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Targeting the gut and tumor microbiota in cancer

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Microorganisms within the gut and other niches may contribute to carcinogenesis, as well as shaping cancer immunosurveillance and response to immunotherapy. Our understanding of the complex relationship between different host-intrinsic microorganisms, as well as the multifaceted mechanisms by which they influence health and disease, has grown tremendously—hastening development of novel therapeutic strategies that target the microbiota to improve treatment outcomes in cancer. Accordingly, the evaluation of a patient's microbial composition and function and its subsequent targeted modulation represent key elements of future multidisciplinary and precision-medicine approaches. In this Review, we outline the current state of research toward harnessing the microbiome to better prevent and treat cancer.

Having originated over 3.5 billion years ago[1], microorganisms represent some of the oldest living organisms on Earth and are responsible for the creation of a more habitable environment through the production of oxygen from photosynthesis and other processes[2,3]. Since those ancient times, microorganisms have continued to shape our planet and our environment, and microorganisms that inhabit individual organisms have a profound influence on physiology—with the ability to impact overall health and states of disease[4–6]. This includes the large proportion of microorganisms that reside in the human gastrointestinal (GI) system that influence numerous physiologic processes including digestion, metabolism, cognitive development and function, and immune system development and function[7,8]. Importantly, microorganisms in the GI tract and other niches can also contribute to the development of disease, including cancer.

One of the most transformative advances in cancer treatment over the past decade involves the use of immunotherapy, with this approach being integrated into the treatment of virtually every cancer type. Specifically, treatment with immune checkpoint blockade (ICB) has markedly improved survival across numerous cancer types[9–13]. However, the full therapeutic potential of ICB remains incompletely realized, as not all patients derive durable benefit, with a substantial proportion demonstrating primary or acquired resistance to treatment[14]. For example, up to 50% of patients with melanoma[15], and 25–44% of patients with non-small-cell lung cancer[16–18], experience primary ICB resistance. This leaves an opportunity for the discovery of novel strategies to improve response and to overcome resistance to this powerful form of treatment.

Studies have identified host-associated genomic and molecular biomarkers associated with response to ICB[19–24], and an emerging body of evidence has now also implicated host-intrinsic microorganisms and their genes (collectively referred to as the microbiome), particularly those microorganisms that reside within the GI tract, in influencing response to ICB[25,26]. Indeed, the composition of the gut microbiome appears to be both predictive and prognostic of therapeutic response to ICB, as distinct gut microbial signatures distinguish healthy individuals from patients with cancer[27] and responders from nonresponders in several ICB-treated cancer cohorts[25,26]. These findings have led to the development and implementation of new microbiome-based treatment strategies aimed at modulating patient gut microorganisms and their function to enhance clinical response to ICB[28–30] and to abrogate toxicity to therapy[31–33]. Currently, several interventional strategies, such as fecal microbiome transplant (FMT), prebiotic, probiotic and antibiotic treatments and dietary interventions, have shown early promise as modulators of the gut microbiome. However, the ultimate therapeutic role of the gut microbiota relates to its functional status and its ability to favorably impact systemic immunity and overall host health. Therefore, characterizing the gut microbiome-associated systemic biological functions and underlying molecular mechanisms will be crucial to discovery of novel, actionable targets for future intervention and clinical evaluation.

Looking beyond the gut microbiome, we are now entering the era of studying and manipulating the intratumoral microbiome to enhance clinical response to cancer treatments[34–36]. The tumor microenvironment (TME) is an attractive niche for microbial growth, and microorganisms have been identified within tumors of patients with cancer for over a century[37], although the breadth of microorganisms and the depth of their influence has been somewhat incompletely appreciated to date, mainly owing to technologic limitations. Now, advances in next-generation sequencing (NGS) techniques are giving rise to a greater appreciation of the local diversity and functional relevance of the intratumoral microbiome in solid malignancies. Its ultimate role, however, remains incompletely characterized, as certain microorganisms seem to promote tumorigenesis, while others aid antitumor immune responses by serving as immune adjuvants. Indeed, unlike their gut-associated counterparts, the comprehensive characterization of response-associated intratumoral microbiome signatures is still in its infancy. Nevertheless, novel investigative and therapeutic opportunities now exist to target intratumoral microorganisms to intercept[38,39], treat[37,40] and perhaps even prevent cancer altogether[41,42].

In this Review, we discuss the relevance of the gut and intratumoral microbiota in cancer development and treatment response, highlighting recent key studies that have moved the field forward. We highlight emerging and future strategies that seek to manipulate these microbiomes to enhance cancer treatment outcomes, and, finally, we discuss potential future strategies to develop microbial

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Table 1 | Influence of gut-based and tissue-based microorganisms across the spectrum of health, disease and cancer

	Health	Inflammation associated disease (non-cancer)	Pre-cancer	Early cancer	Advanced cancer
Systemic immunity	++++	++	++	+	Minimal
Systemic inflammation	Minimal	+	++	++	++++
Tissue immunity	++++	++	++	+	Minimal
Gut microbiome	High diversity with functional redundancy	Intermediate diversity with moderate functional redundancy	Intermediate diversity with moderate functional redundancy	Low diversity with poor functional redundancy	Very low diversity with no functional redundancy
	No/few pathogenic microorganisms	Few pathogenic microorganisms	Few pathogenic microorganisms	Moderate pathogenic microorganisms	High pathogenic microorganisms
Tissue microbiome	Low density	Moderate density	Moderate density	High density	Very high density
	Nonpathogenic	Pathogenic	Pathogenic	Pathogenic	Pathogenic

For systemic immunity, plus symbols indicate the level of immunity observed (with ++++ indicating the highest level); 'Minimal' indicates the lowest level. For systemic inflammation, plus symbols indicate the level of inflammation observed (with ++++ indicating the highest level); 'Minimal' indicates the lowest level. For tissue immunity, plus symbols indicate the level of immunity localized within the tissue (with ++++ indicating the highest level); 'Minimal' indicates the lowest level.

targeting as a pillar of personalized cancer care over the next 5 to 10 years.

The gut microbiome across the spectrum of health, disease and cancer

Beyond the well-established contribution of specific GI bacteria in local carcinogenesis (such as *Heliobacter pylori* in gastric cancer and *Fusobacterium nucleatum* in colorectal cancer (CRC))[34,43,44], it is now quite clear that the microorganisms within the GI tract (gut) can also shape overall immunity and influence states of health and disease (including cancer) at the systemic level[45,46] (Table 1). This is mediated by a dynamic interaction between components of systemic immunity and markers of inflammation, tissue-based and tumor immunity, and the gut and tumor microbiomes. Importantly, microbiota are now identified as an enabling factor in the most recent iteration of the 'Hallmarks of Cancer'[47].

Mechanisms of action on systemic immunity and cancer treatment response. Numerous studies now demonstrate a link between the gut microbiome and ICB response[25,26,28,48–50]. Microorganisms within the gut influence host immunity and response to cancer treatment via several hallmark mechanisms. These include the following: (1) effects on other microorganisms in the gut causing shifts in the ecosystem[51], (2) effects on the intestinal wall including enterocytes (with the induction of autophagy and apoptosis) and the gut-associated lymphoid tissue[52,53], (3) local or systemic stimulation of pattern-recognition receptors perceiving adjuvant signals[54,55], (4) systemic neuroendocrine effects via the secretion of gut hormones[56], (5) systemic metabolic effects through the synthesis of polyamines and B vitamins[57], and (6) the induction of immune responses against microbial antigens that are cross-reactive with tumor-associated antigens[58,59] (Fig. 1)[28,58–83]. While these hallmarks influence both response and resistance to various cancer treatments, it is crucial to note that they are highly context dependent—highlighting the importance of considering patient-specific and tumor-specific factors when evaluating the microbiome's role in individual cancer care.

Preclinical evidence. Preclinical and clinical evidence for the influence of gut microorganisms on systemic immunity is pervasive. For example, gnotobiotic mice (which have been raised in a germ-free environment) bear an immature immune system and lack mature secondary and tertiary lymphoid organs—with an inability to respond to vaccination[84]. Moreover, in mice raised in specific pathogen-free conditions, the reduction of bacterial diversity within the GI tract by a combination of broad-spectrum antibiotics negatively impacts the efficacy of chemotherapeutic agents such as cyclophosphamide[85] or oxaliplatin[86], which both act in part through immunologic mechanisms. The use of broad-spectrum antibiotics also negatively impacts outcomes to treatment with ICB targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4)[50] or programmed cell death protein 1/programmed death ligand 1 (PD-1/PD-L1) in preclinical models[25,26,48]. Notably, FMT from patients with cancer to antibiotic-pretreated mice converts the animals into 'avatars' with the potential ability to predict response or nonresponse of the patient to PD-1 blockade[26,48], although such an approach is not widely used and warrants further investigation and validation.

Clinical evidence. In line with evidence from preclinical models, numerous studies show that patients with cancer treated with broad-spectrum antibiotics shortly before or during ICB treatment have shorter progression-free survival (PFS) and overall survival compared to those not receiving antibiotics[25,26,87–90]. This supports the hypothesis that an unperturbed and diverse microflora is essential for efficient cancer immunosurveillance and, similarly, a disequilibrated microbiota has negative consequences on the cancer–immunity cycle[51]. Indeed, previous work has shown an increased relative abundance of certain microbial taxa in the gut of patients with active cancer versus cancer-free individuals across several cancer types (Fig. 2a)[27], although causal relationships between these gut microorganisms and carcinogenesis are not established, and further investigation is clearly warranted. Additionally, specific microbial taxa have been associated with response to ICB across several cancer types studied—with some unifying bacterial taxa identified across these cohorts (Fig. 2b)[26,29,30,32,91–100].

However, despite some overlap in gut microorganisms associated with response to ICB in published studies across cancer types, this overlap is modest and cannot be explained by differences in sequencing approaches[101]. This highlights opportunities to identify more unifying immune-activating and response-associated characteristics of the gut microbiome from a functional standpoint and/or by more deep and thorough characterization of human cohorts, and via mechanistic studies in preclinical models.

Impact on treatment-related toxicity. In addition to identifying microbial signatures associated with response, specific microbial taxa and dysbiosis in the gut have also been associated with treatment-related toxicity (relating to ICB, stem cell transplant, and other cancer treatments)[32,33,102–107]. These findings have

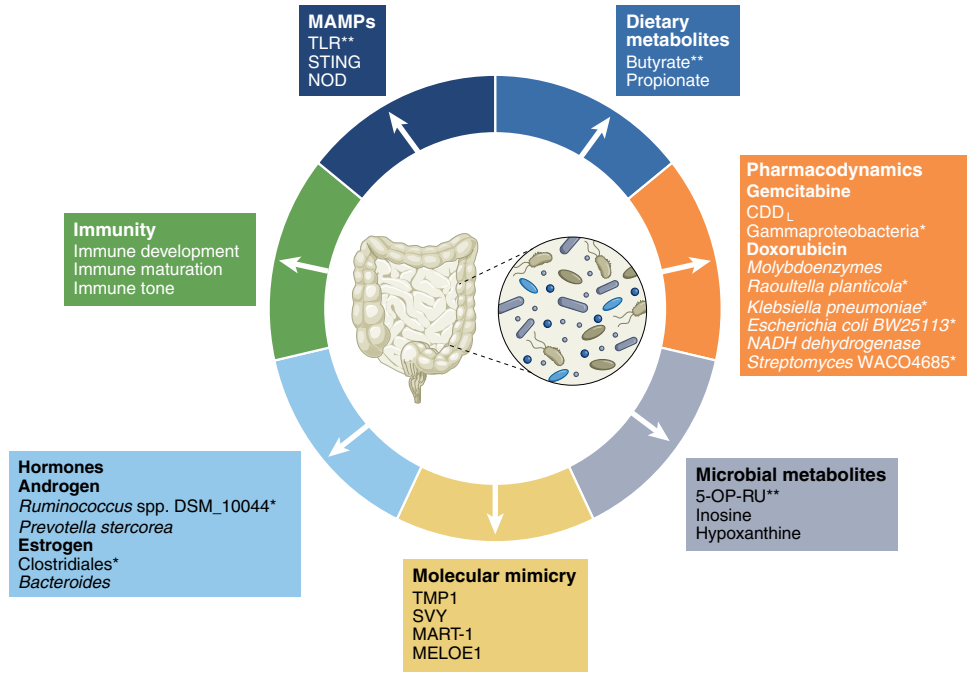

Fig. 1 | Hallmarks of the impact of the gut microbiome on immunity, health and disease. Work over the past decade has demonstrated a multifaceted association between the gut microbiome and health across several domains: immunomodulation, endocrine modulation, molecular mimicry, microbial metabolites, dietary metabolites, alteration of pharmacodynamics, and MAMPs. Each of these plays a role in health and disease, including cancer. Effects or mechanisms associated with a favorable antitumor response are listed without any symbol, while those associated with a pro-tumor state are indicated with an asterisk. Context-dependent effects are indicated with two asterisks. Examples of effector organisms are listed below effects or mechanisms where their activity has been demonstrated.

provided opportunities to restore a healthy microbiota or target specific microorganisms using techniques such as FMT therapy or targeted microbial-modulating therapy, respectively, to reduce therapy-related toxicity. In preclinical models of CRC, for example, FMT has been shown to mitigate adverse events of 5-fluorouracil-based chemotherapy[108]. FMT and indole 3-propionic acid (a microbial metabolite with intracellular signaling activity) have each been demonstrated to protect against radiation-related toxicity in preclinical models[109,110], and *Bifidobacterium* administration has been associated with decreased CTLA-4-associated intestinal toxicity[111].

Beyond bacteria. Although many of the studies to date have focused on bacterial taxa associated with response and toxicity, additional microorganisms (for example, viruses/bacteriophages[112,113] and fungi[114]) must also be considered for study in the future. This would require the incorporation of metagenomic sequencing approaches, as traditional microbiome profiling relies on sequencing 16S ribosomal RNA (rRNA), which is unique to bacteria. Whole-metagenome sequencing may facilitate the identification of several additional microorganisms associated with response[115], and may also be supplemented with additional functional assessments such as RNA sequencing (RNA-seq)[116], small-read sequencing[117], and metabolomic sequencing[118,119]. Such approaches may yield novel and critical mechanistic insight to inform future strategies aimed at targeting the function of these microorganisms, rather than relying solely on phylogenetics. Currently, a lack of standardized approaches for sample preparation, sequencing, data processing and analysis poses a substantial challenge and will need to be addressed in future efforts[120,121]. Despite these challenges, profiling and targeting of gut microorganisms will almost certainly become a part of the very fabric of personalized medicine and health approaches in the next decade.

Tissue-resident and intratumoral microorganisms—friend or foe?

In addition to microorganisms within the gut, microorganisms in other niches may profoundly influence host physiology[122–125]. This includes microorganisms on external surfaces and mucosal sites, and also tissue-resident microorganisms[126]. Microorganisms have also been identified in tumor tissues for over a century, and although some insights have been gained regarding the mechanisms through which they impact carcinogenesis and therapy response, there remains a substantial amount to learn.

Recently, advances in NGS have greatly facilitated the identification of microorganisms in tissues throughout the body and their relationship with health and disease, including cancer[127,128], although complexities exist in the mining of NGS data collected for other purposes[129–131]. For example, poly(A) mRNA capture, which is commonly performed to enrich eukaryotic transcripts before RNA-seq, limits the utility of robustly curated traditional RNA-seq datasets to study the microbiome, as they are devoid of valuable prokaryotic transcripts[132]. Additional complexities exist even when NGS approaches are specifically used to identify microorganisms in tissues and tumors, as the relative biomass (and, therefore, the starting signal) in these peripheral sites is substantially lower than that within the gut[133]. Additionally, contamination from environmental sources during tissue acquisition, processing, and sequencing poses another major challenge in correctly analyzing microbial presence[130], although advanced in silico 'decontamination' algorithms are being developed to address this challenge[134]. Nonetheless, while accurate profiling of microorganisms in carefully curated samples remains critical[37], deep sequencing of microorganisms residing in human tissues represents an area of fruitful investigation with opportunities to target tissue-based and tumor-based microorganisms in cancer and other diseases.

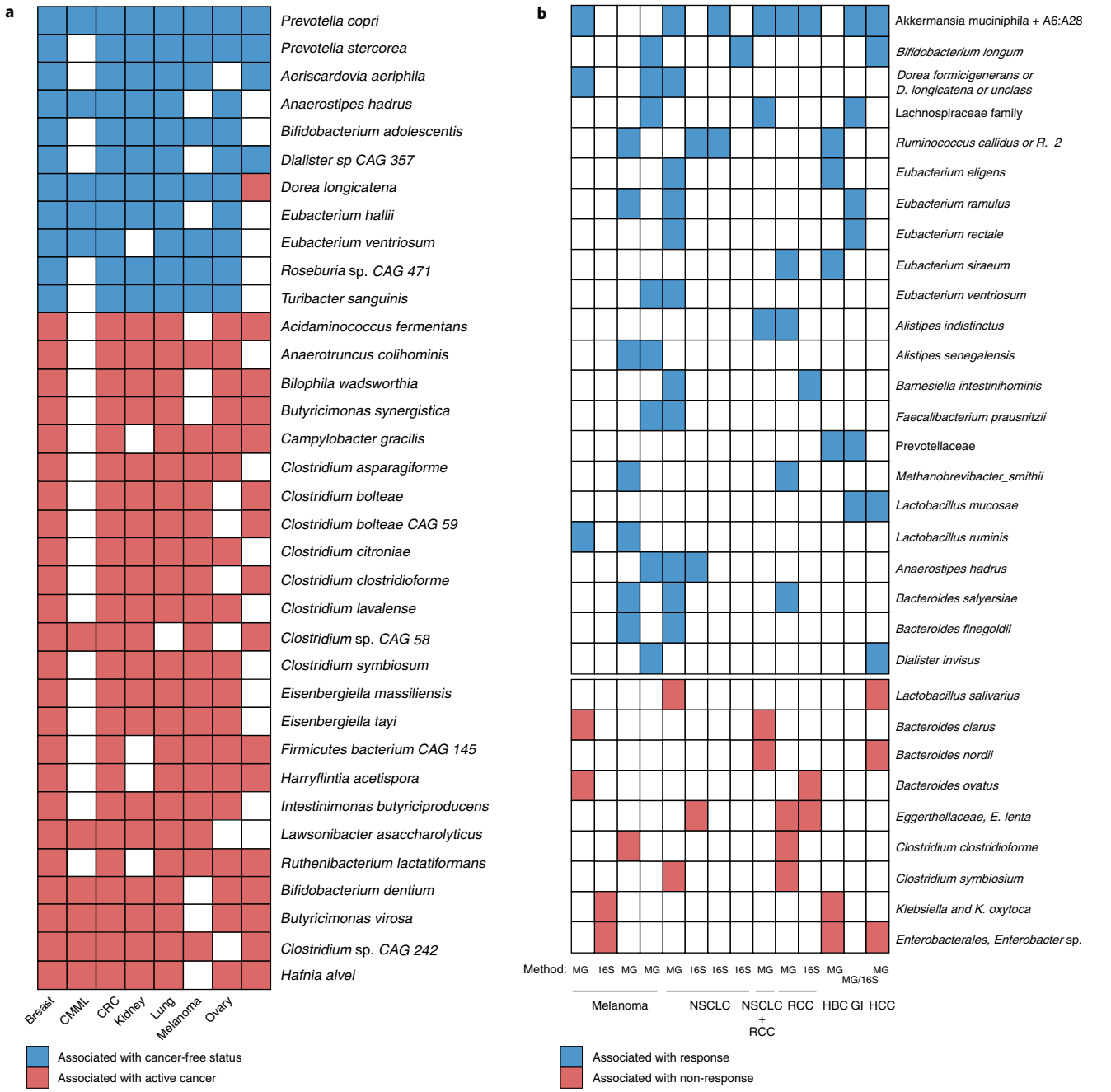

Fig. 2 | Gut oncomicrobiome signatures associated with cancer diagnosis or response to ICB. a, Shotgun metagenomic-based analysis of fecal material collected from over 1,900 individuals with cancer across eight different malignancies of varying stages was compared with that from over 5,500 healthy individuals, as described in recent work by Yonekura et al.[27]. Linear discriminant analysis (LDA) of effect size (LEfSe), together with pairwise comparison of relative taxonomic abundances, was used for this comparison across the selected cohorts. This demonstrated an overrepresentation of selected taxa (blue) in the gut microbiota of healthy individuals, as well as a relative overrepresentation of other taxa (red) in the gut microbiota of individuals with cancer. **b**, Data are shown from 14 studies analyzing the impact of the taxonomic composition of stools from individuals with cancer at baseline on the clinical outcome (ORR or PFS based on radiologic evaluation according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1)) following ICB therapy (in neoadjuvant settings or for advanced disease) across various cancer types and stages (III and/or IV), as well as geographical sites (France, Europe, the United States, Canada, Japan and China). Studies were chosen based on the following criteria: sizeable cohorts, ICB treatment excluding concomitant chemotherapy or tyrosine kinase inhibitors, excluding antibiotic-treated individuals, using 16S rRNA and/or shotgun metagenomic sequencing. Only bacteria or archaea (after false discovery rate (FDR) corrections when indicated) with a significant association with an outcome were retained using LefSe (discriminant logarithmic LDA score > 1.5) to estimate discriminative features in fecal microbiomes at family and/or genus and/or species level(s). Beneficial and harmful microorganisms associated (or not) with clinical benefit are featured in blue and red, respectively. Only families, genera or taxa that were found at least twice in independent studies and were associated with the same general effect (beneficial versus deleterious) are highlighted in **b**. CMML, chronic myelomonocytic leukemia; HCC, hepatocellular carcinoma; NSCLC, non–small-cell lung cancer; HBC, hepatobiliary carcinoma.

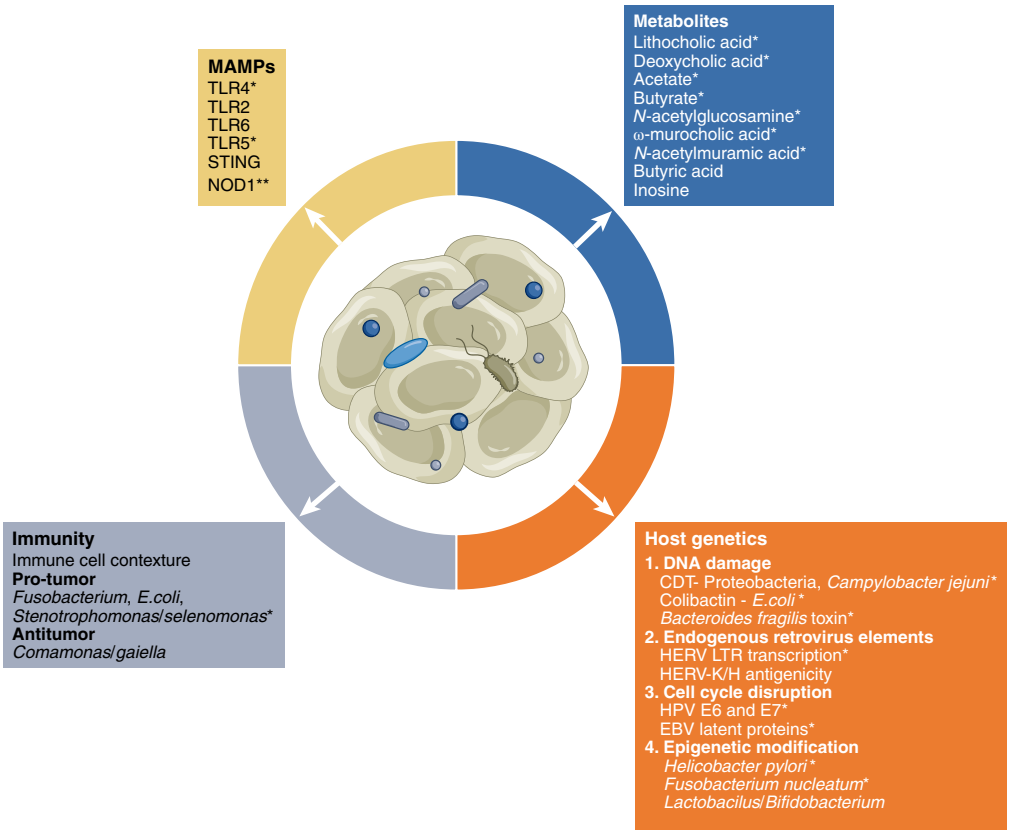

Fig. 3 | Hallmarks of the impact of tissue-based and tumor-based microorganisms in cancer. Increasing evidence has demonstrated an association and interactions between tissue-based and tumor-based microorganisms and host cells across several domains: host genetics, local immunity, metabolites, and MAMPs. Each of these plays a role in tumor progression and response to antitumor therapies. Effects, mediators, or mechanisms associated with antitumor response are listed without any symbol, while those associated with a pro-tumor state are indicated with an asterisk. Context-dependent effects, mediators, or mechanisms are indicated with two asterisks.

Why might microorganisms be found in tissues and tumors within an organism? Indeed, neoplastic/cancerous and precancerous lesions can prove particularly hospitable environments for colonization and persistence of microorganisms. Rapid angiogenesis and tumor necrosis contribute to the development of a highly hypoxic and nutrient-rich TME that can support the specific colonization of facultative and/or anaerobic bacterial strains[36]. These microorganisms can profoundly influence cancer development, progression, therapy response and antitumor immunity via a number of mechanisms[60,62,70,135–163] (Fig. 3).

Tissue-based and tumor-based microorganisms and carcinogenesis. Across tumor types, characterization of the TME has resulted in the detection of a distinct intratumoral microbiome for each cancer type. Intratumoral microorganisms typically colonize intracellularly, within cancer, stromal, and immune cells of the TME[37]. Interestingly, historical evidence has elucidated several mechanisms through which intratumoral microorganisms drive carcinogenesis.

First, tissue-resident microorganisms may promote tumorigenesis by altering various aspects of the host's genome. For example, tissue-resident bacteria can directly induce DNA damage through the genotoxins they express. Notably, colibactin-expressing *Escherichia coli*[164], and cytolethal distending toxin-expressing proteobacteria[165] and *Campbylobacter jejuni*[136], induce double-stranded DNA breaks, while strains such as *Bilophila wadsworthia*, *F. nucleatum,* and *Desulfovibrio desulfurican*[135] induce DNA oxidative stress through production of reactive oxygen species. In addition to directly inducing DNA damage, tissue-resident microorganisms such as EspF-expressing *E. coli*[166] and *H. pylori*[167,168] can disrupt mechanisms of DNA mismatch repair to further exacerbate genomic instability and drive tumorigenesis. Independently, the cell cycle may also be directly disrupted by tissue-resident viruses to promote tumorigenesis, as evidenced by the mechanisms underlying human papillomavirus (HPV)-driven[141] and Epstein–Barr virus (EBV)-driven[142] malignancies. Microorganisms (and their remnants, as in the case of endogenous retroviruses), have also been shown to drive tumorigenesis through other mechanisms related to the host genome, such as by altering the local epigenetic landscape[143,144] or hijacking host transcription[138].

Second, tissue-based microorganisms can promote tumor formation by inducing a pro-tumorigenic inflammatory milieu within the tissue. Several species, such as enterotoxigenic *Bacteroides fragilis* (ETBF)[169], *F. nucleatum*[146], *E. coli*[148], and *Stenotrophomonas*/*S elenomonas*[149], produce toxins capable of initiating inflammatory responses and recruiting immune subsets that collectively contribute to hyperproliferation in the local tissue. In colonocytes, *B. fragilis* toxin initiates a STAT3–nuclear factor-κB (NFkB)-dependent pro-inflammatory signaling cascade to produce cytokines such as interleukin (IL)-17 and IL-23, which has been shown to recruit pro-tumoral myeloid cells[169]. Additionally, *H. pylori* promotes hyperproliferative inflammation in gastric tissue through expression of its cytotoxin-associated gene A (CagA)[170], while *F. nucleatum* achieves the same phenotype in colorectal tissue, through expression of the protein FadA[171]. Select microorganism-associated molecular patterns (MAMPs) have also been shown to promote tumorigenesis. For example, flagellin-dependent Toll-like receptor (TLR) 5 activation in bone marrow-derived leukocytes enhanced carcinogenesis in a model of chemical-induced skin cancer[153].

The impact of microorganisms on tumor progression also extends beyond bacteria. The mycobiome, comprising the fungal components of a given microenvironment, has been associated with the development of pancreatic ductal adenocarcinoma (PDA)[114]. The migration of fungi, particularly *Malassezia* spp. from the gut to the pancreas, promotes PDA development through complement activation by mannose-binding lectin[114].

Finally, byproducts of microbial metabolism can also contribute to carcinogenesis. Secondary metabolites, such as the secondary bile acids lithocholic acid[157,158] and deoxycholic acid[159], and catabolites, such as acetate[160] and butyrate[62], each play a carcinogenic role by enhancing either epithelial–mesenchymal transition and/or cell proliferation in several models of cancer[172]. Deoxycholic acid, which has been implicated in CRC, hepatocellular carcinoma, and esophageal cancer, is produced at increased levels in response to high-fat diets[173], which further provides a mechanistic basis for the association of certain high-risk diets with cancer development in the digestive tract.

Tissue-based and tumor-based microorganisms and their role in the antitumor immune response. Beyond having a role in the tumorigenesis of some tissue types, tissue-based microorganisms can also influence the immune landscape within a developed cancer lesion by altering local cytokine and immune cell profiles. It must be noted that these effects are, however, highly context dependent, as different microorganisms have been shown to polarize contrastingly different types of local immune responses that can either favor or inhibit tumor growth.

In many instances, intratumoral microorganisms drive tumor progression by promoting tolerogenic immunity. First, microorganisms may achieve this through conditional activation of pattern-recognition receptors. For example, bacterial lipopolysaccharide, which has been found within both cancer cells and immune cells in the TME[37], can bind to TLR4 on infiltrating monocytes to skew their differentiation to an immunosuppressive M2 phenotype[174,175], and also on tumor cells to promote recruitment of $CD11b^+Gr1^+$ myeloid-derived suppressor cells and $CD1d^+CD5^+$ regulatory B cells, which can collectively suppress local antitumor T cell responses[176]. Additionally, in CRC, NOD1 activation by microbial peptidoglycans has been shown to induce myeloid-derived suppressor cell-driven immunosuppression in an arginase 1-dependent manner[155].

Intratumoral microorganisms may also mediate immunosuppression by altering the local cytokine milieu or upregulating immunoregulatory ligands on tumor cells. In PDA, *Proteobacteria* promote immune evasion by upregulating immunoregulatory cytokines such as IL-10, thereby decreasing polarization of interferon (IFN)-γ^+ type 1 helper T cells and skewing development of tumor-infiltrating monocytes toward an M2 phenotype[177]. Moreover, recent work has demonstrated an upregulation of immunoregulatory pathways involving PD-1 and CTLA-4 in virally mediated tumors[178]. In head and neck squamous cell carcinoma (HNSCC), for example, HPV integration has been shown to upregulate expression of the genes encoding PD-L1 (*CD274*) and PD-L2 (*PDCD1LG2*), resulting in the suppression of antitumor immune responses[178].

On the other hand, intratumoral microorganisms may also support antitumor immunity. In separate instances, intratumoral activation of TLR2 (ref. [151]), TLR6 (ref. [152]), STING[154], and NOD1 (ref. [156]) by MAMPs has demonstrated enhanced and efficacious immunosurveillance of the cancerous lesion. Recent evidence also suggests that microorganisms can serve as the focal point of local lymphocytic activation, leading to the formation of highly therapeutic tertiary lymphoid structures (TLSs) within the TME. In a mouse model of CRC, experimental colonization with *Helicobacter hepaticus* (Hhep) reduced tumor burden by inducing classical, germinal center-containing TLSs[179]. Strikingly, these classical TLSs harbored both Hhep and Hhep-specific follicular helper T cells, suggesting that intratumoral Hhep was the focal point of TLS-derived antitumor immunity. Microorganism-associated cancers such as HPV-positive HNSCC are characterized by extended survival, increased TLS frequencies and enhanced B cell activity (increased somatic hypermutation and preferential class switching to IgG1 isotypes), as compared to HPV-negative HNSCC, where antibodies derived from TLS-associated tumor-infiltrating B cells also show heightened recognition of HPV proteins E2, E6 and E7 (refs. [180,181]). Furthermore, microbial peptides have also been described to be presented on the surface of melanoma tumor cells and to be recognized by T cells[182]. Together, these results suggest that intratumoral microbial antigens can be found within the TME and may induce robust local immune responses, especially within TLS, where highly therapeutic and cross-functional immunity can be primed.

Targeting gut and tumor microbiota in cancer: current and emerging strategies

Given their integral role in shaping immunity and other physiologic processes, one can anticipate that interventional strategies that manipulate gut and tissue-resident/tumor-resident microbiota through targeted reconstitution, and/or augment current treatment with administration of microorganism-derived products, will become an integral part of cancer treatment—one day evolving into a major pillar of cancer care. Several strategies can now be used to target gut and tumor microorganisms[183], including (but not limited to) FMT, targeted microbial strategies using either single strains or designer consortia, diet-based and prebiotic, probiotic and postbiotic-based interventions, targeted antibiotic approaches, and phage-based approaches. Numerous clinical trials investigating these approaches in patients with cancer are now underway and/or have been completed (Supplementary Table 1), and the different strategies and opportunities to iterate on these approaches to treat, intercept and ultimately prevent cancer altogether are discussed below.

Fecal microbial transplantation. Perhaps the most drastic, yet effective, means by which to modulate the gut microbiome involves FMT—whereby the entire gut microbial complement from a donor (usually a healthy individual or exceptional responder to treatment) is transplanted into a recipient, such as a patient with cancer. This approach has demonstrated success in reversing resistance to treatment with ICB; two recent pivotal studies in patients with metastatic melanoma showed that FMT from patients who experienced a complete response to ICB into patients who were resistant was associated with reversal of ICB resistance in these patients[29,30]. In these studies, successful colonization of the recipient gut by the donor microbiota increased the abundance of bacteria such as Ruminococcaceae and Bifidobacteriaceae in the recipient gut, and these changes were associated with improved clinical responses. Additionally, this improved response after FMT was characterized by enhanced immune infiltration in the tumor and gut of treated patients, as well as enrichment for specific therapy-associated serum metabolites[29,30]. Given these findings, rigorous research efforts are now underway to determine whether the ideal FMT donor in cancer trials is a patient who successfully eliminated their cancer in response to immunotherapy, or a highly fit, healthy individual who has never had cancer. Additional trials combining ICB treatment with FMT from complete responder donors and/or healthy donors are currently underway (NCT03772899, NCT04521075, NCT04924374 and NCT04951583) and are demonstrating promising early results[184].

The clinical relevance of FMT has also extended beyond ICB treatment and has improved other therapeutic applications as well. In clinical trials, preliminary results from an early phase I study have identified FMT as a promising modality to treat

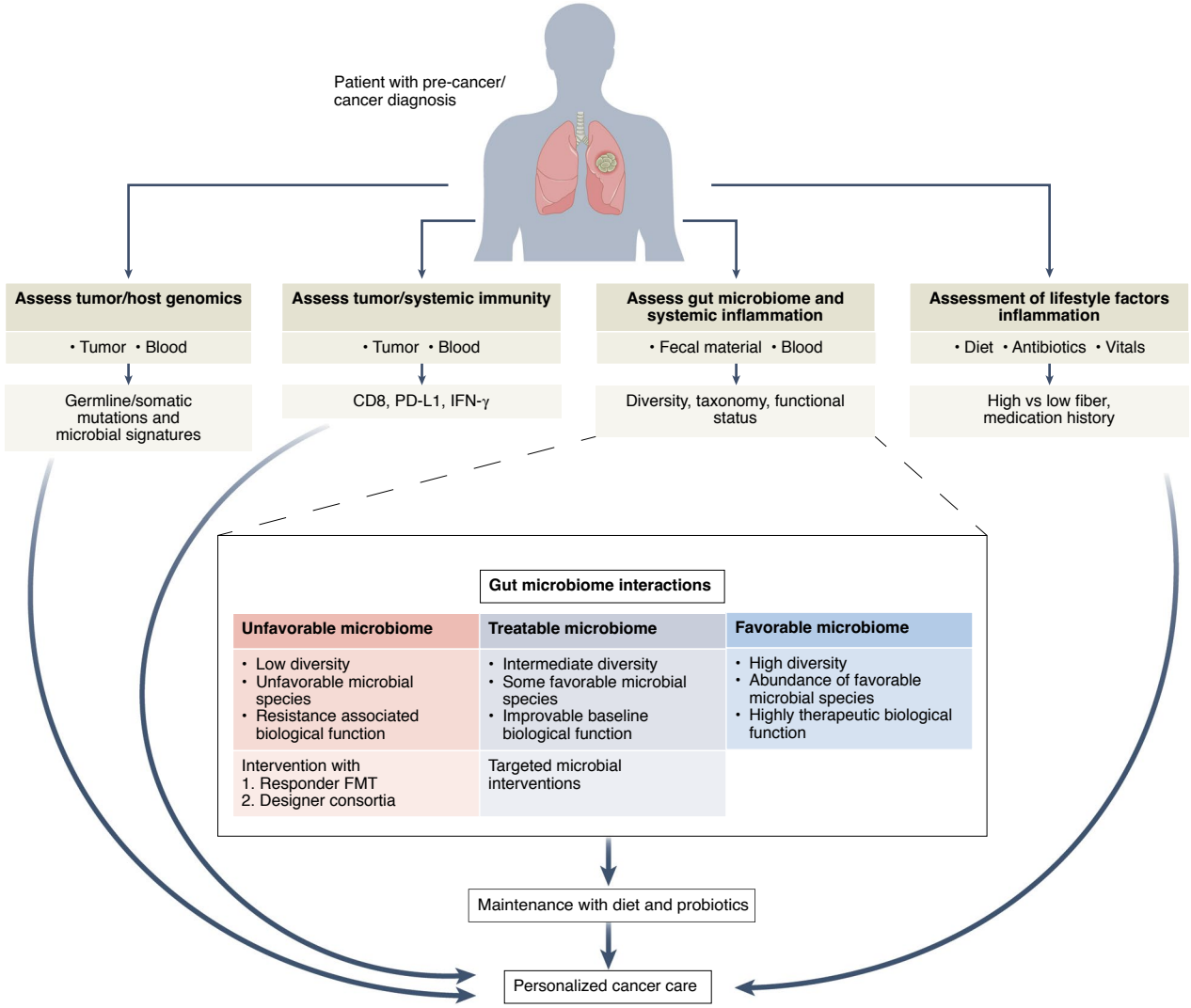

Fig. 4 | Personalized approaches incorporate microbiota-centered interventions: a forward-looking view. In patients with pre-cancer or cancer diagnoses, current approaches for profiling and therapeutic targeting focus mainly on limited genomic and immune profiling, without consistent profiling or targeting of microorganisms in tissues, tumors, or the gut. Profiling of additional modifiable factors, such as diet, stress, and other lifestyle factors, is also limited. Given the emerging role of microorganisms and other factors in influencing carcinogenesis and therapy response, there is an opportunity to incorporate profiling and targeting of these into cancer care and ultimately into overall precision health, which will be greatly facilitated by emerging technologies over the next 5 to 10 years.

steroid-refractory GI tract graft-versus-host disease (a complication of hematopoietic stem cell transplantation to treat leukemia), whereby FMT recipients demonstrated greater clinical remission of graft-versus-host disease[185]. Thus, FMT as an approach to mitigate treatment-related toxicity is also being explored in several clinical trials (NCT03819296, NCT04038619, NCT04721041 and NCT04163289). It should be noted that FMT trials experience several challenges (such as identifying appropriate FMT donors, identifying optimal preparative regimens before transplant, dose and route of administration, among others)[183,186], although the results of these trials are being used to inform the development of more effective next-generation microbiome-based strategies.

Defined microbial consortia and probiotics. While FMT approaches involve transplantation of the entire donor microbiota, recent efforts to modulate the gut microbiota are focusing on the specific transplantation of single microbial species and/or designer microbial consortia to enhance response to ICB and other forms of cancer treatment (Supplementary Table 1). Early results have demonstrated some evidence of success. For example, in a small, open-label trial, 58% of patients with metastatic renal cell carcinoma (RCC) who were treated with CBM588 (a formulation that includes a strain of *Clostridium butyricum*) in combination with ICB responded to treatment, compared to 20% of patients who received only ICB[187]. Additionally, PFS was significantly increased in CBM588-receiving patients to 12.7 months, compared with 2.5 months on ICB alone[187], thus highlighting that the addition of bifidogenic bacterial product can enhance the clinical outcome of patients with RCC. In a similar vein, several hypothesis-generating preclinical and clinical studies suggest that strains of *Akkermansia muciniphila*[32], Bifidobacteria[49], *Enterococcus gallinarum*[188], or a consortium of multiple commensal strains[189], also warrant further clinical evaluation.

However, despite the aforementioned early success with CBM588 (ref. [187]), the feasibility and efficacy of this overall approach is still to be determined. Indeed, several trials are currently underway evaluating microbial consortia or targeted-microbial strategies in conjunction with current cancer therapeutics (for example,

NCT03686202 and NCT05079503), and results from these trials are eagerly awaited. Nonetheless, such approaches have demonstrated remarkable efficacy in non-cancer indications such as *Clostridium difficile* colitis[190,191] and are expected to hold distinct advantages over FMT in the longer-term effort to optimize gut microbiota modulation in the treatment of cancer.

Additional probiotic-based treatments have also been tested, but caution should be taken in considering their use with ICB (and other therapies) in the treatment of patients with cancer. Recent evidence suggests that the use of commercially available probiotics is associated with worse outcomes in preclinical cancer models and in human cancer cohorts treated with ICB[28]. Nonetheless, there are tremendous opportunities to develop informed, 'next-generation' probiotics, as recent studies suggest that specific microorganisms in the gut may enhance antitumor immune responses—in part through the induction of highly therapeutic TLSs in the TME, which have been favorably associated with patients' response to ICB across cancer types[180,192–194]. Together, these lines of investigation open up new possibilities in transplanting and targeting specific therapeutic microorganisms to enhance antitumor immunity in the treatment, interception and prevention of cancer.

Targeted antibiotic, phage-based, small-molecule and CRISPR-based strategies. As previously noted, the use of broad-spectrum antibiotics and massive disruption of the gut microbiota is associated with worse outcomes to ICB[25,26] and other therapies[195–197]. However, it is possible that a carefully selected antibiotic regimen targeting potentially deleterious or pathogenic microorganisms—while facilitating expansion of beneficial microorganisms (with functional redundancy and better community structure)—can promote favorable responses in the host. Along these lines, selective depletion of specific disease-associated taxa using CRISPR–Cas9-encoding phages has been proposed[198], and its promising use in cancer treatments is only in its infancy. Additionally, small-molecule approaches are also being developed to modulate gut microbial composition and function, and these strategies obviate several complexities associated with scalable manufacturing of microbial consortia and skilled administration of these live biotherapeutics to patients. Such approaches have been successfully applied in preclinical models of atherosclerosis, whereby targeted remodeling of the gut microbiome via administration of small-molecule peptides was associated with decreased total cholesterol, reduced atherosclerotic plaques and reduced pro-inflammatory cytokines such as IL-6, tumor necrosis factor, and IL-1β[199]. These types of approaches are expected to expand over the next several years and will greatly shape the therapeutics landscape as we move forward in this field.

Diet and prebiotic strategies. Alongside the aforementioned strategies for directly modulating the gut microbiota, diet is a critical means through which microbial composition and function may be regulated. While the role of diet and dietary interventions have been widely studied in the context of cancer over the past several decades, limitations arise from the lack of rigorous standardization of procedures and the reporting of correlational rather than causal relationships between diet and observed clinical effects[200]. However, strong evidence from preclinical studies suggests that many dietary strategies, including long-term caloric restriction, short-term starvation, ketogenic diets (or oral supplementation with the ketone body 3-hydroxybutyrate), high-fiber diets, and oral administration of specific micronutrients, may improve anti-cancer immunosurveillance in the context of immunotherapy treatment[28,81,201–206]. Additionally, multiple clinical studies evaluating such dietary interventions in patients with cancer have been launched (Supplementary Table 1) and have successfully informed the inclusion of dietary recommendations (such as recommendations to follow a Mediterranean diet) in clinical trials aimed at modulating the gut microbiome using FMT[207] and other strategies. Such studies are critical, as this represents a tractable strategy to modulate the function of gut microorganisms either in the setting of other microorganism-targeting strategies or in the setting of other cancer treatment. There is already evidence that a high-fiber diet is associated with improved clinical outcomes with ICB in preclinical models[28,81] and in clinical cohorts[28] by means of enhancing T cell activation and monocytic reprogramming within the TME. Certainly, this represents a modifiable host factor that can be actively modulated by patients themselves, although critical guidance from physicians and data-driven approaches to monitor and support patient adherence are critical as we integrate such dietary changes into the fabric of cancer care.

In addition to modifying the diet itself, there are tremendous opportunities to incorporate the use of prebiotics. Prebiotics are chemically defined, nondigestible fibers such as inulin or inulin propionate ester, which have been shown to affect the functional status of gut microorganisms in preclinical models[208]. For example, polyphenol supplementation has been associated with increased butyrate production along with increased bifidobacteria and lactobacilli populations[209]. It remains important to note, however, that the effects of these prebiotics depend largely on the bacterial populations present in the gut, as, for example, carbohydrate fermentation differs between *Prevotella*-dominant microbiomes and those in which *Bacteroides* dominate[210]. Nonetheless, the prebiotics have the potential to modulate physiology in myriad ways via modulation of bacterial metabolite production and alteration of the microbial ecosystem—all of which can be harnessed for therapeutic potential[211].

Therapeutic strategies involving tissue-based and tumor-based microorganisms. The notion of treating cancer by directly introducing microorganisms into the cancerous lesion has been around for over a century and is supported by anecdotal observations of tumor regression in patients with cancer who developed infections[212,213]. A century later, rigorous efforts to use microorganisms to treat cancer were underway with the use of microbial strains or toxins such as Coley's toxins (comprising a mixture of toxins derived from *Streptococcus pyogenes* and *Serratia marcescens*)[214], which showed a nominal but promising therapeutic benefit[214,215]. Emerging data in the twenty-first century, many of which have been discussed above, have now renewed enthusiasm in this idea and have birthed several experimental studies aimed at introducing or altering intratumoral microorganisms for the treatment of cancer.

Several microbial agents, such as wild-type or modified live viruses that have the ability to selectively lyse cancer cells (oncolytic viruses, such as the granulocyte–macrophage colony-stimulating factor-producing virus T-VEC)[216] and bacteria modified to induce therapeutic benefit (for example, *Clostridium noyvi*-NT, which has toxin-producing genes removed)[217], to name a few, have been used in clinical trials and have demonstrated promising antitumor efficacy in a number of cancer settings (Supplementary Table 1). For example, the aforementioned T-VEC oncolytic virus in combination with pembrolizumab resulted in a 30% overall response rate (ORR) among patients with sarcoma[218]. Similarly, the Pexa-Vac oncolytic virus, combined with cemiplimab, also yielded a 37.5% response rate among patients with RCC[219]. Modified bacteria have proven effective as well; the engineered Listeria strain ADXS11-001, which secretes the fusion protein HPV-16 E7 to target HPV-positive cells, showed an HPV-specific response rate of 33% in HNSCC[220]. These same engineered bacteria, when used in patients with HPV-positive anal squamous cell carcinoma in combination with radiation and chemotherapy, resulted in 8 of 9 patients (89%) showing no evidence of disease after 34 months[221]. These examples highlight the potential efficacy of microbial agents in treating existing tumors. Other strategies aim to prevent cancer

from occurring, such as vaccination against cancer-associated infectious agents, including EBV and HPV[41,222].

Encouraging findings (including those mentioned above) that demonstrate efficient homing of designer microbial agents, which allows them to accurately target tumor cells or the TME, may serve as a foundation for the development of future therapies in which microbe-based therapies or vaccines yield not only localized tumor treatment but also systemic protection against metastatic disease[223]. Furthermore, the identification and validation of unifying, therapy-associated intratumoral signatures within cancer types could accelerate development of broadly usable off-the-shelf treatments for cancer.

Future outlook: personalized approaches

With the emergence of the microbiota as an important driver of health and disease, there is now a unique opportunity to integrate profiling and targeting of microbiota in the gut and other niches into precision cancer care and, ultimately, into overall precision health (Fig. 4). Currently, personalized cancer care incorporates profiling of the precancerous or cancerous tissue for histopathologic features (visually, by a pathologist) alongside profiling for genomic or proteomic alterations (such as RAS, MSH/MLH, and HER-2) via either targeted genotyping or NGS approaches[13,224], as well as limited immune cell profiling (for PD-L1, CD8 and other markers) at baseline and during treatment to help guide therapy and assess response[225–227]. In the near future, certain markers will likely be analyzed via noninvasive interrogation of tumor-derived, circulating cell-free DNA[228].

Currently, profiling of microbiota in the gut, tissue/tumor, and other niches (including blood-based microbial signatures) is performed solely in the context of research studies, although initiatives to include assessment of microbiota as biomarkers of response[229–231] and even as therapeutic targets[232] are increasing. Assessment of markers of systemic inflammation (such as C-reactive protein and IL-6) and markers of systemic immunity (such as the neutrophil-to-lymphocyte ratio) are being explored but are not part of current clinical standards[233,234]. Additionally, assessment of dietary patterns and other lifestyle factors (such as sleep and stress) is not consistently incorporated when the treatment of pre-cancer, cancer, and other disease states is being considered.

However, the tides are turning. Strategies are now emerging that incorporate a more holistic approach to the prevention and treatment of cancer[235], with opportunities to promote health via advances in monitoring, feedback, and early intervention across multiple fronts. Certainly, in the next 5 to 10 years, one could envision a more comprehensive approach to care of individuals with cancer that incorporates assessment of tissue/tumor and blood for somatic and germline mutations, along with tissue-based and blood-based microbial signatures (Fig. 4). Along these lines, deeper assessment of systemic-based and tissue/tumor-based immunity is warranted beyond present-day markers, with opportunities to target novel immune mechanisms to prevent and treat cancer via improved immunosurveillance[236,237]. Additionally, profiling of microbiota in the gut (and potentially other niches) holds great promise as we move toward a more holistic approach to the treatment of cancer and other diseases, as does interrogation of markers of systemic inflammation (beyond present-day markers), and assessment of lifestyle and other factors[233,238]. Such strategies will be greatly facilitated through the use of emerging technological advances such as artificial gut-on-a-chip models, wearable technologies, ingestible capsules for sampling the microbiome, metabolomic profiling, and smart toilets, to name a few[239,240]. Additionally, the integration of multi-omics data via artificial intelligence[241,242] will offer opportunities for refining treatment, interception, and prevention strategies via mathematic modeling and other approaches[243–245], while iterating and building on current-day approaches[246].

Focusing on the gut microbiota, individuals with a markedly dysbiotic profile, characterized by low diversity and an abundance of 'unfavorable' and/or pathogenic microorganisms with poor functional status may optimally benefit from comprehensive interventions such FMT or a complete designer consortium. In contrast, individuals with a moderately dysbiotic gut microbiota profile, characterized by intermediate diversity and some favorable species with relatively preserved functional status, may benefit from targeted microbial interventions, while those with a favorable microbiome are unlikely to benefit from such interventions and should be supported with dietary intervention (which should be consistently implemented across all treatment groups). Together with other strategies, the approaches described above will result in more optimized personalized cancer care and improved precision health.

Conclusion

Our understanding of the role of host microorganisms on normal physiology and disease has evolved markedly over the past decade, revealing opportunities to target microorganisms in the gut and other niches to treat disease and ultimately to promote overall health. However, the field is in its nascency, and tremendous opportunities exist to further elucidate the mechanisms through which these microorganisms impact physiological and pathological processes, as well as optimal means for targeting them through dietary intervention and other approaches. Although challenges remain, we anticipate that over the next 5 to 10 years, profiling and targeting of microbiota in the gut and other niches will become part of the very fabric of integrated cancer care, as well as the management of other diseases.

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Author contributions

All authors contributed equally to the design, preparation and review of this manuscript. J.A.W. supervised and oversaw all aspects of this work.

Competing interests

J.A.W. is an inventor on a US patent application (PCT/US17/53.717); reports compensation for speaker's bureau and honoraria from Imedex, Dava Oncology, Omniprex, Illumina, Gilead, PeerView, MedImmune and Bristol-Myers Squibb; serves as a consultant/advisory board member for Roche/Genentech, Novartis, AstraZeneca,

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Additional information

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