immunological profiling revealed enhanced immunoactivation of the TME in long-term survivors, which positively correlated with the survivor-enriched microbes, demonstrating a potential role for tumour microbes in altering immune-cell function. Two of the most enriched bacterial species identified in the tumours of long-term survivors have documented immunomodulatory functions. First, *Saccharopolyspora* spp induce hypersensitivity pneumonitis[41 42] and promote a proinflammatory response in lung epithelial cells via activation of protein kinase D1 through the innate immune signal transduction adaptor MyD88.[42] Second, *B. clausii* can mediate immune-cell production of nitrous oxide and IFN-γ, along with increased $CD4^+$ T-cell proliferation in vitro.[43] *B. clausii* spores are very popular probiotics in Europe, branded as Enterogermina.[43] In vitro studies have shown that *B. clausii* can protect enterocytes from rotavirus infection by improving epithelial barrier function and reducing the production of reactive oxygen species and cytokines like interleukin-8 and interferon-β.[44] *B. clausii* can also decreased the toxicity of pathogens like *Clostridium difficile* and *Bacillis cereus* through secretion of a serine protease.[45] No clinical or preclinical studies have assessed a potential role for *B. clausii* in the context of cancer.

Another notable microbial population in pancreatic tumours is that of the fungal mycobiome, which was recently reported to be important for pathogenesis.[46] Aykut *et al*[46] showed that the fungal genus *Malassezia* is abundant in murine and human PDACs and that its depletion with amphotericin B reduced tumour growth in orthotopic and autochthonous pancreatic cancer models. Repopulation of *Malassezia* but not other genera, such as *Candida*, *Saccharomyces* and *Aspergillus*, promoted tumour growth in orthotopic pancreatic tumour-bearing mice given pretreatment with antifungals.[46] Signalling through the mannose-binding lectin (MBL) pathway enabled tumour-associated fungal populations to activate the complement cascade via the C3 molecule.[46]

Further work is needed to determine the route of colonisation of intestinal bacteria in pancreatic tumours, whether through reflux from the duodenum or via circulation through the bloodstream or lymphatic system.[47] Also, it would be important to gain a better understanding of the local conditions that favour a niche for bacteria colonisation in tumours.

ANTIMICROBIALS AS BACKBONE THERAPY FOR PANCREATIC CANCER

Disruption of a dysbiotic microbiota has been associated with beneficial effects and decreased tumour growth in numerous pancreatic cancer murine models.[23 27 48] Sethi *et al*[48] demonstrated that oral treatment with an antibiotic cocktail composed of vancomycin, neomycin, metronidazole, ampicillin and amphotericin B delays the growth of subcutaneous murine PDAC implants by increasing the number of IFN-γ-producing cytotoxic T cells and inhibiting the number of interleukin (IL)-17A and IL-10 producing protumourigenic T cells. Also, Pushalkar *et al*[23] reported that depletion of the gut microbiota with oral antimicrobials led to decreased

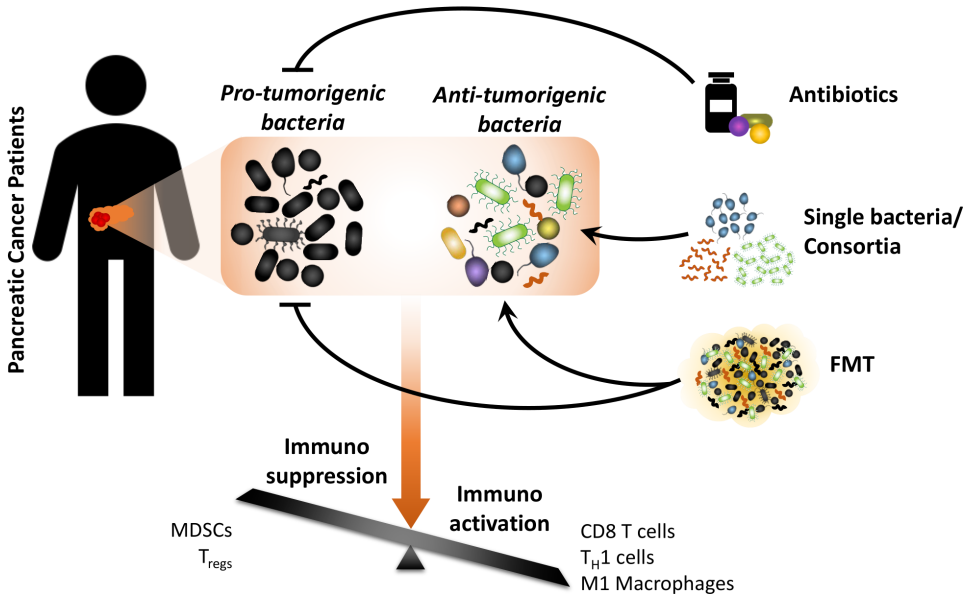

Figure 2 Diagram showing different approaches to microbiota modulation for cancer treatment. The overall goal is to shift the tumour microenvironment from an immunosuppressive to an immune-activated state. FMT, faecal microbial transplant; MDSC, myeloid-derived suppressor cell; T_H1, type I helper; Treg, regulatory T cell.

tumour size and changes in the immune landscape of the TME in orthotopic PDAC mouse models by eliciting both innate and adaptive responses to reduce myeloid-derived suppressor cells (MDSCs) and increase M1-type macrophages tumour infiltration with suppression of TLR 2/5 signalling while enhancing the number of Th1-type $CD4^+$ and cytotoxic $CD8^+$ T cells. In addition, Thomas *et al*[27] found that murine gut microbiota ablation with antibiotics dampened tumour growth in genetic PDAC mouse models ($Kras^{G12D/+}$, $PTEN^{lox/+}$ and Pdx1-Cre). These three independent studies using different PDAC models showed that broad microbial ablation is an effective approach to affect progression of premalignant pancreatic lesions to cancer and PDAC growth.[23 27 48] We envision a clinical trial in which chemotherapy is combined with systemic antibiotics for treatment of PDAC. However, known toxic effects associated with long-term use of broad-spectrum antibiotics as well as rise of multidrug resistant bacteria would certainly limit the enthusiasm for such studies.[49 50]

Elimination of bacteria with targeted local delivery of antibiotics such as ampicillin and chloramphenicol resulted in reduced tumour growth in a subcutaneous colon carcinoma mouse model (MC26) as shown by Geller *et al*.[37] Local intratumoral release of antibiotics was achieved through the use of an implantable microdevice with a standard biopsy needle that can release microdoses of single agents or combinations of therapeutic drugs.[51] Increased levels of apoptosis as measured using cleaved caspase 3 staining were observed in tumours only when the implanted microdevice released a combination of gemcitabine and antibiotics, thus highlighting the specific role of intratumoral bacteria in opposing chemotherapy activity.[37] Analogous to this, in a colon carcinoma xenograft mouse model, abrogation of the protumourigenic *Fusobacterium* load via treatment with metronidazole slowed tumour growth as well.[52] Localised delivery of antibiotics using local release devices would be less toxic and more effective for systemic use, potentially representing a promising local therapeutic strategy to be combined with systemic antitumoral therapies.[37 53]

Despite the promising data on antimicrobial use for PDAC described previously, antibiotics have been associated with worse outcomes for other cancer types. Antibiotic use prior to immune checkpoint inhibitors treatment has been associated with worse survival in melanoma, lung and other cancers.[54–56] Meta-analyses of published clinical data have also shown that antibiotic usage in patients with cancer (melanoma, lung, renal and head and neck carcinomas) receiving immune checkpoint inhibitors leads to poorer survival.[57] Several preclinical studies have also reported on the negative effects of concomitant use of antibiotics and immunotherapy. Iida *et al*[58] reported a detrimental effect of depletion of the commensal microbiota in a subcutaneous colorectal cancer mouse model (MC38), as treatment with an antibiotic regimen consisting of vancomycin, imipenem and neomycin resulted in decreased efficacy of CpG oligonucleotide immunotherapy and platinum-based chemotherapy. Similarly, in a subcutaneous sarcoma mouse model (MCA-205), Vétizou *et al*[59] observed decreased potency of inhibition of the activity of the immune checkpoint molecule cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on ablation of the commensal microbiota with the use of antibiotics such as ampicillin, colistin and streptomycin. Using the same antibiotic cocktail, Routy *et al*[60] observed reduced efficacy of blocking another immune checkpoint target, PD-1 blockade, in reducing tumour growth in subcutaneous mouse models of sarcoma (MCA-205) and melanoma (RET) when combined with antibiotics.

From the information in the previous two paragraphs, antibiotics may have different impacts depending on the tumour type, the concomitant therapies and the presence or absence of specific bacteria in the cancer cells as in the example of patients with colorectal cancer, in whom antibiotics only play an antitumoral effect when tumour cells contain *Fusobacterium*.[52] Therefore, identifying the microbial profiles present across tumours in different patient populations is important to elucidate their differential effects. If antibiotic treatment in patients with cancers like sarcomas and melanoma results in increased tumour growth, microbial populations in these settings may be beneficial for the host and

may be needed for therapeutic responses of tumours.[58–60] In patients with pancreatic cancer, however, because most studies have reported antitumoral effect on antimicrobial treatment, microbial enrichment associated with this cancer type may contribute to its pathogenesis, explaining why its elimination may play a beneficial role.[23 27 48] Future studies must evaluate factors shaping the overall effect of the tumour microbiota, including variables like diversity, location, the microenvironment and host factors.

Additionally, certain microbes can generate cross-reactive T cells owing to molecular mimicry between tumour and microbial antigens which may trigger antitumor immunity through antigen presentation by major histocompatibility complex class I (MHC-I).[61] However, a recent study showed that pancreatic cancer cells have downregulation of MHC-I molecules on their surfaces for evasion of immunosurveillance through upregulation of autophagy, and inhibition of MHC I improves therapeutic outcomes by synergising with immune checkpoint blockade.[62] This highlights the importance of ascertaining the balance between eliminating harmful microbiotas and enriching beneficial microbiotas, which can improve antitumor immunity.

Another emerging strategy for depleting pathogenic intratumoral bacteria is the use of a special class of highly selective viruses known as lytic bacteriophages or phages, which can selectively infect and lyse certain bacteria and have been tested for their capacity to shift the gut microbiota in murine models.[53 63] Phage display libraries have been shown to target specific organs *in vivo*, and they can produce bystander cytotoxicity in tumour cells.[64–66] In preliminary studies, researchers explored the utility of phages for delivery of anticancer drugs to pancreatic cancers and demonstrated early viability of successful targeting of pancreatic tumour cells.[67–69] Whereas substantial data demonstrate the effect of phages on regulation of microbial population dynamics, evidence of their direct effects on human health is limited. Technical limitations in therapeutically using phages, such as dosing, route of administration, tolerance by the host immune system, and specificity to pancreatic cancer microbes and tumour antigens, remain to be addressed before this strategy can be widely adopted.

FECAL MICROBIAL TRANSPLANTS AS BACKBONE THERAPY FOR PANCREATIC CANCER

The potential of the whole gut microbiota to modulate the pancreatic tumour microbiota and outcomes has been explored by several groups using preclinical models.[23 40 46 48]

To approximate the human scenario, Riquelme *et al*[40] performed human faecal microbial transplantation (hFMT) in mice that later received orthotopic tumour cell implantation to generate a PDAC humanised microbial mouse model. This study demonstrated that about a quarter of the human PDAC microbial composition but not the adjacent normal tissue overlapped with the human donors' gut microbiota, suggesting the existence of a microbial cross-talk between the gut and pancreatic tumours.[40] The same study also demonstrated that the human donors' gut bacteria were efficiently transferred to the gut in these mice.[40] Moreover, the tumour microbial composition in humanised microbial mice was also differentially modulated by transplants from different donors.[40] In accordance with previous murine studies, hFMT using samples obtained from healthy controls resulted in slower tumour growth and a more modest reversal of TME immunosuppression than did hFMT with samples from short-term survivors, whereas the most potent reversal of TME immunosuppression and tumour growth was induced by hFMT using samples from long-term survivors of PDAC with no evidence of disease.[40] Of note, this effect was lost when antibiotics were given after the transplant or when cytotoxic T cells were depleted.[40] This demonstrated the ability of hFMT to positively affect PDAC tumours by modulating the gut/tumour microbial axis along with the immune system. All of this evidence demonstrates that FMT can modulate the gut and tumour microbiotas, the immune activation status and outcomes, paving the way for FMT to be used in combination with other treatment modalities, such as chemotherapy and immunotherapy, for pancreatic cancer and perhaps other tumour types.

FMT is effectively used in clinical practice for treatment of conditions like recurrent *C. difficile* infection, and several ongoing clinical trials at different phases of development are using FMT for treatment of autoimmune, inflammatory and metabolic conditions (figure 1A–C and online supplemental table S1).[70 71] The use of FMT as a treatment option for cancer is gradually attracting more attention. Several early-phase clinical trials of hFMT are currently testing its role in cancer treatment responses and to reduce treatment-associated toxic effects (figure 1D and online supplemental table S1). Ongoing and future clinical studies will be required to establish the safety and efficacy of FMT in modulating the human pancreatic tumour microbiome and enhancing the immune response to ultimately improve survival.

SINGLE OR CONSORTIA BACTERIOTHERAPY

Bacteriotherapy, consisting of oral administration of either a single or a consortium of bacterial species, is a potential strategy for more targeted manipulation of the microbiome than FMT. Early studies in animals looked at the toxicity of spores derived from a non-pathogenic strain of *C. novyi*, a bacterium that can germinate in necrotic and hypoxic regions of tumours.[72 73] Other studies evaluated the roles of specific gut bacterial species in augmenting the efficacy of immunotherapy for tumours.[59 74] *Bacteroides* spp, particularly *Bacteroides fragilis* and *B. thetaiotaomicron*, identified in the gut microbiota in a fibrosarcoma mouse model, are capable of enhancing CTLA-4-based immunotherapy.[59] *Bifidobacterium* spp, identified in the gut microbiota of a melanoma murine model, induced antitumourigenic immune responses alone and, more potently, in combination with another immune checkpoint molecule, programmed cell death-ligand 1 (PD-L1).[74] Engineered non-pathogenic bacteria also have been studied for targeted delivery of therapeutic agents like antibodies and chemopreventive metabolites to determine their ability to be enriched in tumours.[75–77] Nonetheless, no bacterial species have emerged as being therapeutic for pancreatic cancer, and bacteriotherapy for this cancer remains largely unexplored. However, being mindful of the scenarios in which addition of a single bacterium may result in decreased gut diversity, which is usually associated with poor cancer outcomes, is important. Thus, supplementation of single-species bacteriotherapy with FMT may be needed to provide the advantages of both methodologies.

CONCLUSIONS

Studies that aim to target the PDAC-associated gut and tumour microbiotas may employ three strategies: (1) elimination of protumourigenic bacteria from the host through the use of antibiotics, (2) enhancement of immunoactivation by delivery of single or multiple microbial species and (3) 'normalisation' of the dysbiotic gut and tumour environment in patients with PDAC using whole FMT (figure 2). Ultimately, all of these strategies have the common goal of shifting the

balance from an immunosuppressive TME to an immunoactivated one. Furthermore, identification and better understanding of the mechanisms employed by downstream effector molecules (eg, host or microbial metabolites) mediating microbial responses as biomarkers and potentially as therapeutic targets may also be very important. In summary, modulation of the microbiome may emerge as a supplement for existing cancer therapies with the main goal of increasing their efficacy by reversing immunosuppression. Because most functional studies reported thus far have been conducted using animal models, we hope to get more information from upcoming clinical trials targeting the microbiome.

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