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The microbiome, cancer, and cancer therapy

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With the advent of next-generation sequencing, we have an unprecedented ability to study tumor and host genomes as well as those of the vast array of microorganisms that exist within living organisms. Evidence now suggests that these microbes may confer susceptibility to certain cancers and may also influence response to therapeutics. A prime example of this is seen with immunotherapy, for which gut microbes have been implicated in influencing therapeutic responses in preclinical models and patient cohorts. However, these microbes may influence responses to other forms of therapy as well and may also affect treatment-associated toxicity. Based on these influences, there is growing interest in targeting these microbes in the treatment of cancer and other diseases. Yet complexities exist, and a deeper understanding of host–microbiome interactions is critical to realization of the full potential of such approaches. These concepts and the means through which such findings may be translated into the clinic will be discussed herein.

Over the last decade, we have seen a plethora of data linking host microbiota with normal physiology and function, and there is now an abundance of literature that demonstrates an association between disruptions in the homeostasis of microbial communities (termed dysbiosis) and pathologic conditions, ranging from neurologic diseases to metabolic and cardiovascular disorders as well as gastrointestinal disturbances[1–5]. In addition, results from numerous studies now suggest there is a link between the commensal microbiota and cancer[6], including recent compelling evidence regarding the role of the gastrointestinal (gut) microbiota in modulating responses to cancer immunotherapy[7–12] and data demonstrating that the microbial communities within the tumor microenvironment can contribute to therapeutic efficacy[13].

Importantly, strategies to target the microbiota are being employed across the spectrum of human disease, with some evidence of success[14]; however, complexities exist, and questions remain on how best to therapeutically target the gut and tumor microbiotas. Herein, we highlight the impact of the commensal microbiota across the continuum of health and disease and discuss the emerging role of the gut and tumor microbiotas in response to cancer therapy and whether their modulation might serve as a viable adjunct to conventional anticancer therapies.

Gut dysbiosis and cancer development

The gut microbiome is increasingly being recognized for its influence in health and disease (Box 1). This is true for cancer as well, where certain bacteria and viruses have been implicated in cellular dysplasia and carcinogenesis (Fig. 1). Known oncogenic gut bacteria include *Salmonella typhi*[15] and *Helicobacter spp.*[16] in biliary cancer and *Helicobacter pylori* in gastric cancer[17,18], among others. In most of these cases, carcinogenesis is believed to be secondary to the creation of a local chronic inflammatory state; however, some bacteria, including *H. pylori*, have direct genotoxic effects and can alter key intracellular signaling pathways that regulate the growth and proliferation of mucosal cells[17]. Notably, *H. pylori* is associated with both gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT lymphoma) and is identified as a class I carcinogen by the World Health Organization (WHO)[17,18].

Additionally, there is also evidence supporting the notion that generalized dysbiosis of the gut microbiota may contribute to carcinogenesis[19,20]. An association between repeated courses of antibiotics and the development of a variety of both gastrointestinal (GI)-tract and non-GI-tract tumors has been demonstrated in large case-control studies[21]. The mechanisms through which dysbiosis is proposed to affect tumorigenesis and/or tumor growth across cancer types are numerous and varied (Fig. 1); yet, comprehensive mechanistic insights are lacking, and studies are underway to better understand how gut microbes may influence carcinogenesis.

The preponderance of evidence supporting a causal role for gut dysbiosis as a modulator of cancer development is in colorectal cancer (CRC)[22–24] (Fig. 1). For one, the tumor microbiota (that is, adenoma- or cancer-associated microbiota) is distinctly different from that of the adjacent healthy mucosa[25–27], and evidence from preclinical models suggests that transplant of stool from patients with CRC can induce polyp formation, induce procarcinogenic signals, and alter the local immune environment in mice as compared with that from healthy controls[28]. Additionally, evidence from various preclinical models and some human studies has implicated dysbiosis as an oncogenic driver in CRC. Certain bacterial species can stimulate an inflammatory state that can promote carcinogenesis via induction of proinflammatory toxins (such as those produced by *Bacteroides fragilis*[29–31]), increased production of reactive oxygen species[32], and alterations in signaling pathways (*Fusobacterium nucleatum*)[33] in human tumors and in mouse models, or they may act to prevent antitumor immune functions (*F. nucleatum*)[33,34]. The chronic inflammatory state may itself propagate dysbiosis, as genetic deficiencies in key inflammation-modulating genes promote the accumulation of certain bacteria, including *Escherichia coli*[35–38]. Alternatively, production of metabolites that are directly genotoxic (such as production of colibactin from *E. coli*[35,39,40] or cytolethal distending toxin by *Campylobacter jejuni*[41]) has been shown to induce to carcinogenesis in mice. Finally, components of *F. nucleatum*, including the FadA adhesion (FadAc) complex, can activate the β-catenin–Wnt signaling pathway in human colon cancer cell lines, resulting in oncogenic transcriptional changes[22,42].

It has been demonstrated that *F. nucleatum* plays a role in the development and progression of colon adenomas and colon cancer[31,33,43–47], and it has also been detected in nodal and distant metastasis in patient samples[48,49]. In many of these studies, detection of *F. nucleatum* has relied on amplification of nucleic acid by PCR, which does not demonstrate presence, viability, or invasiveness of the organisms themselves; however, via non-amplification-based techniques like 16S ribosomal RNA (rRNA) fluorescent in situ hybridization (FISH), *F. nucleatum* has been detected not only in

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Box 1 | The microbiome and disease

Insight into the factors that influence overall 'gut health' is increasing, and there is compelling evidence that the microbiota within our guts (including bacteria, archaea, viruses, fungi, protozoa, and other microbes) can have a profound impact on our overall health and response to disease[145]. In addition, there is a growing recognition that the gut microbiota influences local and systemic immunity[135].

Though we do not yet have a complete understanding of the optimal composition of a core 'healthy' gut microbiome and its influence on the immune system, several studies have provided insight into specific gut microbes that are associated with overall 'health'[146]. This complex ecosystem is also influenced by a large number of factors, including geography, diet, and medications[147,148].

Gut dysbiosis is typically characterized by reduced microbial diversity and/or substantial shifts in resident species. Dysbiosis within the gut may trigger inflammatory signaling pathways with effects that span far beyond the level of the gut, affecting immune function as a whole. There is evidence that dysbiosis of the gut microbiota increases susceptibility to infection and leads to impaired vaccine response, as has been observed in the setting of malnutrition[149–151]. Dysbiosis of the gut microbiota is also implicated in a number of autoimmune and inflammatory conditions, including IBD, multiple sclerosis (MS), type I diabetes, and rheumatoid arthritis (RA)[1].

A prototypic example of a condition associated with dysbiosis is CDI, which is associated with the use of some oral antibiotics and which can cause rapid disruption of gut microbial communities[4,152,153]. The altered microbial profile naturally affects both the host- and microbial-derived metabolic environments and is characterized by an enrichment of primary bile acids and simple carbon compounds, aiding in the germination and growth of *C. difficile*[154]. Conventional treatment of CDI involves carefully coordinated antibiotic therapy; however, recent evidence points to the clinical benefit of modulation of the gut microbiota (particularly via FMT) over the standard antibiotic regimen in patients with recurrent CDI[155,156]. In addition to this, FMT has proven to be more cost effective than traditional antibiotic therapies[157]. Various prospective clinical trials are ongoing.

Another condition characterized by dysbiosis is IBD, which encompasses several chronic inflammatory conditions, including Crohn's Disease (CD), characterized by patchy inflammation found in any portion of the GI tract, and ulcerative colitis (UC), characterized by extensive transmural inflammation and restricted to the colon. These conditions are associated with an inflammatory reaction that is thought to be, in part, triggered by gut dysbiosis[5]. However, host genetics also plays a major role, with almost 200 polymorphisms identified that are associated with the condition[158,159]. Many of these polymorphisms relate to intestinal barrier function and immune response to commensal organisms, lending further credence to the notion of the importance of gut dysbiosis in the pathogenesis of this condition. It is exceedingly difficult to establish causality, however, as IBD itself is associated with profound dysbiosis[160], likely induced by inflammatory changes and substantial alterations in mucosal integrity characteristic of the disease process. Therefore, the altered microbial composition may also be a result of mucosal damage and/or chronic inflammation[161–163].

Given this evidence, scientists have devised strategies to modulate the microbiota in patients with IBD, including both preventive measures and treatment of active disease. This includes the use of antibiotics, which affect the gut microbiota in several aspects, including reducing total bacterial load. Treatment with antibiotics has demonstrated benefit in multiple trials[164]. Studies have also shown that exclusive enteral nutrition can alter the gut microbiota and induce remission in patients with CD[165]. Direct modulation of gut microbiota through FMT or probiotic supplementation has also been successfully employed in these patients[166–169]; however, additional studies are needed to more thoroughly explore this route for microbiota manipulation.

Dysbiosis of the gut microbiota has also been implicated in a number of pathologic conditions outside of the gut, including MS[3]. MS is characterized by the immune-mediated destruction of myelin in the central nervous system; the gut microbiota has been demonstrated to influence this process in genetically susceptible individuals[170,171]. Epidemiological studies of patients with MS to date have demonstrated no major variation of microbial structure in the human gut compared with that of healthy individuals; however, shifts in specific bacterial taxa that have been observed are believed to contribute autoimmune responses[3].

Modulation of gut microbiota in the treatment of MS has mainly been employed in animal models[172], but limited clinical trials investigating probiotic supplementation have yielded promising results[173,174]. Trials incorporating FMT for MS are underway, with anecdotal evidence demonstrating the utility of such an approach[175].

primary tumors, but also in metastatic lymph nodes and liver metastases, and it has been found within tumor cells[48,49]. Importantly, it has also been cultured from a limited number of fresh frozen specimens from patients with colon cancer and liver metastasis (two of three paired specimens for which the specimen was adequate for culture) as well as patient-derived xenograft models[49].

Gut microbiota have also been associated with a number of other malignancies, including hepatocellular carcinoma (HCC)[50] and breast cancer[51] (Fig. 1). Through the portal venous system, the liver is uniquely exposed to intestinal bacterial components and their metabolites and byproducts, which could cause inflammatory changes and hepatotoxicity and/or could potentially directly lead to carcinogenesis. For example, microbial modification of primary bile acids produced by the liver to secondary bile acids, such as deoxycholic acid (DCA), can cause DNA damage, hepatotoxicity, and carcinogenesis[52]. Further, the balance of primary and secondary bile acids alters the concentration of natural killer (NK) T cells in the liver, and these can prevent both primary and metastatic tumor growth in mouse models[53]. The gut microbiota is also associated with the response to infectious hepatitis and obesity and with the development of non-alcoholic steatohepatitis (NASH) as well as other pathologies, all of which can cause cirrhosis contributing to the development of HCC[50].

In the case of breast cancer (Fig. 1), it is hypothesized that the gut microbiota may promote carcinogenesis via its influence on steroid (estrogen) metabolism, specifically through its ability to alter the profile of circulating estrogens and phytoestrogens[54]; via its influence on energy metabolism and obesity; or via antitumor immune function[55]. Studies have been published that demonstrate a link between gut dysbiosis and other malignancies; however, these associations are less well characterized and require further investigation. Further preclinical, clinical, and epidemiological studies will ultimately solidify the relationship between dysbiosis and cancer.

The gut microbiota and cancer therapy

In addition to its role in carcinogenesis, the gut microbiota has now been demonstrated to play a key role in the response to

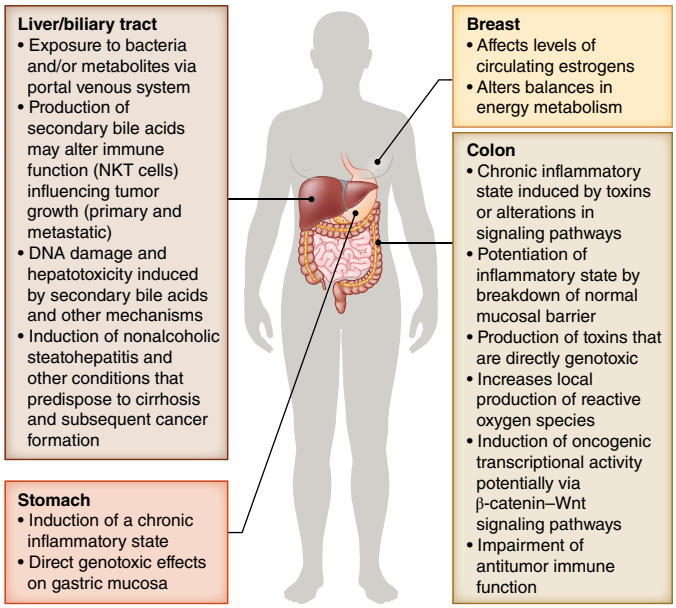

Fig. 1 | The influence of gut microbiota on cancer development. Shown are various mechanisms through which dysbiosis is proposed to affect tumorigenesis and/or tumor growth across cancer types, including colon, hepatobiliary, gastric and breast. Credit: Debbie Maizels/Springer Nature.

cancer therapy. Published studies have implicated the gut microbiota in influencing response, as well as toxicity, across a range of treatments, including chemotherapy, immune checkpoint blockade, and stem cell transplant, via a variety of proposed mechanisms, though a deeper understanding is clearly needed and is the focus of current study.

The role of the gut microbiota in immune checkpoint blockade. Multiple publications have now demonstrated a role for gut microbiota in modulating responses to immune checkpoint blockade across several cancer types[9–12]. These studies were inspired by compelling data in preclinical models[7,8], and proof of principle was provided through the publication of similar observations across numerous clinical cohorts. They broadly demonstrate that differential gut microbiota 'signatures' exist in patients who respond to treatment and that these favorable signatures are associated with enhanced systemic immunity and intratumoral immune infiltrates. Additionally, several of these studies demonstrate that the 'responder' and 'nonresponder' phenotypes could be recapitulated in germ-free or antibiotic-treated mouse models via fecal microbiota transplant (FMT)—and that manipulation of the gut microbiota with specific bacterial taxa could enhance therapeutic response[10–12]. The mechanisms through which the gut microbiota influence response have been investigated in preclinical and clinical studies. The data suggest that gut microbes may impact antitumor immunity via a number of mechanisms, including interaction of microbial components or products (such as pathogen-associated molecular patterns (PAMPs)) with antigen-presenting cells (APCs) and innate effectors (via pattern-recognition receptors (PRRs) like Toll-like receptors (TLRs)), which can help prime an adaptive immune response; induction of cytokine production by APCs or lymphocytes; and even local or distant effects of microbial metabolites (Fig. 2). Notably, there was only modest overlap in these checkpoint-blockade-responsive microbiome signatures across cohorts (Fig. 3), though some phylogenetic commonalities exist among identified bacterial taxa in studies utilizing different checkpoint inhibitors. Based on these published reports, there has been tremendous interest in mining these signatures to devise optimal bacterial consortia to use therapeutically in combination with checkpoint blockade, though this will likely be an iterative approach with numerous considerations regarding both the host and the microbial product administered.

The microbiome and chemotherapy. In addition, there is now evidence that the gut microbiota may shape responses to other forms of cancer therapy. In preclinical models[56–58], microbiotas in the gut and other sites were shown to influence responses to a range of chemotherapies. Beneficial responses to cyclophosphamide were linked to increased intestinal permeability, allowing bacterial translocation resulting in the maturation of T helper 17 (T_H17) cells within the lamina propria and effector lymph nodes, which facilitated a systemic antitumor effect[56]. In contrast, the response to local CpG oligonucleotide therapies and oxaliplatin was dependent on microbiome-dependent changes in the expression of pro-inflammatory genes and the production of reactive oxygen species by myeloid cells within the tumor microenvironment[57].

Impact of cancer therapy on gut microbiota. It is becoming apparent that, in addition to the gut microbiota's impact on response to cancer therapy, all aspects of cancer therapy may in turn impact the microbiota. Chemotherapy can cause profound dysbiosis and affect multiple metabolic pathways[59,60]. Antibiotics are frequently prescribed during the course of chemotherapy that also impact the microbiota; this is important, as concurrent antibiotic administration has now in several studies been shown to negatively impact the outcomes of cancer immunotherapy[61]. In addition to this, in the context of surgery, the gut microbiota may be disrupted with the administration of pharmacologic (antibiotics) and/or osmotic bowel preparations[62]. Radiation therapy may also impact the gut microbiota by damaging intestinal and/or colonic mucosa, thereby altering absorption of bile salts and changing stool frequency[63].

The gut microbiota and therapeutic toxicity. In addition to mediating therapeutic response, the gut microbiota have also been implicated in modulating cancer therapy toxicity. In the setting of allogeneic stem cell transplantation, performed for a variety of hematologic malignancies (including non-Hodgkin lymphoma, Hodgkin lymphoma, multiple myeloma, and leukemia among others), compositional differences in the gut microbiota have been associated with differing rates of development of graft-versus-host disease (GVHD)[64–66]; GVHD occurs when donor cells, most commonly T cells, of the graft cross-react with the patient's major histocompatibility complex, producing immune-related toxicities of the skin, GI tract, and other sites[67]. This has extremely high morbidity and mortality. Interestingly, the most common sites for acute GVHD are those that are highly colonized by bacterial flora, and the development of GVHD has also been linked to TLR signaling, suggesting microbial influences[67,68]. As advances in sequencing technology continue to improve, so has our understanding of the role of specific components of the microbiota in GVHD toxicity. Several studies have pointed to the gut commensal *Blautia* as a potential beneficial player, as patients with a high abundance of this taxa had reduced GVHD-associated mortality[69].

The role of the gut microbiota in toxicity has also been studied in the context of other anticancer therapies. Several gut bacterial taxa may be protective against toxicity to cancer immunotherapy—including Bacteroidetes, which is more abundant in patients resistant to ipilimumab-induced colitis, and *Bifidobacterium,* which can abrogate pathology in a mouse model of immunotherapy-induced colitis[70,71]. Interestingly, some bacterial taxa may also be associated with favorable response as well as toxicity, including Firmicutes in the setting of immunotherapy and immunotherapy-induced colitis[71]; therefore, uncoupling these effects is critical. Similarly, preclinical

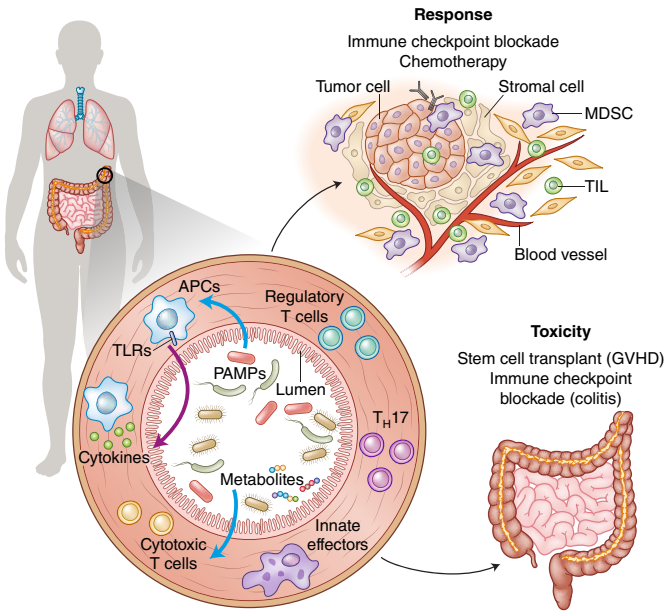

Fig. 2 | The influence of the gut microbiota on different cancer therapies. Gut microbes can impact both the response to various cancer therapies and associated toxicities, such as colitis and GVHD. The gut microbiota is thought to alter systemic immune function via local changes within the gut mucosa and gut-associated lymphoid tissue. The interaction of PAMPs with APCs and innate effectors via PRRs (TLRs) can help prime an adaptive immune response. Cytokines and microbial metabolites produced locally can act systemically. These combined activities lead to increased antitumor immune function with increased numbers of tumor-infiltrating lymphocytes (TILs) and decreased numbers of myeloid-derived suppressor cells (MDSCs). Credit: Debbie Maizels/Springer Nature.

models show that the gut microbiota plays a dual role in the response to oxaliplatin, contributing to both tumor cytotoxicity and mechanical hyperalgesia, a common chemotherapy-related complication, by way of increasing reactive oxygen species and proinflammatory cytokines in the dorsal root ganglion[72]. Radiation has also been shown to alter the composition of gut microbiota in preclinical models, reducing the abundance of Firmicutes and increasing that of Proteobacteria and subsequently increasing susceptibility to radiation-induced colitis[63,73].

We have also recently demonstrated the usefulness of FMT as a therapeutic modality for severe immunotherapy-induced colitis[74] that was refractory to immunosuppressive therapies, such as both corticosteroids and biologic agents, including anti–tumor necrosis factor alpha and anti-integrin therapies. The endoscopic resolution of colitis was commensurate with a durable change in the microbiota, which came to resemble that of the donor, and an alteration in the colonic inflammatory infiltrate to a more anti-inflammatory phenotype[74].

The role of the tumor microbiota

Given that the human body hosts trillions of microbes, it is not surprising that bacteria have been detected within tumors themselves; lung, breast, colon, gastric, pancreatic, cholangiocarcinoma, ovarian, and prostate cancers have all been found to harbor microorganisms[75–81]. While tumors of the enteric tract, respiratory system, or reproductive tract are routinely exposed to microorganisms, it is more difficult to account for the growth of bacteria in tumors of other organs. Systemic seeding from infection or bacterial translocation from the GI tract may happen at a relatively high frequency, even in healthy individuals with normal gut mucosal integrity[82]. These bacteria could selectively home to tumors that have a rich blood supply with relatively leaky aberrant vasculature via a potentially chemotactic gradient of necrotic cellular debris. Once settled, they could selectively thrive within the relatively hypoxic tumor microenvironment (especially anaerobes or facultative anaerobes). In support of this theory, systemically administered bacteria can seed tumors in rodent models[77,83].

While there is less direct evidence that intratumoral bacteria can affect patient outcomes compared with the gut microbiota, it is likely that they confer some effect on response to cancer therapies as well. These intratumoral bacteria are metabolically active; bacteria often found within tumors can alter the chemical structure of common chemotherapeutic agents, changing their activity (by either increasing or decreasing it) and thus their effective local concentration[84,85]. Bacterial taxa, including Gammaproteobacteria (found within pancreatic tumors), express an isoform of cytidine deaminase that can inactivate gemcitabine, thereby lowering local concentrations of the drug and conferring resistance to this potent chemotherapeutic agent that is commonly used to treat malignancies of the GI tract, including the pancreas[13,59,85]. However, the effects of the tumoral microbiota on the response to cancer therapy are not limited to enzymatic activity. For example, *Fusobacterium* may also confer resistance to chemotherapy in CRC through the activation of TLRs on cancer cells and the subsequent loss of certain microRNAs within the tumor and initiation of autophagy[86].

Furthermore, the presence of bacteria within the tumor environment can be, in and of itself, immunomodulatory; some evidence supports an immunostimulatory role for bacteria in the tumor environment, while other studies suggest that intratumoral bacteria create a predominantly immunonsuppressive microenvironment. In preclinical models, recognition of bacteria by intratumoral innate immune cells (via PRRs) can activate proinflammatory cytokine production, driving further influx of a variety of immune cells and improving antigen presentation, thereby augmenting antitumor immune function[87,88]. Intratumoral bacteria can also alter the expression of ligands and receptors on both immune and cancer cells that are current targets of immunotherapy[89–91]. On the contrary, they have also been shown to be immunosuppressive. They can recruit myeloid-derived suppressor cells (MDSCs) and increase the production of immunosuppressive cytokines or the activation of alternative immune checkpoints, conferring a noncytolytic response[77,92]. The Fap2 protein of *Fusobacterium* can prevent T cell immunoreceptor with Ig and ITIM domains (TIGIT)-mediated activation of NK cells, shielding colon adenocarcinoma cell lines from NK cell–mediated killing[34]. Antibiotic therapy that reduces the load of intratumoral bacteria in pancreatic cancer has been shown to decrease recruitment of suppressive cells and increase recruitment of innate effector cells and increase cytolytic T cell activity[77].

The microbiome as a therapeutic target in cancer therapy

Efforts are currently underway to enhance therapeutic responses and/or abrogate treatment-associated toxicity via modulation of the gut microbiota. Parallels may be drawn from efforts in targeting nonmalignant disease; however, unique complexities exist which will be discussed herein (Fig. 4).

Fecal microbiota transplantation. FMT was originally used roughly 2,000 years ago when Chinese researchers orally administered 'yellow soup,' a slurry of stool from a healthy individual, to patients to cure them of severe diarrhea. This approach was also used in Africa during World War II, when German soldiers and nomads in the region reportedly used camel stool as treatment for severe dysentery. Interest in this concept was reinvigorated in 1958 when Eiseman treated patients with fulminant pseudomembranous enterocolitis diarrhea with retention enemas of fecal matter[93]. Within the past decade, FMT has been more widely used in

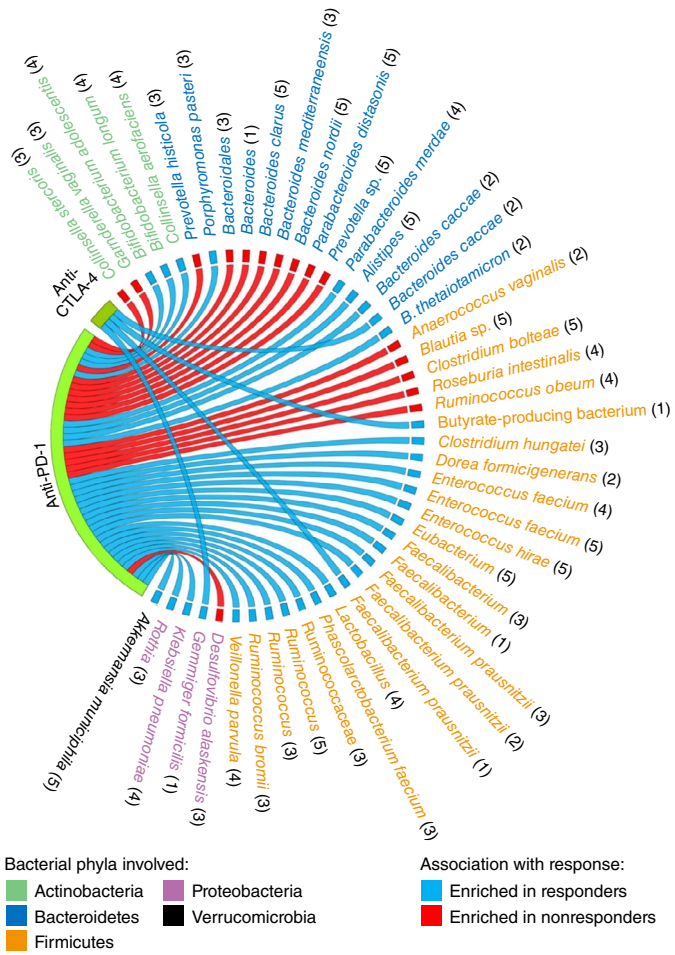

Fig. 3 | Existing evidence for the role of the gut microbiota in cancer development and treatment response. Circos plots illustrating the relationships of unique microbial taxa response to cancer immunotherapy agents (anti-CTLA-4 and/or anti-PD-1). The blue ribbons represent the microbial taxa that are enriched in the context of a beneficial therapeutic response. The red ribbons represent the microbial taxa that are enriched in nonresponders to therapy. These reported associations should be interpreted with caution as they are correlative in nature, and causality has yet to be established. Different taxa are further grouped and colored by their phyla as indicated by the legend. Numbers in parentheses indicate the references that demonstrate these relationships: (1) Chaput et al.[71]; (2) Frankel et al.[9]; (3) Gopalakrishnan et al.[12] (4) Matson et al.[11]; (5) Routy et al.[10]. Credit: Debbie Maizels/Springer Nature.

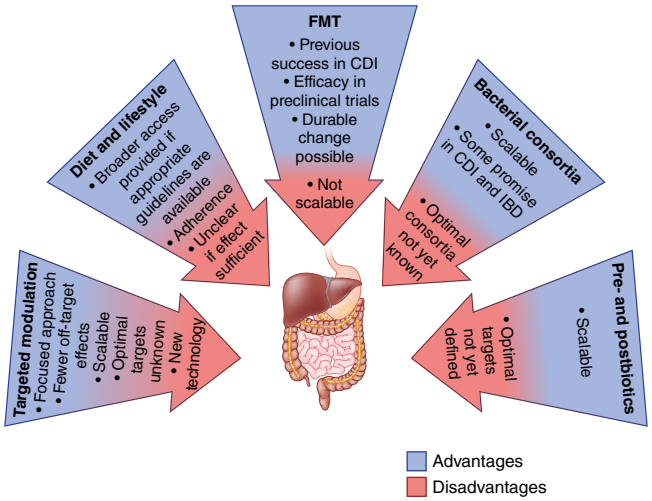

Fig. 4 | Strategies to target the gut microbiota. The gut microbiome can be modified via FMT, the administration of probiotics or specific bacterial species, alterations in diet or lifestyle, and targeted modulation using tailored antibiotic therapies or bacteriophages. There are pros (blue) and cons (red) related to the use of each of these individual techniques to modulate the gut microbiota in the context of cancer therapy. Credit: Debbie Maizels/Springer Nature.

the treatment of resistant *C. difficile* infection (CDI), and incredibly high response rates have been reported[94].

FMT may be delivered via a number of different routes—including through colonoscopy, enema, or oral administration (via nasogastric tube or oral capsules). Response rates to FMT for CDI were thought to vary with the approach, with administration via colonoscopy or enema demonstrating the highest response rate, followed by nasogastric tube administration and oral encapsulated FMT with a first-dose efficacy[94]; however, a more recent randomized control trial suggests that clinical efficacy is equal for oral encapsulated products and delivery via colonoscopy[95]. To date, this approach has an excellent safety profile in the treatment of nonmalignant disease, even in immune-compromised individuals[96,97]. The use of FMT in patients with cancer has been more limited, though there are several current clinical trials incorporating modulation of the gut microbiota via FMT in the context of cancer therapy (Table 1). The majority of these are in the setting of treatment with stem cell transplantation, though there is a growing number of trials using FMT in the context of treatment with immune checkpoint blockade.

Probiotics. The concept of administration of microbial consortia to improve human health is likewise not new, as efforts have been underway to modulate the gut microbiota since the early 1900s when Metchnikoff (a Nobel laureate for his work in immunity) theorized that administration of microbes could have beneficial effects[98]. The term 'probiotic' refers to bacteria or a combination of live bacteria that "confer a health benefit to hosts when consumed in adequate amounts"[99]. An entire industry now focuses on this effort—with sales in the United States totaling $6.8 billion in 2018 and expected to rise to an even more lucrative $12.8 billion by 2026 (http://www.TransparencyMarketResearch.com/).

While probiotics that are used as therapeutics fall under Food and Drug Administration (FDA) regulations, those deemed to be dietary supplements (which is the overwhelming majority) are not subjected to the rigorous FDA review process before being marketed[100]. This begets complexity for patients and healthcare providers, as the potential health claims of these less-regulated consumables may lack robust scientific evidence. The composition of commercially available probiotics has been inadequately studied, though we do know that the content may vary significantly from what is advertised[101,102] and that their impact on the flora of the gut microbiota and overall health may be quite limited[103]. Some commercially available probiotics have also been studied in preclinical models and in a number of clinical trials, with varied results. Many of these have been in the setting of CRC carcinogenesis owing to the evidence implicating the gut microbiota in CRC development and progression. Though some of these studies revealed potentially positive effects of probiotics[104,105], other studies, in some situations using the same probiotic, uncovered a deleterious effect, with increased tumor penetrance and multiplicity[106]. Theoretical explanations for the difference in effect included timing of probiotic administration among other variables.

The impact of administration of probiotics has been studied in several clinical trials in patients with cancer, though most of these

Table 1 | Selected clinical trials modulating the gut microbiome in cancer therapy

NCT number	Malignancy	*n*	Objective	Intervention	Outcome measure(s)	Location
Modulation of the gut microbiome in cancer and cancer therapy						
NCT03341143	Melanoma	20	To study concurrent use of FMT and pembrolizumab in patients with PD-1-resistant melanoma	FMT (donor responder to PD-1 therapy) with pembrolizumab	ORR, change in T cell composition and function; change in innate and adaptive immune subsets	USA
NCT03353402	Melanoma	40	To study use of FMT in patients with stage IV metastatic melanoma for whom immunotherapy failed	FMT (colonoscopy and capsules) (donor responder to immunotherapy)	Incidence of FMT-related adverse events, engraftment, changes in composition of immune cell population and activity	Israel
NCT03358511	Breast cancer	20	To assess the efficacy of presurgical antibiotics to influence antitumor immune function	Primal Defense ULTRA Probiotic Formula	Mean number of cytotoxic $CD8^+$ T cells	USA
NCT00936572	Colorectal cancer[139]	35	To investigate the effect of probiotics on gut microflora and the immune and inflammatory response	Probiotics (La1, BB536)	To perform morphological and microbiological evaluation of the colonic microflora, GI function	Italy
NCT03290651	Breast cancer	40	To determine if oral antibiotics can change the breast flora	Probiotic Natural Health Product — RepHresh Pro-B	Change in breast microbiota, inflammatory markers	Canada
NCT03072641	Colon cancer[107]	20	To reactivate the tumor-suppressor genes using probiotics	ProBion Clinica (*Bifidobacterium lactis*, *L. acidophilus*)	Changes in microbiota composition and DNA methylation	Sweden
NCT01609660	Colorectal cancer	33	To assess the impact of probiotics on patients undergoing colorectal resections	*Saccharomyces boulardii*	To measure mucosal cytokine and SCFA, postoperative complication and hospital LOS	Brazil
Modulation of the gut microbiome to prevent cancer treatment-related toxicities						
NCT00197873	Colorectal cancer	84	To prevent chemotherapy-induced diarrhea	*L. rhamnosus* supplementation	Effect on treatment related toxicity other than diarrhea	Finland
NCT02928523	Acute myeloid leukemia[140]	20	To use FMT to prevent complications associated with dysbiosis in patients undergoing intensive treatment	Auto-FMT	Dysbiosis correction, eradication of multidrug resistant bacteria, definition of dysbiosis biosignature	France
NCT02269150	Malignancies requiring allo-HSCT	59	To assess the utility of FMT in prevention of CDI in patients who underwent allo-HSCT	Auto-FMT	CDI	USA
NCT01410955	Colorectal cancer[141]	46	To prevent irinotecan-induced diarrhea by probiotics	Probiotic formula Colon Dophilus	Prevention of grade 3–4 diarrhea by probiotics in patients treated by irinotecan based chemotherapy	Slovakia
NCT01479907	Colorectal cancer[142]	100	To develop synbiotics to improve quality of life following colectomy	Synbiotic Forte: lactic acid bacteria including *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *L. paracasei*, *and L. plantarum*, and fermentable fibers.	To assess gastrointestinal-function-related quality of life postoperatively	Greece
NCT02021253	Hepatocellular carcinoma	64	To assess the role of probiotics in preventing septic and liver functional complications related to bacterial translocation following surgical resection of HCC	Probiotics — Lactibiane Tolerance (*Bifidobacterium lactis*, *L. acidophilus*, *L. plantarum*, *L. salivarius*, *Bifidobacterium lactis*)	Area under the plasma concentration versus time curve of endotoxin circulating levels	France

Continued

Table 1 | List of selected recent and ongoing clinical trials utilizing strategies to modulate the gut microbiome in cancer and in preventing toxicities related to cancer treatment (continued)

NCT number	Malignancy	*n*	Objective	Intervention	Outcome measure(s)	Location
Modulation of the gut microbiome to prevent cancer treatment-related toxicities						
NCT02944617	Renal cell cancer	20	To prevent diarrhea in patients treated with sunitinib by probiotics	Micronutrient-fortified probiotic yogurt (experimental)	Change in levels of *Bifidobacterium spp.* assessed in stool samples	USA
NCT01790035	GI neoplasms[143]	23	To prevent chemoradiation induced toxicity by probiotics	*L. rhamnosus* GG	Efficacy, safety, diarrhea subscale score	USA
NCT02351089	Gynecologic cancer	200	To assess the efficacy of probiotics in preventing GI toxicity associated with irradiation of gynecologic cancer	Capsules probiotic powder and corn starch	Change in incidence of loose/watery stools	Sweden
NCT03552458	Head-and-neck cancer	50	To assess the role of probiotics in preventing oral mucositis	*Lactobacillus Reuteri* Oral Solution (BioGaia)	Oral mucositis severity, oral bacterial genetic and transcriptional analysis	Singapore
NCT01579591	Rectal cancer	160	To access the role of VSL#3 in reducing acute bowel toxicity	VSL#3	Reduction of acute bowel toxicity	Italy
NCT02771470	Lung cancer[144]	41	To assess the effects of chemotherapy on microbiome and probiotics on chemotoxicity	*Clostridium butyricum*	Composition of microbiome with probiotics, adverse effects of chemo, change in immunity and nutrition index	China
NCT01723592	Breast cancer	27	To improve the quality of the vaginal flora by probiotics	*L. rhamnosus*, *L. jensenii*, *L. crispatus*, *L. gasseri*	Isolation of specific *Lactobacilli* from vagina	Austria

n is the number of patients in the trial. Auto-FMT, Autologous FMT; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; ORR, overall response rate.

studies have focused on assessment of changes in microbiota composition and have not specifically assessed differences in outcomes to cancer therapy (Table 1). Administration of probiotics to patients with CRC can alter the gut microbiota—with an increased abundance of butyrate-producing microbes in mucosal and fecal samples following probiotic administration[107] (NCT03072641). Probiotic administration is also being studied in patients with operable breast cancer before surgery (NCT03358511), with similar plans to assess changes in the microbiota, as well as changes within the tumor microenvironment—focusing on the density of $CD8^+$ T lymphocytes within the tumor microenvironment after a short course of probiotic therapy.

There are some reports that suggest that these compositional changes in the gut microbiota or tumor microenvironment could affect patient outcomes. In patients with superficial bladder cancer who underwent transurethral resection followed by intravesical administration of epirubicin, administration of an oral *Lactobacillus casei* preparation for a year after completing therapy was associated with an improvement in recurrence-free survivalbut no difference in overall survival compared with the control group[108].

In addition to focusing on the impact of probiotics on cancer development and response to treatment, clinical trials are also focusing on the impact of probiotics on treatment-related toxicity (Table 1)[84,109]. Though some studies show success—with decreased diarrhea in patients with colon cancer receiving 5-FU (fluorouracil) with coadmininistration of *Lactobacillus rhamnosus*[110] and improvement of oral mucositis in a patient with head and neck cancer who concurrently received chemotherapy and probiotics[111]—it is clear that additional studies are needed and probiotic formulations must be carefully considered.

Next-generation biotherapeutics and designer microbial consortia. Given the growing evidence regarding the role of microbiota in health and disease, efforts are now underway to develop next-generation biotherapeutics involving single or multistrain bacterial consortia, with strong scientific rationale and evidence regarding their efficacy. Numerous biotech companies are now focusing on this effort, as are branches within larger pharmaceutical companies, with interest and enthusiasm for working with authorities like the FDA, given the therapeutic intent of these compounds. This is in contrast to over-the-counter probiotic formulations, which are often considered to be supplements or 'functional foods' falling under the domain of the European Food Safety Authority (EFSA) in Europe and the Dietary Health and Supplement Education Act (DHSEA) in the United States and are not as tightly regulated.

Initial success in treating CDI and inflammatory bowel disease (IBD) has led to hope that these next-generation biotherapeutics may become the method of choice for modulating the microbiota. Early studies have demonstrated some success[112,113] as well as some limitations[114]. Significant insights have been gained through analysis of longitudinal samples in these carefully planned clinical trials, such as optimal dosing, engraftment, and participant recruitment. These insights have informed subsequent trials to treat IBD[115], with promising early results.

As there are numerous publications now substantiating the presence of microbial signatures within the gut microbiome of patients

responding to immune checkpoint blockade[9–12], efforts are now underway to design an 'optimal' microbial consortia to augment therapeutic responses to these agents. This effort has been met with some complexities as there is modest overlap in the bacterial taxa associated with response in the studies carried out thus far. Several factors likely contribute to this, including differences in sequencing techniques and pipelines used for analysis, differences between the patient cohorts (such as different cancer types or being from geographically distinct areas with differences in diet and other environmental factors), as well as the purported importance for combined microbial function over phylogeny. This certainly highlights the need to standardize approaches for microbiome sequencing and analysis to facilitate comparisons between cohorts. Incorporation of metabolomic profiling is also important and should be considered in future analyses.

Diet, prebiotics, and postbiotics. The notion of food as medicine dates back to the famous Hippocrates quote "Let food be thy medicine and medicine be thy food," recorded around 431 BC, and the gut microbiota is thought to mediate many dietary benefits within the human body. Indeed, various microbial communities are intimately involved in every step of human digestion and nutrient extraction, with the gut microbiota playing the largest role[116]. The nutritional availability of food is altered by the gut microbiota, which releases a number of nutrients that the human body is incapable of digesting[117]. In addition to this, diet can impact the composition of the gut microbiota (not only bacteria, but also viruses, fungi, protozoa, and bacteriophages) as well as their transciptomic and metabolomic profiles[118,119].

Intense dietary changes can cause detectable changes in the gut microbiome community structure in a relatively short timeframe[120]. Several examples of the influence of dietary modification on specific gut microbiota composition exist, with elimination of animal fat associated with a decrease in Bacteroidales[121] and a high-fiber diet associated with an increase short-chain fatty acid (SCFA)-producing bacteria[122,123]. Such changes have also been shown to influence immune responses in mice and metabolism in humans[123]. Given these findings, dietary influences may ultimately be considered in the effects of cancer therapies[118,124,125].

Beyond diet, prebiotics and postbiotics may also be used to modulate gut microbiota. Prebiotics consist of specific chemicals that promote the growth of a selective group of bacteria and thereby a diverse and 'healthy' microbiota. These include substances such as fructans (including oligofructose and inulin), which have been demonstrated to selectively stimulate growth of specific bacterial taxa and alter SCFA levels within the gut[84,126]. These agents have been incorporated into the diets of mice and shown to augment the effects of multiple common chemotherapeutic agents as well as radiotherapy in murine models[126]. Postbiotics have also been studied, with a focus mostly on downstream products, such as SCFAs like buyrate that have been studied in murine models, as a component of high-fiber diets or administered in drinking water for the prevention of CRC[127]. However, studies with a large number of patients are lacking, and it is clear that such approaches will need to be carefully studied and may need to be combined with administration of microbiota to enhance their potential.

Targeted modulation. Though use of broad-spectrum antibiotics has been associated with profound changes in the gut microbiota and worse outcomes in some studies[61,128], approaches that are better-targeted to modulate gut and other microbiota using targeted antibiotics and/or bacteriophages to impact human health and disease may be useful.

Perhaps the most striking recent data regarding the deleterious effects of antibiotics on treatment responses is a study in which it was demonstrated that patients with metastatic renal cell carcinoma (RCC) or non-small-cell lung cancer (NSCLC) had significantly worse survival outcomes if they received antibiotics just before or just after initiation of treatment with immune checkpoint blockade. Additional data regarding the deleterious effects of antibiotics also exist for hematologic malignancies, with a study demonstrating that patients who received anti-Gram-positive antibiotics in the context of treatment with cyclophosphamide for chronic lymphocytic leukemia (CLL) or cisplatin for relapsed lymphoma had a lower overall response rate, earlier progression, and reduced overall survival[129]. Though indiscriminate use of broad-spectrum antibiotics may be deleterious, 'tailored antibiotic therapy' to selectively deplete select microbial communities in the setting of disease could potentially improve responses to therapy.

In addition to antibiotic approaches, bacteriophages are also now being explored for use in therapeutic strategies to modulate microbiota to treat disease. Bacteriophages are viruses that infect bacteria and are the most abundant and diverse members of the gut virobiota[130], and they can be used to kill bacteria in a very selected fashion. In preclinical models, bacteriophages have been shown to have equal efficacy to antibiotics in targeting specific bacterial taxa with less disruption of commensal, nontargeted bacteria[131]. Interestingly, recent studies have shown that bacteriophages may actually shape the gut microbiota in other ways as well, and may, in part, contribute to the efficacy of FMT for CDI[132]. However, further studies are needed to understand the mechanism of action and possible development of resistance, safety, and product preparation of these agents as well as potential effects on the normal flora and the immune system[133].

Targeting the tumor microbiota. In addition to modulating the gut microbiota to impact cancer therapy, efforts are now underway to target the tumor microbiota to impede cancer progression and enhance responses to cancer therapy. The rationale for this is well-founded, as several studies have now demonstrated a deleterious effect of intratumoral bacteria on therapeutic response in the context of CRC and pancreatic cancer[13,77,86]. Importantly, bacterial ablation in these studies via antibiotic use was associated with improved responses to chemotherapy as well as immune checkpoint blockade, and clinical trials are now being designed to target these bacteria in combination with conventional cancer therapy. Nonetheless, complexities exist with such approaches, as the impact of systemic antibiotic administration on the gut microbiota must also be taken into consideration.

An alternative, creative strategy to target the tumor microbiota takes advantage of the predilection of some bacteria to home to tumors and utilizes bioengineering techniques to enable these bacteria to either directly kill the tumor cells or to produce an immune microenvironment conducive to antitumor immune responses. In this regard, attenuated *Salmonella* strains designed to express the TLR5 ligand flagellin derived from *Vibrio* incited an immune response that facilitated antitumor immune activity against orthotopic human CRC[87] lines in mice. Several highly attenuated strains of *Salmonella* in phase I human studies have been shown to home to melanoma and RCC tumors[134]. In these studies, there was little to no efficacy with regards to the effect on cancer growth, perhaps secondary to the degree of attenuation. Furthermore, there remains some concern in using an infectious agent as a therapeutic modality in patients.

A more developed understanding of the full range of bacteria within a given tumor type as well as of the specific enzymatic and cellular activities of each bacterium is far from complete, but, in the future, will likely enable basic and translational scientists to further impact the therapeutic effects of known anticancer therapies and potentially allow for the development of novel anticancer agents.

Future directions for microbiota in clinical care. It is becoming increasingly clear that commensal microbiota play a major role in overall health by contributing to overall immunity[135] and that disruptions in the microbiota may contribute to various disease states and their treatment—including cancer[6]. Additionally, evidence now suggests that these microorganisms may confer susceptibility to certain cancers, either through a direct effect by local presence within the tumor microenvironment or via the systemic impact of distant microbiota (such as the gut and the skin). The latter is particularly relevant to the ability of gut microbiota to moderate the response to and potentially affect the toxicity of both traditional chemotherapeutic agents as well as immunotherapy—ultimately affecting patient outcomes. Interestingly, this ecosystem is both protean and inherently modifiable, offering the potential for practical application of these findings.

Furthermore, external forces (such as diet, antigen exposure, medications, and stress) are also important in contributing to states of health or disease through their interaction with factors inherent to the host; a significant proportion of these impact the microbiota, leading to the hypothesis that the microbiota may be a common link. Notably, there are also recent, unexpected findings that suggest that our understanding of the effects of the human microbiota at this time is far from complete; for example, there is extensive crosstalk between the gut microbiota and the nervous and endocrine systems with bidirectional feedback[136–138].

This field is young, and we are left with many unanswered questions—especially regarding the mechanism of action as well as the exact bacterial species or group of bacterial species that are most important in mediating antitumor effects and overall health. There is tremendous opportunity for research at every level, from basic and translational research to clinical studies and epidemiological analyses, to cooperatively advance our understanding of this complicated ecosystem. However, as we move forward in treating cancer and other diseases, it is important even now that we take all these factors into account—from a prognostic as well as therapeutic angle. Multifaceted strategies will need to be devised to monitor and to modulate these factors to optimize health and to effectively treat disease. It is only through such an approach that we may realize the full potential of precision medicine.

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Competing interests

J.A.W. and V.G. are inventors on a US patent application (PCT/US17/53,717), submitted by The University of Texas MD Anderson Cancer Center, that covers methods to enhance checkpoint blockade therapy by the microbiome. J.A.W. reports compensation for speaker's bureau and honoraria from Imedex, Dava Oncology, Omniprex, Illumina, Gilead, MedImmune and Bristol-Myers Squibb. J.A.W. serves as a consultant / advisory board member for Roche/Genentech, Novartis, AstraZeneca, GlaxoSmithKline, Bristol-Myers Squibb, Merck, Biothera Pharmaceuticals and Microbiome DX. J.A.W. also receives research support from GlaxoSmithKline, Roche/Genentech, Bristol-Myers Squibb, and Novartis. A.C-H. and M.A.W.K. report no relevant conflicts of interest or financial disclosures. B.A.H. is supported by National Institutes of Health T32 CA 009599 and the MD Anderson Cancer Center support grant P30 CA016672.

Additional information

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