



## Cancer as microenvironmental, systemic, and environmental diseases: opportunity for transdisciplinary microbiomics science

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**Use of standardised official symbols:** We use HUGO (Human Genome Organisation)-approved official symbols (or root symbols) for genes and gene products, including ADIPOQ, ANXA1, APC, ARID1A, ATG7, BRAF, CD3, CD4, CD8, CD47, CD274, CDKN2A, CEACAM1, CRP, CTLA4, CTNNA1, CXCL1, CXCR2, FOXP1, FOXP3, GDF15, HAVCR2, IFNG, IL1B, IL2, IL33, IL6, IL10, IL17A, JAK2, KRAS, MYD88, NOTCH1, PBRM1, PDCC1, PDX1, PIK3CA, PTPRC, STING1, TGFB1, TIGIT, TLR, TLR4, TNFRSF1B, TP53, ULK1, and WNT; all of which are described at [www.genenames.org](http://www.genenames.org). The official gene symbols are italicised to differentiate from non-italicised gene product names (and other colloquial names). Names of non-human genes and gene products have the first Capital letter followed by small case letters.

Note added in proof

A recent study has shown a positive relationship between long-term Western-style diets and increased incidence of colorectal cancer containing high amounts of *pkcs<sup>+</sup> E. coli*,<sup>216</sup> which further attests to the power of the MPE approach in deciphering dietary influences on tumourigenic processes via the microbe.

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## Abstract

Cancer is generally regarded as a localised disease, with the well-established role of the tumour microenvironment. However, the realm of cancer goes beyond the tumour microenvironment, and cancer should also be regarded as a systemic and environmental disease. The exposome (*i.e.*, the totality of exposures), which encompasses diets, supplements, smoking, alcohol, other lifestyle factors, medications, *etc.*, likely alters the microbiome (inclusive of bacteria, viruses, archaea, fungi, parasites, *etc.*) and immune system in various body sites and influences tumour phenotypes. The systemic metabolic / inflammatory status, which is likely influenced by exposures and intestinal physiological changes, may affect tissue microenvironment of colorectum and any other organs. Germline genomic factors can modify disease phenotypes *via* gene-by-environment interactions. Although challenges exist, it is crucial to advance not only basic experimental research that can analyse the effects of exposures, microorganisms, and microenvironmental components on tumour evolution but also interdisciplinary human population research that can dissect the complex pathogenic roles of the exposome, microbiome, and immunome. Metagenomic, metatranscriptomic, and metabolomic analyses should be integrated into well-designed population research combined with advanced methodologies of artificial intelligence and molecular pathological epidemiology. Ideally, a prospective cohort study design that enables biospecimen (such as stool) collection before disease detection should be considered to address reverse causation and recall biases. Robust experimental and observational research together can provide insights into dynamic interactions between environmental exposures, microbiota, tumour, and immunity during carcinogenesis processes, thereby helping us develop precision prevention and therapeutic strategies to ultimately reduce the cancer burden.

## Keywords

biobank; bioinformatics; computational biology; microbiology; precision medicine

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## Introduction and Purpose of the Article

While a tumour evolves with the accumulation of genomic and epigenomic aberrations in neoplastic cells, it generates its intrinsic microenvironment, where neoplastic cells interact with immune and other non-neoplastic cells. Among various components of the tumour microenvironment, increasing attention has been devoted to microorganisms that encompass viruses, bacteria, fungi, archaea, *etc.* Microorganisms, which are ubiquitously present in and around the human body and particularly abundant in digestive tracts, not only influence oncogenesis in various organs but also shape the host's antitumour immunity in the local and systemic environment.<sup>1-4</sup>

To better understand cancer, we should also account for the exposome, *i.e.*, the totality of exposures including diets, supplements, alcohol, smoking, medications, microorganisms, *etc.* Many exposures have been established as either risk or protective factors for cancer. In addition, various exposures may influence tumour development through alterations of the tumour microenvironment.<sup>5</sup> Systemic physiological statuses such as immune, inflammatory, metabolic, and hormonal conditions are also influenced by exposures (including the microbiota), and in turn, influence local tumour development.<sup>5,6</sup> Taken together, cancer can be regarded as a microenvironmental, systemic, and environmental disease (Figure 1). Therefore, we need to examine not only tumour cells and the surrounding microenvironment but also the effects of various exposures and systemic factors on tumours. However, there have been technical and practical hurdles to performing such integrative analyses of these factors in human populations. To date, large-scale human population studies have rarely been conducted to elucidate the complex interactions between the exposome, microbiota, and cancer.

This article aims to provide a viewpoint that cancer is an environmental, systemic, and microenvironmental disease where the microbiome plays a pivotal role in the interplay of various factors. We emphasise the increasing need for transdisciplinary multi-modal research to assess the interactive effects of the exposome, microbiome, and the tumour microenvironment on tumours, using experimental models and human populations. Such integrative research approaches can help us develop better strategies for precision cancer prevention and therapeutics.

This article uses the standardised nomenclature system for genes and gene products according to the Human Genome Organisation Gene Nomenclature Committee (HGNC),<sup>7</sup> to increase clarity and reduce ambiguity associated with colloquial protein names.

## Tumour Microenvironment

### Tumour arises through a dynamic network

Tumour arises with the accumulation of cellular genomic and epigenomic alterations. Emerging evidence indicates that the expansion of clones harbouring somatic mutations in cancer-associated genes is common in non-neoplastic tissues, especially in aging individuals or individuals with chronic inflammation.<sup>8</sup> Somatic driver mutations for colorectal cancer (CRC) were documented in approximately 1% of normal colorectal crypts in middle-aged individuals.<sup>9</sup> Most newly-forming clones are destined to be well controlled or eliminated.<sup>10</sup> Some mutant clones in phenotypically normal epithelium may purge other clones through cell competition, thereby exerting an antitumorigenic effect and preserving tissue integrity.<sup>10</sup> Eventually, one of these mutant clones may proliferate enough to form a benign neoplasm. A benign tumour may further accumulate genomic and epigenomic alterations and progress to malignancy.

A neoplasm generates its microenvironment, where tumour cells, microorganisms, immune cells, other cells, and extracellular matrix components (*e.g.*, collagen, fibronectin) interact *via* dynamic crosstalks.<sup>11,12</sup> The intercellular communications are regulated by direct cell-to-cell contact and through biomolecules (*e.g.*, cytokines, chemokines, growth factors).

Tumours harbour distinct microbial communities, which vary by tumour types<sup>13–15</sup> and may persist during the metastatic process.<sup>16</sup> As such, polymorphic microbiomes have recently been noted as a new cancer hallmark.<sup>17</sup> Intracellular bacteria may exist within tumour cells and/or immune cells.<sup>14</sup> Peptides derived from intracellular bacteria can be presented by tumour cells and influence immune responses.<sup>18</sup> Intratumoural microbes interact with tumour cells, immune cells, and other cells / components. In mice bearing inflammation-induced colon tumours, neutrophil depletion increased intratumoural microbes, induced IL17A-mediated inflammatory response, and promoted tumour growth.<sup>19</sup> Certain microbes may promote carcinogenesis through producing tumourigenic molecules or inducing a pro-inflammatory microenvironment, whereas other microbes may exert tumour-suppressive properties through maintaining vigorous antitumour immune responses.<sup>1,3,20</sup>

### Microbes involved in tumourigenesis across various organs

Accumulating evidence supports the involvement of specific microbes in tumourigenesis of various organs (Table 1).<sup>1–4</sup> Herein, we discuss not only bacteria but also fungi, archaea, and viruses that have been implicated in gastroenterological tumours. Presumably, enormous amounts of data on the pro/anti-carcinogenic roles of microorganisms and microbial dysbiosis will emerge in the next decade.

The colorectum hosts the largest load and diversity of bacterial species among all organs; therefore, the dysregulated microbiota has been examined extensively in the development of colorectal diseases, including CRC. Metagenomic analyses demonstrated enrichment of *Fusobacterium nucleatum* in CRC tissues compared to adjacent normal tissues.<sup>21,22</sup> *F. nucleatum* appears to exert carcinogenic effects on the colorectal epithelium by activating myeloid-derived suppressor cells,<sup>23</sup> suppressing NK and T cells *via* interaction with TIGIT and CEACAM1 inhibitory immunoreceptors,<sup>24,25</sup> and activating the CTNBN1 (beta-catenin)-WNT signalling pathway *via* ANXA1 (annexin A1) upregulation.<sup>26</sup> CRC containing *F. nucleatum* is characterised by proximal tumour localisation, *BRAF* mutation, high-level microsatellite instability, high-level CpG island methylator phenotype,<sup>27–29</sup> decreased CD3<sup>+</sup>CD4<sup>+</sup>CD45RO(PTPRC)<sup>+</sup> cells,<sup>30</sup> and increased tumour-associated macrophages.<sup>31</sup> Specifically, *F. nucleatum* subspecies *animalis* may play a role in most of these associations.<sup>32</sup>

In addition to bacteria, non-bacterial microorganisms, including viruses, fungi, archaea, and parasites, likely play pathogenic roles in various cancer types, including CRC.<sup>33,34</sup> Viruses represent an essential component of the intestinal microbial community and have been implicated in inflammatory bowel diseases<sup>35,36</sup> and CRC.<sup>34,37,38</sup> The faecal virome of CRC patients appeared more diverse than that of CRC-free individuals and enriched for bacteriophages that are expected to be bacterium-virus community hubs,<sup>37,38</sup> suggesting a role of the virome in colorectal carcinogenesis *via* its modulating effect on the bacterial community. Regarding the mycobiome, CRC patients exhibited faecal fungal dysbiosis with an increased Basidiomycota:Ascomycota ratio.<sup>39</sup> Additionally, the faecal microbiota was characterised by increased co-occurring fungal intrakingdom correlations and disappearance of co-occurring bacterial–fungal correlations (*e.g.*, fungal Ascomycota and bacterial Proteobacteria), indicating that synergistic intrafungal and antagonistic bacterial–fungal

associations may contribute to colorectal carcinogenesis.<sup>39</sup> The faecal archaeome of CRC patients was characterised by enrichment of halophilic archaea (*e.g.*, *Natrinema* species *J7-2*) and depletion of methanogenic archaea.<sup>40</sup> Multi-kingdom microbiota analyses of CRC metagenomic datasets identified 16 microbial biomarkers (including 11 bacterial, 4 fungal, and 1 archaeal feature) that achieved better performance than single-kingdom markers in diagnosing CRC patients.<sup>34</sup> Moreover, exploration of the metagenomic functions indicated that bacterial–fungal interactions might contribute to colorectal carcinogenesis *via* upregulation of D-arginine and D-ornithine and stimulation of butanoate metabolism.<sup>34</sup>

Chronic infection with *Helicobacter pylori* is a leading cause of gastric cancer.<sup>41–43</sup> *H. pylori* typically resides in the gastric mucus layer and promotes chronic inflammation, mucosal atrophy, and intestinal metaplasia.<sup>42,44</sup> *H. pylori* infection can induce infiltrations of immune cells that produce inflammatory mediators such as TGF $\beta$ 1 (transforming growth factor- $\beta$ ), thereby contributing to gastric tumourigenesis.<sup>45</sup>

Epstein-Barr virus (EBV) is another pathogenic microbe associated with certain forms of gastric cancer. EBV-associated gastric cancer, which comprises 7–10% of gastric cancer cases, is characterised by male predominance, young-onset, proximal tumour localisation, abundant tumour-infiltrating lymphocytes, *PIK3CA* and *ARID1A* mutations, *CDKN2A* promoter hypermethylation, and *CD274* (PD-L1) overexpression.<sup>46</sup> EBV-encoded microRNAs BART11 and BART17-3p appear to promote immune escape by increasing the enhancer-mediated *CD274* transcription.<sup>47</sup>

Pancreatic cancer tissue harbours greater amounts of bacteria and fungi than normal pancreatic tissue.<sup>48,49</sup> Intratumoural microbes in pancreatic cancer may have migrated from the gastrointestinal tract *via* the pancreatic duct system, as illustrated by the observation that fluorescently labelled bacteria and fungi migrated into the pancreas in a retrograde manner.<sup>48,49</sup> Bacterial translocation to the pancreas may be caused by the biliary infection, as *Enterococcus* species were commonly detected in bile juice and pancreatic cancer tissue.<sup>50</sup> Pancreatic intratumoural microbes may create an immunosuppressive microenvironment by activating distinct Toll-like receptors (TLRs) in monocytic cells.<sup>48</sup> In mice, bacterial ablation decreased myeloid-derived suppressor cells and increased antitumour M1 macrophages.<sup>48</sup> Intratumoural bacterial composition in pancreatic cancer influences patient outcomes, as indicated by observations that a signature of three bacterial genera (*Pseudoxanthomonas*, *Streptomyces*, *Saccharopolyspora*) and high alpha-diversity of intratumoural bacteria were both associated with better patient outcomes. Intratumoural fungi also play a crucial role in pancreatic carcinogenesis. Intratumoural *Malassezia* appears to augment the progression of pancreatic cancer by attaching to mannose-binding lectin and thereby activating the complement cascade.<sup>49</sup> Furthermore, in response to the intratumoural mycobiome, pancreatic cancer cells appear to produce IL33 (interleukin 33) as a chemoattractant for type 2 immune cells, which can stimulate tumour growth by secreting pro-tumourigenic cytokines.<sup>51</sup>

Emerging evidence suggests a feasibility of non-invasive faecal microbiota-based screening for the early detection of pancreatic cancer.<sup>52,53</sup> Pancreatic adenocarcinoma could be predicted robustly and accurately by metagenomic classifiers based on faecal microbial

species.<sup>52,53</sup> *Veillonella* species (e.g., *V. atypica*) and *Streptococcus* species were enriched, and *Faecalibacterium prausnitzii* was depleted in faecal samples of pancreatic adenocarcinoma patients.<sup>52,53</sup>

The liver is chronically exposed to intestinal microbes and their metabolites because of its anatomical connection with the gut *via* the portal vein and bile duct systems. The microbes and their metabolites may produce pro-inflammatory or immunosuppressive conditions, which may result in liver carcinogenesis. Gram-negative commensal gut bacteria can induce hepatocytes to form a tumour-promoting environment by recruiting immunosuppressive CXCR2<sup>+</sup> polymorphonuclear myeloid-derived suppressor cells through TLR4-dependent CXCL1 production, eventually promoting the development of intrahepatic cholangiocarcinoma.<sup>54</sup> *Enterococcus faecalis*, a species enriched in faecal samples of patients with HCV (hepatitis C virus)-related chronic hepatitis, appears to promote liver carcinogenesis *via* the expression of the metallopeptidase *gelE*.<sup>55</sup> In mice, *gelE*-positive *E. faecalis* promoted liver carcinogenesis in a TLR4-dependent manner by increasing gut permeability *via* its gelatinase activity and elevating plasma lipopolysaccharide that acts on hepatocytes.<sup>55</sup> The composition of intratumoural microbiota in hepatocellular carcinoma (HCC) differs according to aetiological factors.<sup>56,57</sup> *Ruminococcus gnavus* was characteristically enriched for virus-related HCCs.<sup>57</sup> As for intrahepatic cholangiocarcinoma, four subgroups characterised by proteogenomic profiling had distinct intratumoural microbiota diversity, compositions, and functions.<sup>58</sup> As viral exposure history differs between HCC patients and HCC-free individuals, a viral exposure signature, determined by serological profiling, could identify HCC prior to a clinical diagnosis.<sup>59</sup> Exposure to HBV (hepatitis B virus), HCV, two influenza strains (H1N1 and H3N2), and cytomegalovirus correlated with increased HCC risk.<sup>59</sup>

## Systemic Conditions as a Component of Tumour

Cancer should be recognised as a systemic disease, as systemic conditions can influence a tumour and *vice versa*. Persistent local and systemic inflammation is a hallmark of cancer. Systemic immune, inflammatory, metabolic, and hormonal statuses may contribute to oncogenesis through their effects on cellular genomic and epigenomic aberrations as well as local tissue microenvironment. Systemic antitumour immunity suppresses tumour initiation, progression, and metastasis.<sup>60–62</sup> Diabetes mellitus, a metabolic syndrome characterised by hyperglycaemia, hyperinsulinaemia, and insulin resistance, increases cancer risk.<sup>63–65</sup> Higher levels of GDF15, CRP, IL6, and TNFRSF1B (HGNC:11917; TNF receptor superfamily 1B) and lower levels of ADIPOQ (HGNC:13633; adiponectin) and 25-hydroxyvitamin D in blood have been associated with cancer risk and mortality.<sup>66–73</sup> Vitamin D is an immunomodulator that helps maintain immune homeostasis and induces tumour-suppressive immune responses.<sup>74,75</sup> The inverse association of vitamin D levels with CRC risk appeared stronger for tumours exhibiting higher lymphocytic infiltrates.<sup>76</sup>

Conversely, localised or metastatic cancer can alter systemic immune and metabolic conditions.<sup>60</sup> In preclinical models, *TP53* loss in breast carcinoma cells induced the secretion of WNT ligands that stimulate tumour-associated macrophages to produce IL1B, thereby causing systemic inflammation and tumour metastasis.<sup>77</sup> In an analysis



of The Cancer Genome Atlas (TCGA) cohorts of 33 cancer types, unique microbial communities were detected in tumour tissues and blood samples, indicating that microbes may migrate between tumour tissue and bloodstream.<sup>13</sup> Intratumoural microbes may move to distant organs, creating a microenvironment (“premetastatic niche”) where tumour cells can implant, survive, and proliferate.<sup>78</sup> In a preclinical study using CRC-bearing mice, intratumoural *Escherichia coli* disrupted the gut vascular barrier and created a premetastatic niche in the liver, promoting CRC metastasis.<sup>78</sup> Tumour microenvironment may favour local bacterial implantation and growth from circulating microbes.<sup>4</sup> Rapidly formed vasculature due to tumour growth is characterised by irregular organisation and leakiness, which may permit microbial migration between the tumour microenvironment and bloodstream.

## Exposome as a Component of Tumour

The exposome (the totality of exposures), which includes the microbiome, influences tumour phenotypes *via* its complex effects on neoplastic cells, tumour microenvironment, and systemic physiological states. The systemic conditions are also conceptually a part of the exposome. Certain exposures may predispose individuals to cancer development as well as influence its disease course and outcomes.<sup>63,79,80</sup> Cigarette smoking is the leading cause of cancer.<sup>79,81</sup> Inhaled carcinogens in cigarette smoke directly damage DNA and produce mutations in epithelial cells.<sup>81</sup> Smoking also appears to induce colorectal carcinogenesis *via* its modulating effects on systemic and local immune reactions.<sup>81,82</sup> The association between smoking and CRC incidence was stronger for tumours containing fewer T cells and macrophages, supporting immunosuppressive effects of smoking.<sup>80,83</sup> Higher physical activity was associated with decreased CRC incidence and mortality through its influences on energy balance, cellular prostaglandin biosynthesis, and systemic inflammatory statuses.<sup>84</sup> The beneficial association of exercise with CRC prognosis was stronger in CRC with fewer tumour-infiltrating CD3<sup>+</sup> lymphocytes, supporting interactive effects of physical activity and immune response on clinical outcomes.<sup>85</sup> Essentially, the exposome, which is one of the determinants of tumour evolution and phenotypes, can be regarded as an extended component of the tumour. This notion is helpful in increasing the recognition of exposure modifications as effective preventative and therapeutic strategies for cancer.

## Microbiota as a Pivot of Interplay of the Exposome and Tumour

Emerging evidence indicates a mediating and modifying role of the microbiota (which is itself a component of the exposome) in the effects of other exposures on tumour cells and the microenvironment (Figure 2). The gut microbiota plays a pivotal role in the association between diets and cancer.<sup>86–89</sup> High intake of red meat and low dietary fibre intake were correlated with enrichment of *Fusobacterium* in faeces of healthy individuals.<sup>90</sup> The abundance of CRC-related bacteria or bile acid-metabolising bacteria (*e.g.*, *Bilophila wadsworthia*) was correlated with a high intake of red meat and a low intake of fruits and vegetables.<sup>91</sup> Processed and animal-derived foods were associated with *Firmicutes*, *Ruminococcus* species of the *Blautia* genus, and endotoxin synthesis pathways.<sup>92</sup> In contrast, plant foods and fish were linked to short-chain fatty acid (SCFA)-producing microbes and nutrient metabolism pathways. These diet-microbiota associations

are consistent across healthy individuals and patients with chronic inflammatory bowel diseases (*e.g.*, Crohn's disease, ulcerative colitis).<sup>92</sup> Further research has characterised metabolomic and metagenomic profiles of stool specimens from patients with colorectal tumours.<sup>90,93</sup> Compared to plant-based foods, animal-based foods contain abundant taurine, which increases taurocholic acid in the liver and gut.<sup>94</sup> Taurocholic acid is metabolised to genotoxic H<sub>2</sub>S by *B. wadsworthia* and tumour-promoting deoxycholic acid by *Clostridium scindens*.<sup>95</sup> H<sub>2</sub>S-producing pathways were upregulated in CRC patients based on faecal examinations.<sup>90</sup> Notably, African Americans harbour higher amounts of sulfidogenic bacteria and *B. wadsworthia* than non-Hispanic Whites in the U.S., suggesting that these microbial differences might explain the higher incidence of CRC in African Americans.<sup>96</sup>

Physical and physiological conditions (*e.g.*, obesity, surgery) can influence the gut microenvironment, thereby promoting tumourigenesis.<sup>86</sup> Gastrointestinal surgery influenced the gut microbiota with increased species diversity and enrichment of deoxycholic acid and branched-chain amino acids.<sup>97</sup> Metagenomic and metabolomic analyses of faecal samples suggested that patients with a history of gastrectomy had higher amounts of oral microbes, aerobes, or facultative anaerobes, as well as higher levels of deoxycholic acid and branched-chain amino acids in the intestine.<sup>97</sup> Interestingly, patients with a history of total gastrectomy had abundant CRC-related bacteria (*e.g.*, *F. nucleatum*),<sup>97</sup> which might explain an increased risk of developing metachronous CRC in those patients.<sup>98</sup>

## Role of Basic Experimental Research

Studies based on epidemiological or clinical cohorts have demonstrated associations of specific bacterial taxa with clinical and molecular characteristics of tumours.<sup>27,28,30,31,99–101</sup> Functional analyses using preclinical model systems (*e.g.*, organoids, animal models) can provide biological evidence for tumourigenic roles of specific bacterial species or communities and thereby support findings of population-based studies. Such experimental studies are essential to moving the cancer-microbiome research from a discovery phase to a translation phase, and finally to an implementation phase for cancer prevention, diagnosis, and treatment.

### Organoids

Patient-derived organoids are *ex vivo* tissue cultures that form three-dimensional organ-like structures. Organoids can be genetically manipulated to recapitulate specific genetic mutations observed in patient tumours. Certain *E. coli* strains harbour the genetic island *pks* (polyketide synthase), which encodes a set of enzymes required for colibactin synthesis. The *pks*<sup>+</sup> *E. coli* appear to inhabit the colorectal tissues in approximately 20% of healthy individuals, 40% of patients with inflammatory bowel diseases, and 60% of patients with familial adenomatous polyposis or CRC.<sup>102–105</sup> A study using human intestinal organoids and CRC tissues indicated that *pks*<sup>+</sup> *E. coli* might directly contribute to specific mutational signatures of CRC.<sup>102</sup> These *pks*<sup>+</sup> *E. coli*-induced mutational signatures were closely matched with those in healthy human colon crypts and suggested to be induced during early childhood,<sup>9</sup> implying that the exposure to *pks*<sup>+</sup> *E. coli* may occur during early childhood and predispose individuals to CRC development in later life.



## Mouse models

Genetically engineered mouse models (GEMMs) serve as a valuable tool in preclinical cancer research and allow for microbiome studies of conventional microbiota (conventionally housed or specific pathogen-free) or those of gnotobiotic GEMMs. In particular, germ-free mice facilitate examinations of animals without microbes or gnotobiotic animals exclusively colonised by defined microbial species or communities.<sup>106</sup>

In a study using *Apc*<sup>Min/+</sup> mice,<sup>23</sup> oral administration of *F. nucleatum* increased intestinal tumour formation, supporting its carcinogenic role. However, exposure to other *F. nucleatum* strains did not increase tumour formation in germ-free or specific pathogen-free *Apc*<sup>Min/+</sup> and *Apc*<sup>Min/+</sup>;*IL10*<sup>-/-</sup> mice,<sup>107</sup> suggesting the existence of tumour-promoting virulence factors in specific *F. nucleatum* strains. A study utilising patient-derived xenografts of CRC demonstrated that *Fusobacterium* and co-existing cancer-specific microbes persist following serial implantation,<sup>16</sup> suggesting that the microbiota is an intrinsic component of the tumour microenvironment. This study also serves as a proof-of-principle work of microbe-targeted treatment, demonstrating that bacterial ablation reduced tumour burden in mice harbouring *Fusobacterium*-positive human tumours.<sup>16</sup>

A synergistic role of microbial members in the initiation of hereditary CRC was assessed by examining colonic mucosal biofilms composed of enterotoxigenic *Bacteroides fragilis* (ETBF) and *pks*<sup>+</sup> *E. coli* in GEMMs.<sup>103</sup> Co-colonisation of *pks*<sup>+</sup> *E. coli* and ETBF led to faster tumour onset, greater mortality, and higher levels of colonic inflammation than infection with either bacterial strain alone. ETBF enhanced *pks*<sup>+</sup> *E. coli* colonisation through mucus degradation and subsequently increased cellular DNA damage and IL17A production with the aid of *pks*<sup>+</sup> *E. coli*. Interestingly, mucosal biofilms from CRC patients or even healthy individuals were tumourigenic in germ-free *Apc*<sup>Min 850/+</sup>;*IL10*<sup>-/-</sup> or *Apc*<sup>Min 850/+</sup> and specific pathogen-free *Apc*<sup>Min 716/+</sup> mice, suggesting a carcinogenic potential of bacterial biofilms.<sup>108</sup>

Population-based data indicate associations of the intratumoural microbiota with clinical outcomes of pancreatic cancer patients.<sup>109</sup> To validate these associations, antibiotic-pretreated C57BL/6 mice which received faecal microbiota transplantation (FMT) from pancreatic cancer patients were orthotopically implanted with KPC (*Pdx1-Cre*;*LSL-Kras*<sup>p.G12D/+</sup>;*LSL-Tp53*<sup>p.R172H/+</sup>) pancreatic cancer cells. FMT from long-term survivors resulted in decreased tumour burden with increased antitumour T cell infiltrates.<sup>109</sup> Collectively, intratumoural and intestinal microbiome data at the time of therapy initiation may guide treatment strategies, including microbial manipulation.

## Challenges in basic experimental research

A major challenge of the current preclinical models is the difficulty in accurately recapitulating the complexity of tumour microenvironment along with varieties of microbial populations and immune cells in humans. Bacterial culture has been a fundamental method of analysing microbes, which enables the reproduction of microorganisms in a predetermined culture medium under controlled laboratory conditions. However, approximately 70–80% of the intestinal bacterial species cannot be cultured.<sup>110</sup> It has been

difficult to examine bacterial populations in the human body, retarding our understanding of complex microbial communities in humans. To overcome these challenges, mechanistic approaches should be sophisticated at both reductionist and community levels.

## Human Population Research with Innovative Microbiomics Technologies

To overcome the limitations of the conventional microbiology assays, including bacterial culture, next-generation sequencing (NGS) emerged as a culture-free technology in the early 2000s. NGS-based high-throughput technologies allow for analyses of unculturable or previously unidentified microbes and thereby facilitate examinations of an entire spectrum of microbial populations at the nucleic acid level (*i.e.*, the metagenome).<sup>111</sup> Metagenomic approaches have been increasingly utilised to assess taxonomic and functional characteristics of the microbiota.<sup>112</sup>

In recent meta-analyses,<sup>93,113</sup> CRC-related microbial alterations were noted consistently across three continents, despite considerable differences not only in environmental, dietary, and lifestyle factors between the populations but also in approaches of taxonomic profiling and statistical analyses.<sup>93,113</sup> A core set of intestinal microorganisms was strongly associated with CRC (*e.g.*, *F. nucleatum*, *Parvimonas micra*, *Gemella morbillorum*). Most of those core species decreased dramatically after tumour removal.<sup>90</sup>

Metabolomics analysis is another omics approach that has expanded our understanding of complex interactions between microbiota, metabolites, and the host. SCFAs play pivotal roles in modulating inflammation and tumorigenesis.<sup>114</sup> Amounts of metabolites can be measured comprehensively using mass spectrometry and/or nuclear magnetic resonance spectroscopy. Especially, various charged metabolites can be quantified using capillary electrophoresis time-of-flight-mass spectrometry.<sup>115</sup> These assays have revealed dynamic alterations of metabolomic profiles during colorectal tumorigenesis, including increased levels of deoxycholic acids and branched-chain amino acids in early-stage CRC.<sup>90</sup> Secondary bile acids (*e.g.*, deoxycholic acids) might promote colorectal carcinogenesis through generating reactive oxygen and nitrogen species, which potentially damage DNA and promote resistance to apoptosis.<sup>116</sup> Stool specimens from CRC patients showed increased amino acids and decreased SCFAs.<sup>117</sup> Recent advances in metabolomics technologies have provided novel insights into intestinal metabolic dynamics in the complex microbial ecosystem.<sup>1</sup>

## Integration of Microbiomics into Exposome Research

Epidemiology is a fundamental scientific field that studies the aetiology and consequence of a disease of interest in human populations. However, the importance of epidemiological studies in addressing the complex roles of tumour microenvironment has not been fully recognised. A substantial gap remains between basic experimental and epidemiological research.

It is conceivable that risk factor exposures may contribute to tumorigenesis at least some time points during possibly decades-long latency from normal cells to clinically-detectable cancer.<sup>118,119</sup> Certain exposures (*e.g.*, strong radiation), even for a short time,

can directly cause cellular alterations and increase cancer risks for a lifetime. Other exposures (*e.g.*, obesity) may help altered cells clonally expand and accumulate additional molecular changes over a long time period *via* mechanisms including promotion of cellular proliferation and suppression of antitumour immunity.<sup>120</sup> Therefore, it is desirable to examine various exposures in each individual longitudinally over time. Furthermore, the relationships between exposures and tumour development may differ by tumoural characteristics. Based on this notion, molecular pathological epidemiology (MPE) research examines associations of exposures with disease subtypes classified by molecular and pathological signatures.<sup>121,122</sup> For microbiome-related cancer, utilising microbiomic data from biospecimens (tumour/normal tissue, stool, blood, saliva, *etc.*), MPE research can provide evidence for the association of an exposure with development and consequence of tumour subtypes with specific microbial features (Figure 3). For example, the inverse relationship of fibre-rich “prudent diets” with CRC incidence appeared stronger for tumours containing abundant *F. nucleatum*.<sup>101</sup> Hence, the MPE approach can link prudent diets with specific intratumoural bacteria, thereby supporting causality and uncovering a strong association that is otherwise masked in the traditional epidemiological analysis of overall CRC. Another study showed that a positive association between inflammatory diets and CRC incidence was stronger for tumours containing abundant *F. nucleatum*.<sup>100</sup> These findings suggest that the *F. nucleatum*-rich tumour subtype may be affected by dietary factors and that dietary interventions may help control microbiota-related CRC.<sup>122,123</sup> Moreover, it is of particular interest to examine microbial features in the intestine or other organs (as exposures) in relation to tumours subtyped by intratumoural microbial characteristics. With its unique strengths, MPE research can contribute to developing microbe-targeted strategies for cancer prevention and treatment.

## Translational Potentials for Microbe-targeted Preventive and Therapeutic Strategies

### Microbiome-modulating strategies

Substantial evidence supports microbial manipulation as a promising strategy for cancer prevention and treatment.<sup>124–127</sup> Potential microbial interventions include dietary modifications, pre/probiotics, antimicrobial agents, FMT, and bacterial cocktails. Microbial interventions can be applied as an adjunct to traditional cancer therapeutics or stand-alone therapy and may mitigate the adverse effects of anticancer therapies.<sup>128,129</sup>

Evidence supports dietary alterations as a microbiota-modifying intervention.<sup>130–132</sup> High-fibre, less western-style diets can prevent gut microbes from consuming mucous glycoproteins, and strengthen the mucus barrier function.<sup>133–135</sup> Mediterranean diets may induce SCFA production and exert anti-inflammatory properties, potentially reducing the risk of chronic inflammation-related diseases, including CRC.<sup>136</sup>

Oral administrations of pre/probiotics are easy-to-implement ways to modulate microbial populations.<sup>137</sup> Prebiotics are non-viable substances that facilitate the growth or activity of certain bacterial species, whereas probiotics are individual or combinations of bacteria. Administration of probiotics containing *Bifidobacterium lactis* and *Lactobacillus acidophilus*

to CRC patients increased butyrate-producing bacteria and decreased CRC-related bacteria in the intestine.<sup>138</sup>

Antimicrobial agents, including antibiotics, have an appreciable influence on the development and consequence of cancer by modulating the microbiota. Antibiotics may eliminate favourable microbes and decrease microbial diversity, resulting in microbial dysbiosis. Developing narrow spectrum antimicrobial or anti-virulence approaches with little disruption to the human microbial ecosystem would be an ideal strategy for cancer control. For example, fidaxomicin selectively targets *Clostridium difficile* with minimal effects on gut commensals, as supported by findings that a fidaxomicin-binding determinant of RNA polymerase is present in *C. difficile* but is absent in intestinal bacteria.<sup>139</sup> Another example is sequence-specific antimicrobials based on programmed CRISPR-Cas13a packaged into a bacteriophage capsid, which potentially target antimicrobial-resistant bacteria.<sup>140</sup> This technology would facilitate the development of antimicrobials that can selectively eliminate carcinogenic microbes with minimal dysregulation of the commensal microbial flora for cancer control. The duration of trials investigating antimicrobial approaches for cancer prevention and treatment needs to be determined based on data on numbers of outcome and adverse events in exposed and unexposed populations in previous studies.

The FMT approach, where favourable microbial ecosystem of a donor is transplanted to a recipient, has been investigated in *C. difficile* infection<sup>141–143</sup> and ulcerative colitis.<sup>144,145</sup> Emerging evidence suggests the effectiveness of FMT for cancer control.<sup>146</sup> This approach may be more beneficial than the administration of limited microbial species, given that an appropriate microbial ecosystem comprising various microbes enables the host to maintain normal physiological function and homeostasis.<sup>125,147</sup> Recent studies have reported utilities of FMT to enhance immunotherapeutic efficacy.<sup>148–154</sup> However, FMT has some risks, as illustrated by a report of antibiotic-resistant bacteraemia after receiving FMT in patients with *C. difficile* infection<sup>155</sup> and a preclinical study that showed inflammation-associated carcinogenesis in FMT-treated mice.<sup>156</sup>

Bacterial cocktails are a mixture of purified bacteria with a presumably better safety profile compared to FMT. Bacterial cocktails, including *Firmicutes* species extracted from the stool of healthy human donors, relieved symptoms of *C. difficile* infection as effectively as FMT.<sup>157</sup> Bacterial cocktails can also enhance immunotherapeutic efficacy. For example, a mixture of 11 bacterial strains could expand the populations of CD8<sup>+</sup>IFN $\gamma$  (interferon- $\gamma$ )<sup>+</sup> T cells and boost their activity to kill tumour cells, thereby augmenting the efficacy of anti-PDCD1 (PD-1) or anti-CTLA4 treatment.<sup>158</sup> Most healthy individuals did not harbour these beneficial microbes, and in a minority of individuals who did, the microbial abundance was low.<sup>158</sup> This study emphasises that highly abundant microbes in certain niches are not necessarily functionally important and that specific microbes with experimentally proven functions should be targeted for cancer control.

### Microbial interventions for cancer therapeutics

Microbiota potentially affects the efficacy of anticancer therapies. Several studies have reported differential effects of cancer therapeutics by the microbiota (Table 2).<sup>159–163</sup> Certain microbes appear to enhance chemotherapeutic efficacy, while others may have the

opposite effect.<sup>159–163</sup> The antitumour effect of oxaliplatin was reduced without the innate gut microbiota that stimulates tumour-infiltrating myeloid-derived cells to produce reactive oxygen species.<sup>164</sup> In CRC, *F. nucleatum* may provoke tumour resistance to oxaliplatin and 5-fluorouracil by upregulating ULK1 and ATG7 expressions.<sup>165</sup> Similarly, in oesophageal cancer, intratumoural *F. nucleatum* may promote autophagy by increasing ATG7 levels, thereby conferring chemoresistance to oesophageal cancer.<sup>166</sup> Cyclophosphamide impairs the intestinal mucosal barrier and promotes bacterial translocation to the spleen and lymph nodes, where the microbes provoke antitumour Th17-mediated immune responses.<sup>167</sup> In tumour-bearing mice, ablation of gram-positive bacteria decreased Th17 cells and induced resistance to cyclophosphamide.<sup>167</sup> Through their drug-metabolising activities,<sup>159</sup> gut microbes can influence chemotherapeutic efficacy. In pancreatic cancer, intratumoural bacteria directly provoke gemcitabine resistance by metabolising gemcitabine into its inactive form, and antibiotics (ciprofloxacin) can reverse the chemoresistance and facilitate the apoptosis of cancer cells when administered with gemcitabine.<sup>168</sup> The microbial profile also influences chemotherapeutic toxicity. Indeed, several microbes increase the risk of chemotherapy-induced adverse events by producing drug-metabolising enzymes, and elimination of those microbes ameliorates the adverse effects.<sup>125,159</sup> Irinotecan is detoxified in the liver and transfers through the bile duct to the intestine, where microbe-derived beta-glucuronidases can reactivate it and thereby cause diarrhoea and other toxicities.<sup>169,170</sup>

Recent studies support the effects of the intestinal microbiota on responses to immune checkpoint inhibitors such as anti-PDCD1 (PD-1), anti-CD274 (PD-L1), and anti-CTLA4 monoclonal antibodies.<sup>148–152,163,171–173</sup> The gut microbiota may influence immunotherapeutic efficacy through its complex interactions with the host, which modulate antitumour immunity.<sup>125,172,173</sup> Administration of specific microbes to tumour-bearing mice enhanced the efficacy of anti-CTLA4 treatment by triggering Th1-dependent immune reactions in tumour-draining lymph nodes and shifting dendritic cells towards a pro-inflammatory state.<sup>148</sup> Similar enhancement was observed in the blockade of the CD274-PDCD1 axis with increased CD8<sup>+</sup> cytotoxic T cells and decreased FOXP3<sup>+</sup> regulatory T cells (Tregs) in the tumour microenvironment.<sup>149,151</sup> Patients with favourable microbiota who responded to immune checkpoint inhibition had higher levels of effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells and lower levels of Tregs and myeloid-derived suppressor cells in blood, compared to non-responding patients with unfavourable microbiota.<sup>152</sup> In mouse models of various tumour types, FMT or oral administration of favourable bacteria enhanced the efficacy of immune checkpoint blockades.<sup>148–151</sup> Furthermore, increased diversity of the gut microbiota may augment the effectiveness of this treatment strategy.<sup>151,152</sup> Benefits from antibiotics in patients receiving immunotherapy depend on tumour types. Antibiotic treatment reduced the efficacy of immune checkpoint inhibition in patients with lung, kidney, or bladder cancer,<sup>151,174</sup> while patients with pancreatic cancer benefitted from antibiotic administration.<sup>48</sup> Microbes can travel to the distant tumour microenvironment and, on-site, enhance immunotherapeutic efficacy. Tumour microenvironment is often hypoxic, and therefore may enable anaerobic microbes to preferentially proliferate.<sup>175</sup> In a preclinical model, systemic or oral administration of *Bifidobacterium*, an anaerobic commensal gut bacterium, led to its accumulation in the tumour microenvironment and enhanced the local effect of anti-CD47 immunotherapy *via* STING1 signalling.<sup>175</sup> Engineered microbial

therapies may enable metabolic modulation in the tumour microenvironment, leading to enhanced immunotherapeutic efficacy. In mice, colonisation of tumours with probiotic *E. coli* Nissle 1917 strain increased intratumoural arginine concentrations and tumour-infiltrating T cells, enhancing the efficacy of PDCD1 (PD-1) blockade.<sup>176</sup> Diets and over-the-counter probiotic supplements may have differential effects on immunotherapeutic efficacy.<sup>177</sup> In melanoma patients, high fibre consumption was associated with a better response to anti-PDCD1 (PD-1)-based therapy, with the most pronounced benefit observed in patients with sufficient dietary fibre and no probiotic use.<sup>177</sup>

The gastrointestinal microbiota influences adverse events of immune checkpoint inhibition.<sup>128,129,178,179</sup> Certain microbes elicit immunotherapy-related toxicity, whereas others counteract it.<sup>128,129</sup> In melanoma patients, the abundance of *Bacteroidetes* species was associated with decreased risk of CTLA4 blockade-induced colitis.<sup>180</sup> In patients with urological cancer, FMT ameliorated immunotherapy-induced refractory colitis with decreased CD8<sup>+</sup> T cells and increased anti-inflammatory FOXP3<sup>+</sup> Tregs in colonic mucosa.<sup>181</sup>

Preclinical *in vivo* studies have demonstrated that FMT can enhance immunotherapeutic efficacy.<sup>148–152</sup> Two first-in-human trials reported the safety and feasibility of FMT combined with anti-PDCD1 (PD-1) therapy for therapy-refractory metastatic melanoma.<sup>153,154</sup> Both studies suggested that FMT might help overcome immunotherapy resistance by increasing antitumour immune responses locally and systemically.<sup>153,154</sup>

Despite accumulating evidence indicating immune- and microbiome-modulating effects of various exposures, it remains unknown whether (and if so, how) the association between the microbiota and immunotherapeutic efficacy (or toxicity) is modified by exposures. Integrative MPE research strategies are needed to address this research gap. Previous studies have suggested differential effects of aspirin use, vitamin D level, physical activity, cigarette smoking, and coffee intake on clinical outcomes of CRC patients by levels of lymphocytic reaction or tumour CD274 (PD-L1) expression.<sup>85,182–185</sup> Investigations of the effects of modifiable exposures on clinical outcomes and underlying mechanisms (presumably through microbiota and immunity) can have substantial implications in the development of precision medicine.

### Microbial interventions for cancer prevention

Growing evidence for the role of microbiota in oncogenesis supports microbial manipulation as a promising strategy for cancer prevention. For instance, pharmacological eradication of *H. pylori* can decrease gastric cancer risk and is recommended as a preventative treatment for gastric diseases.<sup>186,187</sup> While the gastric microbiota resembles the oral microbiota in *H. pylori*-uninfected persons, *H. pylori*, when present, dominates the gastric microbial population and reduces the microbial diversity.<sup>188</sup> In a population-based study, successful elimination of *H. pylori* restored gastric microbiota to a similar status as found in uninfected individuals.<sup>189</sup>

Chronic infection with HCV causes chronic hepatitis, cirrhosis, and HCC. Recent advances in combination therapy with direct-acting antiviral drugs have provided a dramatic increase



in the rate of sustained virologic response.<sup>190</sup> Despite adverse effects associated with the antiviral drugs, this treatment strategy has no substantial effects on bacterial communities in the body. Therefore, these classes of agents would be a good candidate for selective antimicrobial strategies for cancer prevention and treatment.

*F. nucleatum* is a potential target to prevent colorectal carcinogenesis. Prudent and anti-inflammatory diets have been associated with a lower incidence of *F. nucleatum*-enriched CRC,<sup>100,101</sup> suggesting the usefulness of diet-modifying preventative strategies. In a preclinical model, aspirin effectively killed *F. nucleatum* strain Fn7-1 and inhibited *F. nucleatum*-potentiated colonic tumorigenesis.<sup>191</sup> In humans, daily aspirin intake was associated with a lower abundance of *F. nucleatum* in colonic adenoma tissues.<sup>191</sup>

Studies also pointed to a link between sulfur microbial diets (associated with abundant sulfur-reducing bacteria in stool) and the development of CRC<sup>192</sup> and early-onset colorectal adenomas.<sup>193</sup> Decreasing animal fat consumption appeared to suppress detrimental *Bacteroidetes* species,<sup>194</sup> while high-fibre diets increased beneficial SCFA-producing bacteria.<sup>195</sup> Therefore, diet-induced microbial alteration may influence the carcinogenesis processes and serve as effective cancer prevention strategies.

Two randomised controlled trials assessed the effect of probiotics and prebiotics on preventing colorectal tumours.<sup>196,197</sup> One trial of 80 participants revealed that administration of synbiotics (*i.e.*, combined prebiotics and probiotics) changed faecal microbiota and blood IL2 and IFNG levels.<sup>196</sup> Another trial of 380 participants observed adenoma risk reduction by probiotics use.<sup>197</sup> However, large-scale trials investigating microbial interventions for cancer prevention have not been conducted. Therefore, costs and potential adverse events should be considered when designing trials using healthy populations (Table 3).

## Challenges and Future Directions

Technical difficulties exist in microbiomic profiling of clinical specimens, including tissue, stool, blood, saliva, urine, *etc.*<sup>198,199</sup> As pre-analytical and analytical factors change microbial compositions, it is essential to standardise methods of specimen collection, processing, storage, and analyses. Although formalin-fixed paraffin-embedded (FFPE) tissue specimens are often used in human population studies, microbial profiles differ between FFPE and fresh tissue specimens. Intratumoural heterogeneity of microbial populations may pose another challenge.<sup>200</sup> Multiple biopsies from each tumour should be conducted when feasible.<sup>200</sup> *In situ* approaches (*e.g.*, immunofluorescence) and spatial transcriptomic profiling enable spatial analyses of specific microbes in relation to tumour, immune, and other cell types.<sup>16,201,202</sup>

A gap remains between microbiomic analyses and epidemiological research. To address this gap, prospective cohort studies that examine the microbiome are needed. Most investigations that assessed the microbiome and cancer risk have used case-control or cross-sectional study designs, which have inherent limitations (Table 4). In typical case-control or cross-sectional studies, exposure information and biospecimens are collected at or after disease diagnosis.

However, disease processes often influence individuals' physiological states and exposures, likely altering biospecimen analytes (microbiome, metabolome, *etc.*). Hence, a difference in any analyte between cases and controls may be a consequence rather than a cause of the disease. Because of this phenomenon (*i.e.*, “reverse causation”), results from case-control studies may not be helpful for aetiological inference or future risk assessments. Therefore, such results need to be tested in prospective cohort studies or randomised controlled trials that collected biospecimens long before disease detection.

Given the multi-factorial processes of tumour development and progression, it is imperative to comprehensively integrate and analyse diverse types of data on exposures, the microbiome, and immune status in research on human cancer. Although conducting such comprehensive analyses is challenging, there is no alternative way. Experimental research using model systems under controlled environment is important and can shed light on pathogenic mechanisms. However, ultimately, we need to validate experimental findings from model systems in real human tumours. Otherwise, we cannot fully understand cancer as microenvironmental, systemic, and environmental diseases. Despite the enormous amounts of resources that have been invested in cancer research, our understanding of cancer currently remains limited because most research efforts have been focused on short-term goals with rather limited data collection and analyses, which cannot adequately decipher cancer (as depicted in Figure 4). It is time to shift our minds to judiciously invest our finite resources for well-designed studies with comprehensive data collection and integrated analyses.

Mounting evidence indicates a significant role of gene-by-environment interaction in various diseases including cancer.<sup>203</sup> Hence, integrative multi-level analyses of genetic, environmental, systemic, and tumoural factors combined with microbiomic analyses of various biospecimens will drastically increase the values of microbiome studies.

A limited number of transdisciplinary education / training programmes has led to a paucity of investigators with transdisciplinary expertise. Conducting integrative microbiomic research necessitates expertise in various scientific disciplines including microbiology, immunology, pathology, epidemiology, bioinformatics, genetics, statistics, *etc.* Transdisciplinary research approaches will contribute to the generation of new research ideas and may lead to new fields of investigation.<sup>204,205</sup>

In recent decades, the incidence of various early-onset cancer types (tumours arising in bone marrow, breast, colorectum, endometrium, extrahepatic bile duct, gallbladder, head and neck, kidney, liver, oesophagus, pancreas, prostate, stomach, and thyroid) among individuals under age 50 has been increasing worldwide.<sup>206,207</sup> Among these, the rise of prostate and thyroid cancers appears to be largely attributable to increased screening and early detection. Notably, eight of the remaining 12 early-onset cancer types arise in the aerodigestive system, implying the critical pathogenic role of the microbiome in this phenomenon. Integrative microbiomic research will likely shed light on the aetiologies of rising early-onset cancer.

## Conclusions

Cancer is a complex condition that should be recognised as an environmental, systemic, and microenvironmental disease. A neoplasm evolves under the influence of various exposures that affect the local and systemic status of immune and microbial activities in the host. Therefore, effective research approaches should account for the interplay between the exposome, microorganisms, immune and other host cell populations, and neoplastic cells. Taking advantage of mechanistic evidence from basic experimental research, integrative microbiomic research can serve as a unique methodological framework and potentially provides novel insights into the host-tumour-microbiome interactions, thereby guiding microbe-targeted strategies for cancer control. Given the increasing availability of multi-omics analysis platforms to interrogate tumour, microbial, and immune signatures, the integrative approach would improve our understanding of the complex cancer pathogenesis. Despite the substantial challenges, there are ample opportunities for integrative microbiomic research to advance cancer science and ultimately reduce the cancer burden through effective precision prevention and treatment.

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## Abbreviations:

<b>aka</b>	also known as
<b>CRC</b>	colorectal cancer
<b>EBV</b>	Epstein-Barr virus
<b>ETBF</b>	enterotoxigenic <i>Bacteroides fragilis</i>

<b>FFPE</b>	formalin-fixed paraffin-embedded
<b>FMT</b>	faecal microbiota transplantation
<b>GEMM</b>	genetically engineered mouse model
<b>HCC</b>	hepatocellular carcinoma
<b>HBV</b>	hepatitis B virus
<b>HCV</b>	hepatitis C virus
<b>HGNC</b>	Human Genome Organisation Gene Nomenclature Committee
<b>MPE</b>	molecular pathological epidemiology
<b>NGS</b>	next-generation sequencing
<b>NSCLC</b>	non-small cell lung carcinoma
<b>PD-1</b>	programmed cell death 1
<b>PD-L1</b>	programmed cell death 1 ligand 1
<b>PI3K</b>	phosphatidylinositol-4,5-bisphosphonate 3-kinase
<b>pks</b>	polyketide synthase
<b>SCFA</b>	short-chain fatty acid
<b>Treg</b>	regulatory T cell

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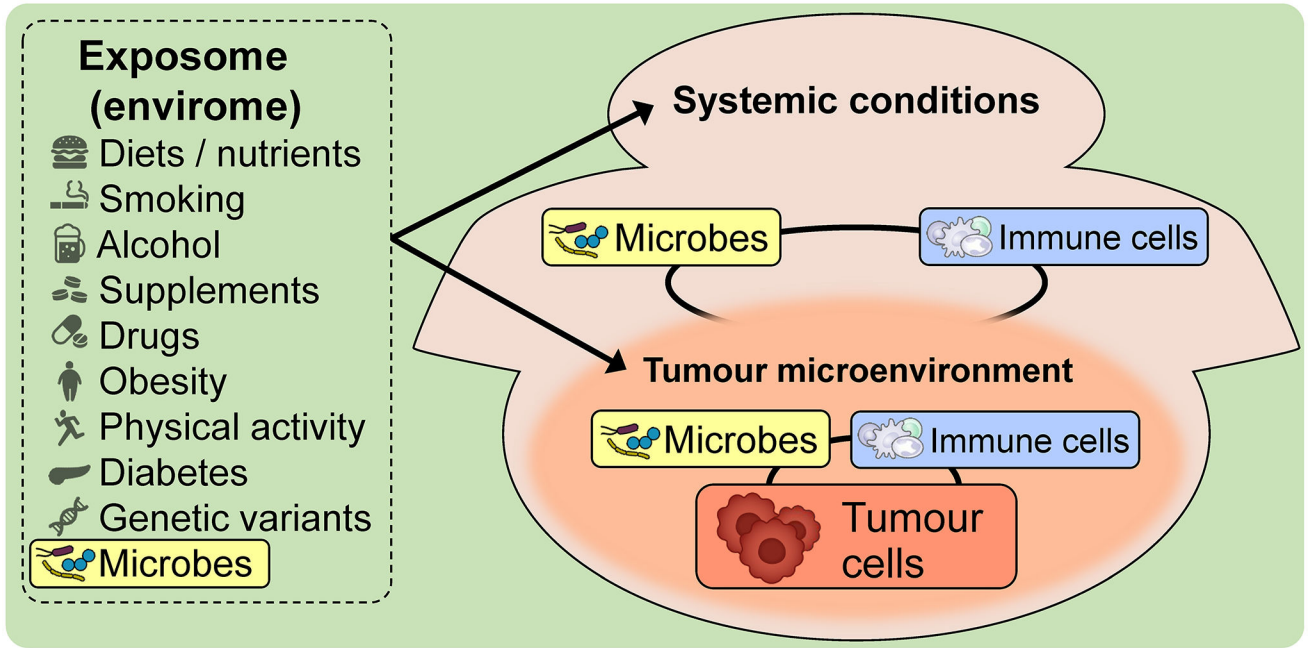
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### Key messages

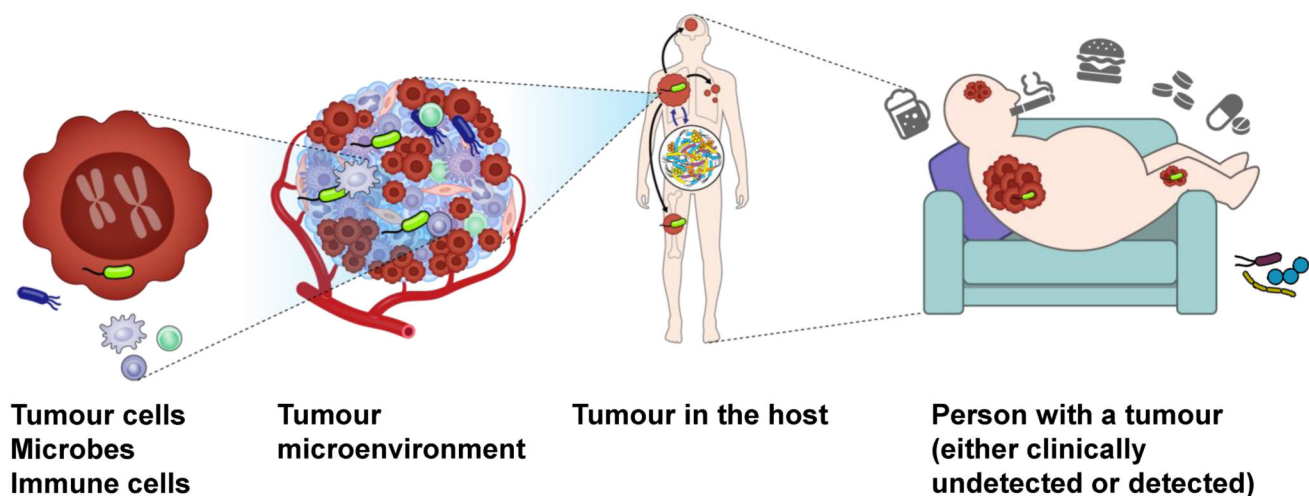
- Cancer is a complex condition that should be recognised as an environmental, systemic, and microenvironmental disease.
- The exposome (the totality of exposures including diets, supplements, smoking, alcohol, medications, obesity, physical activity, *etc.*) influences tumour phenotypes *via* its complex effects on tumour cells, tumour microenvironment, microorganisms, and systemic conditions.
- Microorganisms, which ubiquitously exist in the tumour microenvironment and around the whole human body, play a pivotal role in shaping tumour phenotypes *via* complex host-tumour-microbiome interactions.
- Transdisciplinary research integrating analyses of the exposome, microbiome, and tumour microenvironment based on experimental models and human populations is needed to examine the dynamic interplay of these factors and develop targeted cancer prevention and therapeutics.

### Cancer as a Microenvironmental, Systemic, and Environmental Disease



**Figure 1.** Cancer as a microenvironmental, systemic, and environmental disease. Tumour generates its intrinsic microenvironment, where tumour cells interact with immune cells, microbes, and other cells as well as non-cellular components. Notably, there are no clear boundaries of the tumour microenvironment, which rather blends into tissue outside of the tumour. Systemic conditions, especially systemic immunity, affect the local tumour microenvironment and contribute to tumourigenic processes. The exposome, which encompasses diets, alcohol, medications, lifestyle factors, *etc.*, influences tumour phenotypes by modulating systemic conditions and the tumour microenvironment. Microbes, which may exist in the tumour microenvironment, in distant organs, or around the human body, potentially influence tumour phenotypes directly or indirectly by modulating the host’s local and systemic antitumour immunity.

## Cancer as a Microenvironmental, Systemic, and Environmental (Lifestyle) Disease

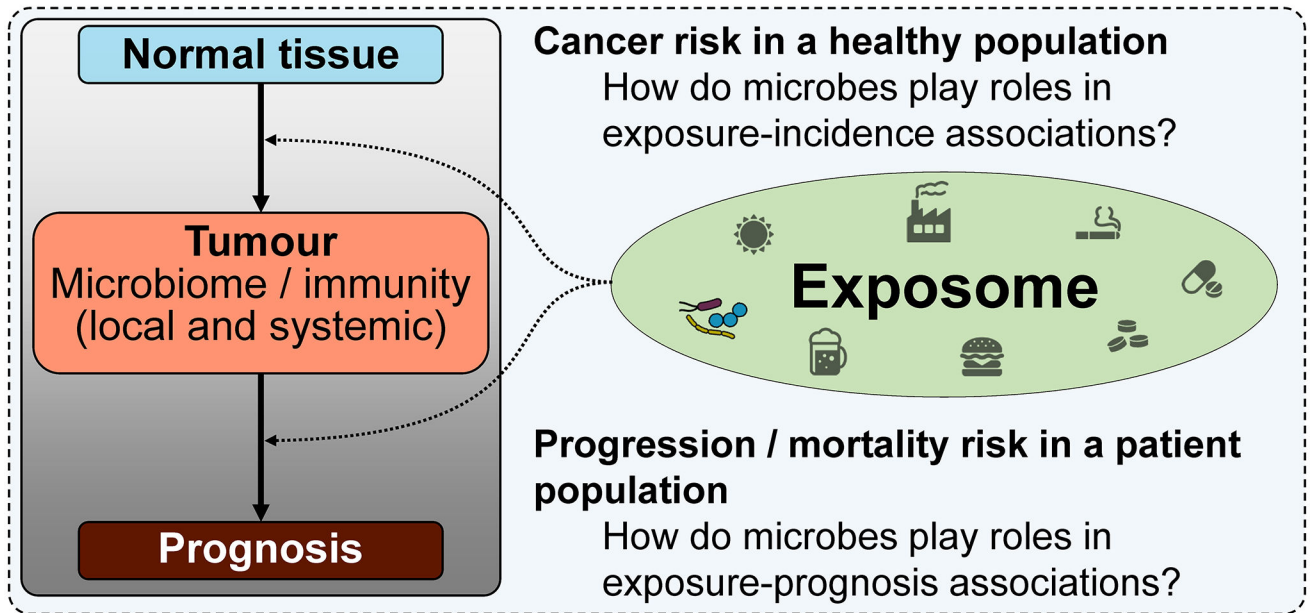


**Figure 2.**

Multi-level perspectives of cancer-microbe associations. Tumour cells, microbes, and immune cells are major constituents of the tumour microenvironment. Cancer may metastasise to other organs. Microbiota, especially the gut microbiota, influences cancer phenotypes *via* systemic host-tumour-microbiome interactions. The exposome (*e.g.*, diets, smoking, alcohol, supplements, medications, obesity, physical inactivity) influences tumour phenotypes and clinical outcomes of cancer patients *via* its complex effects on tumour cells, tumour microenvironment, and systemic conditions.



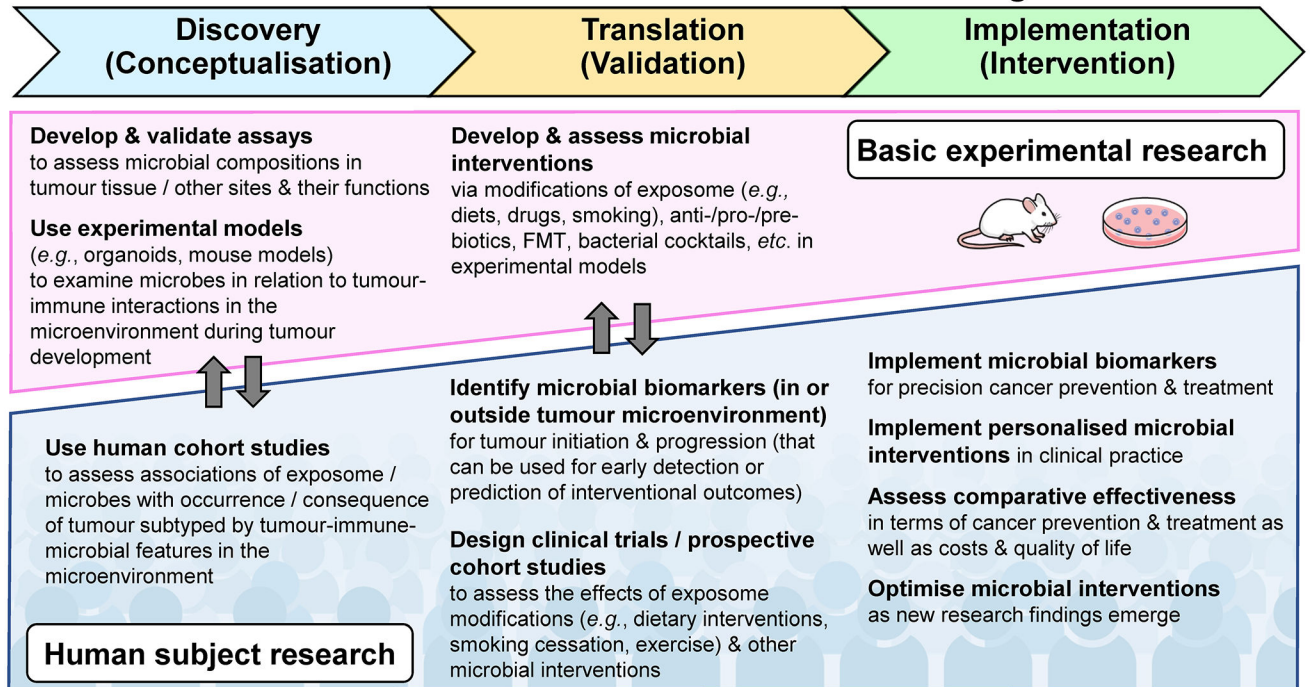
# Molecular Pathological Epidemiology Framework



**Figure 3.**

Analytical framework of molecular pathological epidemiology (MPE) in cancer-microbiome research. MPE research examines associations of an exposure of interest with the development and consequence of tumour subtypes with specific microbial / immune features, potentially providing evidence for complex interactions between exposome and tumour during cancer development and progression. Notably, the “tumour” in this figure may be a benign (pre-malignant) or malignant tumour, which can be analysed for its microbial, immune, and other characteristics.

## Translational Research on Microbiome & Cancer: Strategies & Goals



**Figure 4.**

Roadmap of transdisciplinary cancer-microbiome research from a discovery phase to a translation phase and a final implementation phase for targeted cancer prevention and treatment. The exposome represents the totality of exposures (including but not limited to diets, drugs, and smoking), which can be examined individually or collectively in relation to cancer development and progression. In contrast to research on non-neoplastic diseases, cancer research is characterised by the availability of tumour tissue specimens for examinations of the microenvironment where tumour cells, immune cells, and microorganisms form a dynamic interactive network. Tumour tissue research plays a key role in discovering and validating new insights into the mechanism through which microorganisms may influence cancer initiation and progression by interacting with the exposome and immune cells. FMT, faecal microbiota transplantation.

Specific microorganisms implicated in gastroenterological tumourigenesis by organ site

Table 1.

Specific microbes in a spectrum of organs	Findings
<b>Colorectal cancer</b>	
<i>Atopobium parvulum</i> <i>Actinomyces odontolyticus</i>	<i>Atopobium parvulum</i> and <i>Actinomyces odontolyticus</i> were characteristically enriched for multiple polypoid colorectal adenomas and intramucosal colorectal carcinomas. <sup>90</sup>
<i>Bacteroides fragilis</i>	Enterotoxigenic <i>Bacteroides fragilis</i> (ETBF) is enriched in colorectal serrated lesions and adenoma as well as colorectal cancer. <sup>208</sup> ETBF and <i>pkS<sup>+</sup>E. coli</i> appear to synergistically induce colorectal tumourigenesis. <sup>103</sup>
<i>Escherichia coli</i>	Polyketide synthetase ( <i>pkS</i> )-producing <i>E. coli</i> appear to inhibit colorectal tissues in approximately 20% of healthy individuals, 40% of patients with inflammatory bowel disease, and 60% of patients with familial adenomatous polyposis or colorectal cancer. <sup>103–105</sup> The <i>pkS<sup>+</sup>E. coli</i> appears to directly induce DNA damage and mutagenesis. <sup>102</sup>
<i>Fusobacterium nucleatum</i>	<i>F. nucleatum</i> is implicated in the initiation and progression of colorectal cancer. <sup>21–26</sup> Intratumoural <i>F. nucleatum</i> appears to promote colorectal carcinogenesis by activating anti-inflammatory myeloid cells, <sup>23</sup> suppressing NK and T cells, <sup>24,25</sup> and activating the CTNNB1 (beta-catenin)-WNT signalling pathway. <sup>26</sup> The presence of <i>F. nucleatum</i> in colorectal cancer is associated with decreased CD3 <sup>+</sup> CD4 <sup>+</sup> CD45RO (PTPRC) <sup>+</sup> cells <sup>30</sup> and increased tumour-infiltrating macrophages <sup>31</sup> in the tumour microenvironment. Colorectal cancer containing <i>F. nucleatum</i> is characterised by proximal (caecal) tumour localisation, <i>BRAF</i> mutations, microsatellite instability-high status, CpG island methylator phenotype-high status, and poor prognosis. <sup>27–29</sup>
Viral microbiome (virome)	Faecal specimens of patients with colorectal cancer were characterised by increased viral diversity and enrichment of bacteriophages (e.g., <i>Siphoviridae</i> , <i>Myoviridae</i> , <i>Inovirus</i> , <i>Tumalikevirus</i> ) that were expected to be bacterium-virus community network hubs. <sup>37,38</sup>
Fungal microbiome (mycobiome)	Faecal specimens of patients with colorectal cancer were characterised by increased Basidiomycota:Ascomycota ratio, high amount of Malasseziomycetes, low amount of Saccharomycetes and Pneumocystidomycetes, increased co-occurring fungal intrakingdom correlations, and disappearance of some co-occurring bacterial–fungal correlations (e.g., fungal Ascomycota and bacterial Proteobacteria). <sup>39</sup>
Archaeal microbiome	Faecal specimens of patients with colorectal cancer had enrichment of halophilic and depletion of methanogenic archaea. <sup>40</sup> The halophilic <i>Natrinema</i> species <i>J7-2</i> increased progressively in faecal specimens of healthy individuals, to patients with colorectal adenomas, to patients with colorectal cancer. <sup>40</sup>
Multi-kingdom microbiome	Faecal 16 multi-kingdom microbiome markers, including 11 bacterial (e.g., <i>F. nucleatum</i> , <i>Parvimonas micra</i> , <i>Gemella morbillorum</i> ), 4 fungal (e.g., <i>Talaromyces islandicus</i> , <i>Aspergillus rambellii</i> ) and 1 archaeal ( <i>Pyrobaculum arsenaticum</i> ) feature, achieved good performance in diagnosing patients with colorectal cancer. <sup>34</sup> Bacterial–fungal interactions may contribute to CRC pathogenesis via upregulation of D-arginine and D-ornithine and stimulation of the butanoate metabolism pathways.
<b>Gastric cancer</b>	
<i>Helicobacter pylori</i>	Chronic infection with <i>H. pylori</i> is a leading cause of gastric cancer. <sup>41–44,209</sup> <i>H. pylori</i> induces multistep carcinogenesis, namely progression from chronic gastritis, tissue atrophy, intestinal metaplasia, and benign tumour to carcinoma. <sup>42</sup> The eradication of <i>H. pylori</i> is an established strategy to prevent gastric cancer. <sup>187</sup>
Epstein-Barr virus (EBV)	EBV-associated gastric cancer is characterised by male predominance, early-onset, proximal tumour localisation, less tumour differentiation, poorly differentiated morphology with marked lymphocytic infiltration, <i>PIK3CA</i> and <i>ARID1A</i> mutations, lack of <i>TP53</i> mutation, 9p24.1 amplification, hypermethylation of <i>CDKN2A</i> promoter, <i>CD274</i> overexpression, <sup>46</sup> EBV-encoded microRNAs BART11 and BART17-3p inhibit FOXP1 and PBRM1, respectively, and enhance the transcription of <i>CD274</i> , resulting in the promotion of tumour immune evasion. <sup>47</sup>
<b>Liver cancer</b>	
Bacterial microbiome	The composition of intratumoural microbiota in hepatocellular carcinoma differed according to aetiological factors. <sup>56,57</sup> <i>Ruminococcus gnavus</i> was characteristically enriched for virus-related hepatocellular carcinoma. <sup>57</sup> Four proteogenomic subgroups of intrahepatic cholangiocarcinoma had distinct intra-tumoural microbiota diversity, composition, and functions. <sup>58</sup>
Faecal microbiome	Butyrate-producing bacterial genera (e.g., <i>Ruminococcus</i> ) were decreased, while lipopolysaccharide-producing genera (e.g., <i>Kebsiella</i> ) were increased in faecal samples from patients with early-stage hepatocellular carcinoma than those from controls. <sup>210</sup>

Specific microbes in a spectrum of organs	Findings
	Gram-negative commensal gut bacteria induced hepatocytes to generate an immunosuppressive environment by recruiting CXCR2 <sup>+</sup> polymorphonuclear myeloid-derived suppressor cells through TLR4-dependent CXCL1 production, ultimately promoting the development of intrahepatic cholangiocarcinoma. <sup>54</sup> <i>Enterococcus faecalis</i> was abundant in faecal samples from patients with hepatitis C virus-related chronic liver disease. <sup>55</sup> <i>GelE</i> -positive <i>E. faecalis</i> appears to promote liver carcinogenesis by expressing the metalloproteinase gelE, which increased gut permeability, leading to elevated plasma lipopolysaccharide and activation of TLR4-MYD88 proliferative signalling in hepatocytes. <sup>55</sup>
<b>Oesophageal cancer</b>	
<i>Fusobacterium nucleatum</i>	The presence of <i>F. nucleatum</i> in oesophageal cancer is associated with the low efficacy of chemotherapy. <sup>166</sup> Intratumoural <i>F. nucleatum</i> may contribute to chemoresistance by promoting autophagy. <sup>166</sup>
<b>Pancreatic cancer</b>	
Bacterial microbiome	Pancreatic cancer tissue contains more bacteria than normal pancreatic tissue. <sup>48</sup> <i>Proteobacteria</i> , <i>Bacteroidetes</i> , and <i>Firmicutes</i> were highly colonised and prevalent bacterial phyla in pancreatic cancer. <sup>48</sup> A signature of three intratumoural bacterial genera ( <i>Pseudoxanthomonas</i> , <i>Streptomyces</i> , <i>Saccharopolyspora</i> ) and high alpha-diversity of intratumoural bacteria were associated with better patient outcomes. <i>Proteobacteria</i> , <i>Bacteroidetes</i> , and <i>Firmicutes</i> were highly colonised and prevalent bacterial phyla in pancreatic cancer. <sup>48</sup>
Fungal microbiome (mycobiome)	Pancreatic cancer tissue contains more fungi than normal pancreatic tissue. <sup>49</sup> <i>Malassezia</i> promoted tumour progression by attaching to mannose-binding lectin, thereby activating the complement cascade. <sup>49</sup> In response to the intratumoural mycobiome, pancreatic cancer cells produced IL33 as a chemoattractant for type 2 immune cells, leading to tumour progression. <sup>51</sup>
Faecal microbiome	<i>Veillonella atypica</i> , <i>Fusobacterium nucleatum/hwasookii</i> , and <i>Allocardovia omnicoiens</i> were enriched in faeces of patients with pancreatic adenocarcinoma, whereas <i>Romboutsia timonensis</i> , <i>Faecalibacterium prausnitzii</i> , <i>Bacteroides coprocola</i> , and <i>Bifidobacterium bifidum</i> species clusters were depleted. <sup>52</sup> Enrichments of <i>Streptococcus</i> species and <i>Veillonella</i> species ( <i>V. parvula</i> and <i>V. atypica</i> ) and a depletion of <i>Faecalibacterium prausnitzii</i> were common signatures for faecal specimens of patients with pancreatic adenocarcinoma. <sup>53</sup>

Abbreviations: EBV, Epstein-Barr virus; ETBF, enterotoxigenic *Bacteroides fragilis*; pks, polyketide synthase.

Table 2.

Studies investigating microbial manipulation in relation to the effectiveness of cancer therapeutics with microbial data

	Microbial intervention	Preclinical / clinical	Findings
<b>Chemotherapy</b>			
Colon cancer <sup>2,11</sup>	Antibiotics	Preclinical	In mice, antibiotics dysregulated the gut microbiome and reduced anti-tumour effect of 5-fluorouracil.
Colon cancer, lymphoma, and melanoma <sup>164</sup>	Antibiotics	Preclinical	Antibiotics-treated or germ-free mice responded poorly to CpG-oligonucleotide immunotherapy or oxaliplatin with reduced production of reactive oxygen species and immune-related cytokines.
Hepatocellular carcinoma <sup>212</sup>	Antibiotics	Clinical	Shorter progression-free / overall survival times were observed among patients receiving carbapenem before or during systemic interferon injection and hepatic arterial infusion of 5-fluorouracil with or without cisplatin. Antimicrobial drugs were also associated with poor prognosis. The abundance of <i>Bifidobacterium</i> in faecal microbiota was associated with favourable survival outcomes.
Lung cancer <sup>167</sup>	Antibiotics	Preclinical	In antibiotics-treated or germ-free mice, tumours were resistant to cyclophosphamide with reduced reactivity of T helper 17 cells. Transfer of T helper 17 cells partially restored the antitumour effect of cyclophosphamide.
<b>Immunotherapy</b>			
Colon cancer <sup>176</sup>	Engineered probiotics	Preclinical	In mice, an engineered <i>Escherichia coli</i> strain colonised in the tumour and increased local L-arginine levels, resulting in increased tumour-infiltrating T cells and synergistic effects with anti-CD274 (PD-L1) therapy.
Colon cancer <sup>175</sup>	Probiotics	Preclinical	In mice, <i>Bifidobacterium</i> accumulated within the tumour, when administered systemically or orally, and restored tumour response to anti-CD47 therapy through upregulation of the STING1 signalling.
Colon cancer <sup>213</sup>	Antibiotics	Preclinical	In mice, antibiotics enhanced antitumour immune responses and antitumour effects of neoantigen cancer vaccines delivered by DNA electroporation.
Colon cancer and melanoma <sup>214</sup>	Antibiotics and probiotics	Preclinical	In mice, antibiotics attenuated antitumour effect of the HAVCR2 (Tim-3) blockade. The therapeutic efficacy was restored by oral gavage of faecal bacteria.
Colon cancer, melanoma, NSCLC, and sarcoma <sup>148</sup>	Antibiotics and FMT	Preclinical and clinical	In mice and patients, T cell response to <i>Bacteroides thetaioamicron</i> or <i>Bacteroides fragilis</i> was correlated with the effectiveness of ipilimumab (anti-CTLA4 antibody). Antibiotics-treated mice did not respond to the CTLA4 blockade. In mice, FMT from patients with gut microbiota related to a good response to ipilimumab promoted the outgrowth of <i>B. fragilis</i> and enhanced the antitumour effect.
Melanoma <sup>177</sup>	Diets and oral probiotics	Clinical	In patients, high fibre consumption was associated with better response to anti-PDCD1 (PD-L1)-based therapy, with the most pronounced benefit observed in patients with sufficient dietary fibre and no probiotic use.
Melanoma <sup>150</sup>	FMT	Preclinical and clinical	<i>Bifidobacterium longum</i> , <i>Collinsella aerofaciens</i> , and <i>Enterococcus faecium</i> were more frequently identified in stool samples of patients responding to anti-PDCD1 (PD-L1) or anti-CTLA4 therapy. In mice, FMT from the responders restored T cell-mediated immune response and antitumour effect of anti-CD274 (PD-L1) therapy.
Melanoma <sup>153,154</sup>	FMT	Clinical	In patients with melanoma refractory to anti-PDCD1 (PD-L1) therapy, FMT from responders restored tumour sensitivity to the PDCD1 blockade. The FMT resulted in favourable immune and microbial profiles in the gut and tumour microenvironment.
Melanoma and bladder cancer <sup>149</sup>	Oral probiotics	Preclinical	In mice, oral <i>Bifidobacterium</i> administration improved tumour control and, when combined with the CD274 (PD-L1) blockade, almost eliminated the tumour. The key mediators were enhanced dendritic cell function and recruitment of CD8 <sup>+</sup> T cells to the tumour microenvironment.
NSCLC, renal cell carcinoma, and urothelial carcinoma <sup>151</sup>	Antibiotics and FMT	Preclinical and clinical	In patients with advanced cancer, antibiotics use was associated with poor response to anti-PDCD1 (PD-L1) or anti-CD274 (PD-L1) therapy. In mice transplanted with sarcoma cells, FMT from responding patients improved the effectiveness of the immune checkpoint blockade.

	Microbial intervention	Preclinical / clinical	Findings
Pancreatic cancer <sup>48</sup>	Antibiotics	Preclinical	In mice, antibiotics upregulated PDCD1 (PD-1) expression in CD4 <sup>+</sup> cells and CD8 <sup>+</sup> cells in the tumour and synergised with anti-PDCD1 therapy in tumour suppression.
<b>Hormone therapy</b>			
Prostate cancer <sup>2,15</sup>	Antibiotics	Preclinical	In mice, antibiotics delayed castration resistance of the tumour. FMT from patients with hormone-sensitive prostate cancer and <i>Prevotella stercora</i> administration promoted tumour suppression.

Abbreviations: FMT, faecal microbiota transplantation; NSCLC, non-small cell lung carcinoma.



Consideration and challenges ahead to modulate the microbiome for cancer prevention in healthy populations

**Table 3.**

Intervention type	Cost	Potential disadvantages and considerations	Considerations across all intervention types
Diets, nutrient supplements	Low	Varied effects by individuals; low compliance	
Prebiotics ( <i>i.e.</i> , food ingredients that support beneficial microbes in the gut)	Relatively low	Varied effects by individuals; unknown pernicious effects	<ul style="list-style-type: none"> <li>• Which population should we target?</li> <li>• Which method should we use?</li> </ul>
Probiotics ( <i>i.e.</i> , microorganisms that provide health benefits)	Relatively low	Variation of quality control, bioavailability, and standardisation; known and unknown pernicious effects	<ul style="list-style-type: none"> <li>• What dose is needed for target modulation?</li> <li>• How do we optimally monitor participants?</li> </ul>
Antibiotics	Relatively high	Depletion of commensal / symbiotic bacteria; introduction of antibiotic-resistant bacteria; short-term and long-term side effects	<ul style="list-style-type: none"> <li>• How long do we continue the intervention?</li> <li>• How do we assess the effect and safety?</li> </ul>
Medication	Relatively high	Short-term and long-term side effects	<ul style="list-style-type: none"> <li>• How durable is the effect?</li> </ul>
Faecal microbial transplantation	High	Potential to affect the risk of other diseases; procedural risks; unknown safety profiles; donor selection is crucial but difficult	<ul style="list-style-type: none"> <li>• Can the intervention affect the risk of diseases other than cancer?</li> </ul>

**Table 4.** Comparison of case-control versus prospective cohort study designs for the study of cancer aetiologies

	Case-control study design	Prospective cohort study design
Source of bias		
1. Selection bias due to differential background populations that have given rise to cancer cases from controls	Yes (D)	No (A)
2. Referral bias	Often large (D)	Small to moderate (A)
3. Recall bias in long-term exposure measurements	Often large (D)	Small if the information is repeatedly obtained during follow-up (A)
4. Differential recall bias between cancer cases and controls	Yes (D)	No (A)
Sample size of cancer cases	Can be large; enables to study rare cancer types (A)	Determined by cohort size (D)
Measurement of the incidence rate across different generations	Difficult (D)	Possible (A)
Collection of detailed clinical information	Relatively easy (A)	Relatively difficult (D)
Collection of biospecimens including tumour tissue	Relatively easy (A)	Relatively difficult (D)
Collection of biospecimens years before cancer diagnosis	Difficult or impossible (D)	Possible (A)
Time needed for follow-up	No (A)	Yes (D)
Overall cost	Relatively low (A)	Very high (D)
Generalisability (given the same statistical power)	Low to moderate (D)	Moderate to high (A)

Abbreviations: A, advantage; D, disadvantage.