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The Potential of the Gut Microbiome to Reshape the Cancer Therapy Paradigm
A Review

Longsha Liu, BA; Khalid Shah, MS, PhD

IMPORTANCE The gut microbiome, home to the vast kingdom of diverse commensal bacteria and other microorganisms residing within the gut, was once thought to only have roles primarily centered on digestive functions. However, recent advances in sequencing technology have elucidated intricate roles of the gut microbiome in cancer development and efficacy of therapeutic response that need to be comprehensively addressed from a clinically translational angle.

OBSERVATIONS This review aims to highlight the current understanding of the association of the gut microbiome with the therapeutic response to immunotherapy, chemotherapy, radiotherapy, cancer surgery, and more, while also contextualizing possible synergistic strategies with the microbiome for tackling some of the most challenging tumors. It also provides insights on contemporary methods that target the microbiota and the current progression of findings being translated from bench to bedside.

CONCLUSIONS AND RELEVANCE Ultimately, the importance of gut bacteria in cancer therapy cannot be overstated in its potential for ushering in a new era of cancer treatments. With the understanding that the microbiome may play critical roles in the tumor microenvironment, holistic approaches that integrate microbiome-modulating treatments with biological, immune, cell-based, and surgical cancer therapies should be explored.

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Author Affiliations: Center for Stem Cell and Translational Immunotherapy (CSTI), Harvard Medical School, Boston, Massachusetts (Liu, Shah); Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts (Liu, Shah); Harvard Stem Cell Institute, Harvard University, Cambridge, Massachusetts (Shah).

Corresponding Author: Khalid Shah, MS, PhD, Center for Stem Cell and Translational Immunotherapy (CSTI), Harvard Medical School, Boston, MA 02115 (kshah@bwh.harvard.edu).

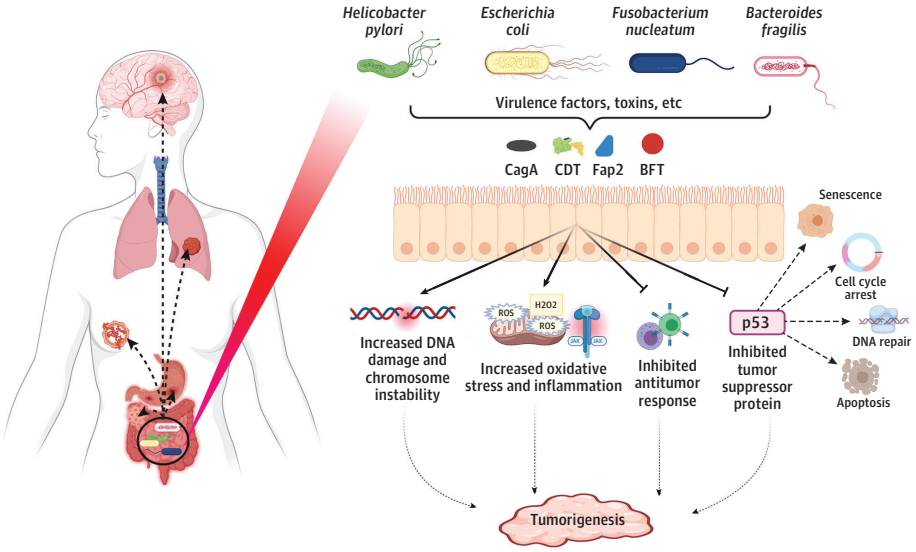
The human microbiota is a vast collection of bacteria, archaea, fungi, and viruses that exhibit enormous intrapopulation variation among individuals. It was long believed that the role of the gut microbiota was limited predominantly to aiding in food digestion and nutrient acquisition; however, with the advent of novel molecular techniques (ie, 16S ribosomal RNA [rRNA] sequencing, DNA sequencing, and metagenomics) and the use of gnotobiotic mice, larger roles of the gut microbiota in systemic homeostasis were able to be unraveled. For one, the microbiome contributes substantially to the balance of circulating nutrient and metabolite levels via production or transformation of neurotransmitters, short-chain fatty acids (SCFAs), amino acids, and secondary bile acids that can either be absorbed or reabsorbed by the host to then enter circulation.[1] Moreover, the microbiota plays an important role in the training and development of the immune system. Certain commensal bacterial species, for example, are able to induce distinct immune cell populations to polarize and play either proinflammatory or anti-inflammatory roles,[2-4] with some ultimately leading to immune suppression.[5] Consequently, there is an increasing need to contextualize the microbiota's importance in cancer development, diagnosis, and remediation.

Gut Microbiome Dysbiosis and Cancer Development

When the delicate balance of bacteria residing in the gut becomes disrupted, a less diverse, less stable, and often more pathogenic microbiota results, contributing to various disease pathologies by negatively influencing either host metabolism or host immune responses and functionalities.[6] Notably, dysbiosis of the gut microbiome is increasingly being associated with tumorigenesis (**Figure 1**). Although associative studies comprise a substantial portion of current cancer microbiome studies, causative roles have been strongly supported through emerging evidence from *Helicobacter pylori*. *H pylori* infections and the subsequent induced development of gastritis have been strongly linked and considered a precursor of cancer.[7] In gastric epithelial cells, *H pylori* induces the degradation of p53, a tumor suppression protein, and subsequently leads to the formation of gastric cancer; furthermore, the CagA protein derived from *H pylori* interacts with epithelial E-cadherin, promoting cell proliferation with an increased possibility of cancerogenic cell transformation.[8] Notably, *H pylori* has recently been classified as a class I carcinogen.[9]

H pylori aside, several other bacterial species are now known to play oncogenic roles. Toxins released by bacterial pathogens, such as colibactin and cytolethal distending toxin from *Escherichia coli*, possess DNase activity that contributes to genomic instability that ultimately could lead to tumor formation and progression.[10,11] Alternatively, pathogenic bacteria such as *Shigella flexneri* and *Bacteroides fragilis* could lead to accumulation of DNA damage and genomic variations via host DNA damage response interference or oxidative stress generation via spermine oxidase activity, respectively.[12-14] Moreover, certain species can even assist cancer

Figure 1. Microbiota in Cancer Development



Dysbiosis of the gut microbiome is increasingly being associated with the development of tumors both near and far away from the gut. Microbial pathogens such as *H pylori, E coli, F nucleatum,* and *B fragilis* are now known to be implicated in tumorigenesis via released virulence factors and toxins that increase chances of DNA damage and chromosome instability, promote oxidative stress and inflammation, and suppress immune responses.

genesis via inhibiting host antitumor immune response; in the case of *Fusobacterium nucleatum*, host natural killer (NK) cells are inhibited via the bacterial virulence factor Fap2 that arrests the ability of NK cells to attack tumor cells.[15,16]

Although the preponderance of information relates to gastric and colorectal cancer, gut bacteria have also been associated with other cancers. *Salmonella typhi* and *Helicobacter* species are known to be implicated in the development of biliary cancer,[17-19] and it is hypothesized that gut microbiota may also influence the development of liver and breast cancer. The liver is uniquely exposed to communications of gut bacteria, metabolites, and by-products through the portal venous system.[20] Through microbial-induced transformation of primary bile acids to secondary bile acids, there could be DNA damage, hepatotoxicity, altered concentration of NK T cells, and carcinogenesis.[20,21] In the case of breast cancer, it is speculated that steroid metabolism may be associated with gut bacteria, causing changes in profiles of phytoestrogens and estrogens, which may play a role in fighting cancer.[22] Brain tumors represent another emerging field speculated to be influenced by the microbiota as more becomes known about the microbiota–gut–brain axis.[23]

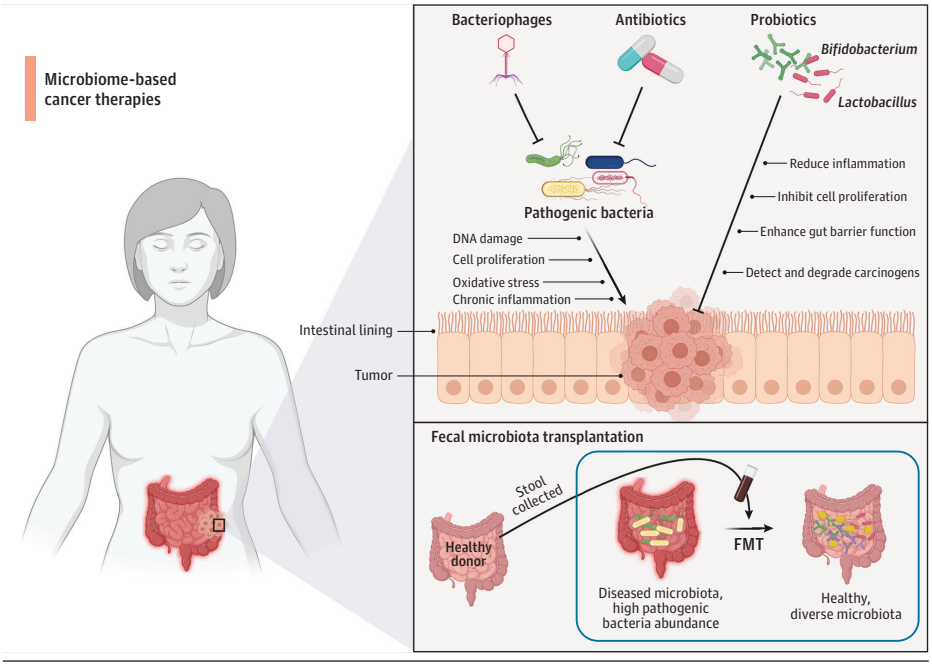
An Emerging Role: Gut Microbiota and Immunotherapy

Treatment of various cancers using immune checkpoint blockade (ICB) therapy has considerably advanced with the targeting of programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4).[24] However, although promising, these novel ICB strategies have considerable interindividual variation, and the effect on treatment of tumors has been inconsistent among tumor types.[25,26] As such, there is a critical need for a predictive biomarker for response and a better understanding of why such heterogeneity exists in order to boost treatment efficacy and expand the respondent population.

Interestingly, several studies have noted important differences in microbial diversity and composition between fecal samples of responders and nonresponders for ICB therapy,[27,28] suggesting that variation in clinical response could be attributed to the gut microbiome. For example, a clinical study noted a significant positive correlation between administration of *Faecalibacterium* and progression-free survival; patients with higher abundance of *Faecalibacterium* had higher infiltration of cytotoxic $CD8^+$ T cells in the tumor bed and other preexisting antitumorigenic immune responses.[29,30] Although the separation of correlation from causation is still unclear, ICB therapy has been notably less successful in germ-free and antibiotic-treated mice, impairing efficacy of PD-1 blockade therapy and overall survival time in patients with epithelial cancer.[31] Moreover, manipulation of gnotobiotic mice with certain bacterial taxa and transplantation of fecal materials from responders have been found to enhance therapeutic response to ICB.[30,32]

Though gradual, new studies are emerging that experimentally demonstrate the therapeutic efficacy of microbiome modulation in attenuating ICB therapies (**Figure 2**). Certain live bacterial species, or probiotics, are known to play key roles in cancer remediation, including influencing anti-inflammatory cytokine levels, detecting and degrading potential carcinogens, activating phagocytes for eliminating early-stage cancer cells, and producing SCFAs that affect cell death and proliferation.[33] *Bifidobacterium* and *Lactobacillus*, in particular, have been associated with reduced incidence of cancer and are known to induce other health benefits as well owing to their known roles in immunomodulation.[34] One study, conducted in germ-free mice, identified *Bacteroides thetaiotaomicron* and *B fragilis* in enhancing CTLA-4 blockade therapy efficacy via elevated IL-12–dependent Th1 immune responses.[29,35] *Bifidobacterium* administration has also been shown to aid in response via antitumor roles through stimulating cytotoxic $CD8^+$ T cells, inducing maturation of dendritic cells, recruiting other immune cells, and activating type I interferon (IFN) signaling.[28] Lastly, oral administration of *Lactobacillus rhamnosus* GG was experimentally noted to augment the antitumor activity of PD-1 immunotherapy also through inducing type I IFN production in dendritic cells and shifting the gut composition to an enrichment of both *Lactobacillus murinus* and *Bacteroides uniformis*, 2 well-known species that increase tumor-infiltrating den-

Figure 2. Microbiome-Based Cancer Therapies



Moving forward, it will be important to note that there are numerous ways to modulate the gut microbiome for cancer therapy. Most broadly, an entire diseased gut microbiota could be replaced via fecal microbiota transplantation (FMT) from a healthy donor. Strategies such as antibiotics and bacteriophages could also be leveraged to remove pathogenic bacteria from the gut that otherwise promote DNA damage, cell proliferation, oxidative stress, and chronic inflammation. Alternatively, certain bacteria strains can be orally consumed via diet or probiotics that have beneficial effects on host physiology, such as reducing inflammation, inhibiting cell proliferation, enhancing gut barrier function, and detecting and degrading carcinogens. Notably, 2 probiotic strains actively being used in many preclinical and clinical studies are *Bifidobacterium* and *Lactobacillus*.

dritic cells and T cells.[36] Importantly, that same study also mechanistically characterized how *L rhamnosus* GG was able to trigger IFN production in dendritic cells through simulating the cGAS/STING/TBK1/IFN-regulatory-factor-7–dependent signal pathway.

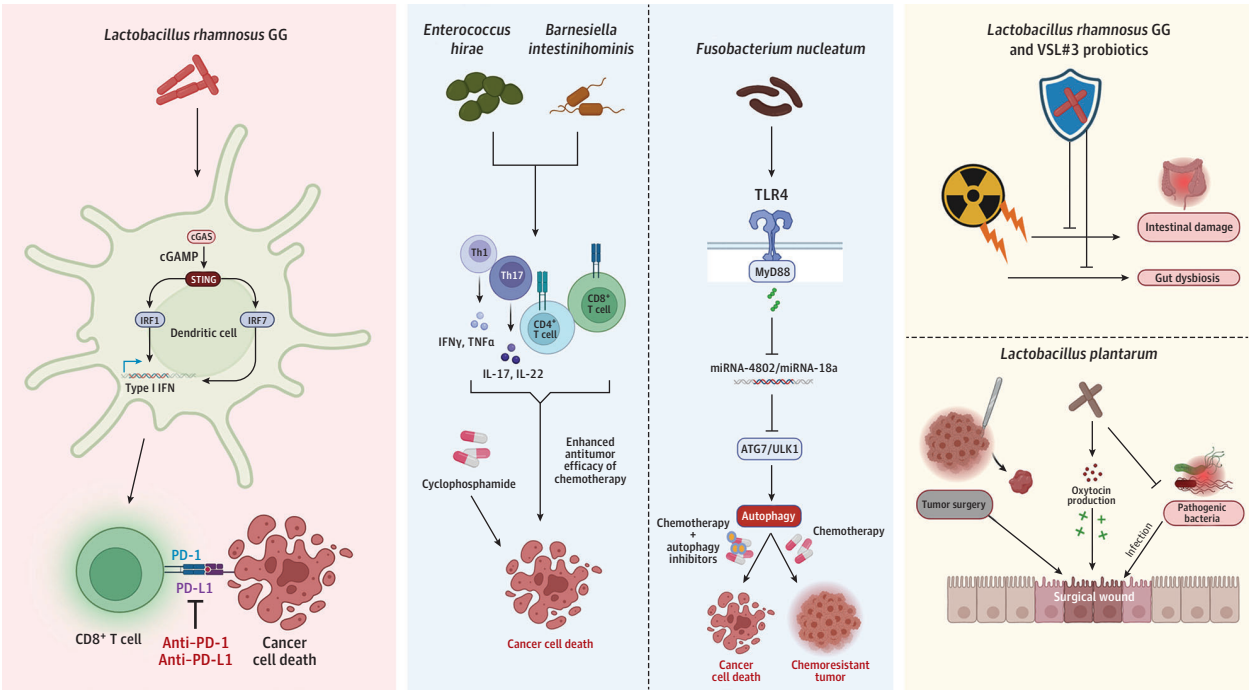
Targeting gut populations through antibiotic medications and bacteriophages also bears additional attention for immunotherapies. Vancomycin, for example, enhances the efficacy of CTLA-4 blockade therapy via decreasing harmful gram-positive bacteria while not affecting gram-negative *Burkholderiales* and *Bacteroidales*.[29] However, owing to imprecise targeting of bacterial strains of similar types, antibiotic medications may, in some cases, do more harm than good through reducing bacterial diversity. One study, for example, found that coupled antibiotic administration with immunotherapy resulted in shorter progression-free survival and overall survival in patients with cancer.[32,37] Thus, in lieu of antibiotics, bacteriophages can be designed to specifically target only detrimental, pathogenic bacteria.[38] For example, a study identified anticolorectal cancer modification of the tumor microenvironment by a bioinorganic hybrid bacteriophage that targeted and killed *Fusobacterium nucleatum*, which is known to increase immunosuppressive myeloid-derived suppressor cells in the tumor microenvironment. The combination of *Fusobacterium nucleatum*–binding M13 phage with silver nanoparticles,[39] which has superior antibacterial properties, led to a significant reduction in myeloid-derived suppressor cells as well as significantly prolonged overall survival time in mouse models when coupled with checkpoint inhibitors or chemotherapies.[40]

Overall, there is promise in leveraging the microbiome to augment the efficacy of immunotherapies. However, while we can conclude that antitumor immune responses are positively associated with a healthy and diverse microbiota, there is a notable disparity in identifying overlapping bacterial species across clinical cohorts. For example, in similar studies examining stool samples of patients with cancer who responded positively to PD-1 checkpoint blockade immunotherapy, 1 study identified an increased abundance of *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*,[41] while another noted a greater abundance of the *Ruminococcaceae* family,[30] and a third commented on greater abundance of *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii*.[42] There is a high likelihood that there are even more bacteria involved in this process that will vary among different cancers and gut ecology. As such, there is a need to understand such disparity in taxa and search for a common functional output and the metabolites that are produced. A recent study provided evidence supporting this point, showing how SCFAs produced by a variety of gut bacteria can promote the memory potential of antigen-activated $CD8^+$ T cells.[43] Moreover, the secondary effects of SCFAs vary depending on environmental and host-specific factors, providing a plethora of cascading phenotypes and effects on the immune system. Taking all these factors into consideration, immunotherapies may need to be redesigned to incorporate a microbiome-modulating therapeutic element, such as supplementation of a probiotic or microbial-derived metabolite, as a standardized approach to minimize heterogeneity of response and boost antitumor immune efficacy.

Gut Microbiota and Chemotherapy

The microbiota has also been implicated in mediating therapeutic response to chemotherapeutic compounds. Cyclophosphamide, an immunostimulatory alkylating agent for chemotherapy, has been noted, for example, to have attenuated antitumor efficacy in germ-free or antibiotic-treated mice owing to lack of relevant memory Th1 and pathogenic Th17 immune responses.[44] Interestingly, efficacy can be restored by administration of *Enterococcus* and *Barnesiella*, which are necessary and sufficient to mount effective immune responses, including induction of Th1, Th17, and tumor-specific $CD4^+$ and $CD8^+$ T cells.[45] Some bacteria species, such as *Fusobacterium*

Figure 3. Microbiome in Immunotherapy, Chemotherapy, and Posttherapeutic Toxicity



The microbiota is implicated in numerous frontline cancer therapies as well as their associated posttherapeutic toxicity. A, Immunotherapy: administration of the probiotic *Lactobacillus rhamnosus* GG triggers interferon (IFN) type I production in dendritic cells through activating the cGAS/STING/TBK1/IFN-regulatory-factor-7–dependent signal pathway; this ultimately enhances the antitumor activity of programmed cell death protein 1 (PD-1) immunotherapy. B, Chemotherapy: administration of *Enterococcus hirae* and *Barnesiella intestinihominis* induces the production of Th1 and Th17 and activates tumor-specific $CD4^+$ and $CD8^+$ T cells that, together, enhance the efficacy of cyclophosphamide. Moreover, knowledge of how *Fusobacterium nucleatum* activates autophagy via a TLR4-MY88 signal pathway that consequently results in chemoresistance can be leveraged to enhance therapeutic response via coupling chemotherapies with autophagy inhibitors. C, Posttherapeutic toxicity: Administration of probiotics such as *Lactobacillus rhamnosus* GG and VSL#3 formulation (consisting of *Streptococcus*, *Lactobacillus*, *and Bifidobacterium* species) can protect intestinal mucosa against radiotherapy-induced toxicity. Moreover, administration of *Lactobacillus plantarum* can promote wound healing following tumor surgery via promoting oxytocin secretion that modulates the gut–brain–skin axis and also prevent surgical-site colonization of pathogens commonly associated with the development of surgical-site infections. cGAMP, cyclic guanosine monophosphate–adenosine monophosphate; cGAS, cyclic guanosine monophosphate–adenosine monophosphate synthase; PD-L1, PD-1 ligand 1; STING, stimulator of IFN gene.

nucleatum, have even been recently found to promote chemoresistance to colorectal cancer through activating cancer autophagy. Specifically for *F nucleatum*, this was mechanistically done via the targeting of the TLR4-MYD88 immune signaling pathway that significantly downregulated levels of miRNA-4802 and miRNA-18a, which subsequently increased ATG7 and ULK1 expression, 2 autophagy signaling elements.[46] Consequently, knowledge of how *F nucleatum* induces autophagy can be leveraged to overcome chemoresistance. For instance, autophagy inhibitors such as chloroquine or 3-methyladenine[47] or *ATG7* gene knockdown/silencing can be used to prevent or reverse *F nucleatum*–mediated cell death.[48] Lastly, the microbiome is involved in attenuating posttherapeutic toxicity in chemotherapy, where some adverse effects of the compounds are so severe that they inhibit patients from receiving proper dosage or duration of treatment.[49] A primary example is irinotecan, a chemotherapeutic agent often used for treatment of colon cancer, which introduces an active chemotherapeutic agent SN38 that can cause serious diarrhea when excreted into the gastrointestinal tract.[49] This results in patients often needing to de-escalate or adjust dosages and coincides with a reduced abundance in *Bifidobacterium* and *Lactobacillus* species.[50] Interestingly, an intact microbiota is responsible for increasing SN38 levels through reactivating SN38 via secretion of β-glucuronidase enzymes after the liver glucuronidates SN38 into an inactive form. Preclinical experiments have indicated that more doses of irinotecan can be received with less gastrointestinal damage in germ-free mice than conventional mice with intact microbiota.[51] Using this knowledge, subsequent preclinical studies coadministered irinotecan with β-glucuronidase inhibitors in mice with healthy microbiota and noted a promising absence of irinotecan-induced diarrhea.[52] Moving forward, it will be important to further investigate this intersection of the microbiome with chemotherapy; therapeutic paradigms may evolve in coming years that incorporates the growing body of knowledge in microbiome-mediated mechanisms that either attenuate or enhance therapeutic response (**Figure 3**).

Gut Microbiota and Other Conventional Cancer Therapy–Induced Toxic Effects

It is crucial to consider how cancer therapy may also reciprocally affect the microbiome, leading to a cascade of complications; thus,

understanding the microbiome's composition at the time of therapy will be important in anticipating and finding countermeasures to posttreatment complications. One of the earliest pieces of evidence supporting the influence of the gut microbiota on cancer therapy response and toxicity was in allogeneic hematopoietic stem cell transplant (HSCT) for hematologic cancers. Clinical studies demonstrated that dysbiosis and loss of microbial diversity during and after HSCT correlated with shortened overall survival and higher transplant-related mortality rates compared with those with greater microbiota richness.[53] Further clinical studies on responders and survivors of HSCT linked higher abundance of the *Blautia* genus[54] with improved survival and higher abundance of *Eubacterium limosum* with reduced relapse risk.[55] *Blautia* was further found to be associated with reduced graft-vs-host disease lethality, a serious adverse effect of HSCT that is characterized by loss of microbial diversity, especially of health-promoting obligate anaerobes (eg, *Lactobacillus* and *Blautia*), and exacerbated by colonization of pathogenic strains such as *Clostridioides difficile*. Importantly, fecal microbiota transplantation (FMT) from a healthy donor may be an effective way to compensate for adverse effects associated with the loss of bacterial diversity and an increase in pathogenic species. Indeed, in a clinical trial of recipients of allogeneic HSCT experiencing posttransplant complications, FMT from healthy donors restored microbial diversity, significantly reduced diarrhea frequency and volume, completely regressed abdominal pain syndrome, and eradicated pathogenic impurities, including *C difficile* infection.[56] It is imperative to note, however, that the investigation of FMT for nondigestive system cancers is still in its infancy and that among healthy donors used for FMT, there is enormous heterogeneity in microbial diversity, therefore posing limitations on the therapy's use owing to lack of standardization.

Ionizing radiation therapy, another double-edged cancer therapy, is known to cause radiation-induced bystander effect and genomic instability.[57] Although there is a gaping paucity in the understanding of how the gut microbiota influences radiosensitivity, some clinically translational information has been elucidated in the recent years. For example, several studies have demonstrated how probiotics including *L rhamnosus* GG and VSL#3 formulation (consisting of *Streptococcus*, *Lactobacillus*, and *Bifidobacterium* species) can protect intestinal mucosa against radiotherapy-induced toxic effects.[58-60] In addition, for patients with head and neck cancer undergoing radiotherapy and chemotherapy, coupled oral administration of *Lactobacillus brevis* CD2 lozenges was found to enhance treatment efficacy and reduce risk for therapy-induced mucositis.[61] Meanwhile, a separate preclinical study showed how the use of the antibiotic vancomycin potentiates the antitumor activity of radiotherapy and halted tumor growth.[62] Interestingly, the same study noted that subsequent administration of butyrate, a metabolite produced by the depleted vancomycin-sensitive bacteria, reversed the antitumor effects, thereby reinforcing the importance of searching for common functional outputs and produced metabolites in future studies.

Lastly, cancer surgery, or surgical resection, is well known to have a host of postoperative complications; in colorectal cancer for instance, a substantial portion of patients often experience surgical-site infections (SSIs) or anastomotic leak (AL). Notably, several key perioperative interventions aimed toward minimizing the risk of fecal contamination during surgery, including antibacterial therapy and mechanical bowel preparation, greatly reduce microbial diversity, thus posing an inquiry whether the gut diversity or composition may be implicated in postoperative complications. Indeed, SSIs and ALs have been associated with lower microbiota biodiversity; in fact, a translational study went as far to suggest that an intestinal microbiome consisting of 60% or more of the mucin-degrading microbiome families, *Lachnospiraceae* and *Bacteroidaceae*, is a predictive indicator of AL.[63] Meanwhile, a preclinical study demonstrated that colonization of 2 collagenase-producing bacteria, *Serratia marcescens* and *Pseudomonas aeruginosa*, could induce AL in mice following colorectal anastomosis.[64] However, oral administration of local phosphate—which is known to control the virulence of bacteria—markedly decreased *S marcescens* and *P aeruginosa* colonization and collagenase activity at anastomotic tissues, thus subsequently preventing anastomotic abscess formation and leak.[64] Moreover, probiotics are also becoming increasingly well represented in influencing postsurgical outcomes. *Lactobacillus plantarum*, for example, has been shown to promote wound healing via promoting oxytocin secretion that modulates the gut–brain–skin axis and prevent surgical-site colonization of pathogens commonly associated with the development of SSIs.[65]

Overall, these findings support the potential of leveraging the gut microbiota to diminish adverse effects of therapy-induced toxicity from HSCT, radiotherapy, and cancer surgery. As the preponderance of information currently stems from colorectal cancer, future studies should aim to better characterize how translatable microbiota-targeting strategies are across numerous cancer types for remediating posttherapeutic toxic effects or complications.

Cancer Microbiome in Clinical Trials

There are many ongoing clinical trials translating findings from bench to bedside (**Table**).[66-76] The Preventing Toxicity in Renal Cancer Patients Treated With Immunotherapy Using Fecal Microbiota Transplantation (PERFORM) study,[73] for example, is one of several trials actively seeking ways to reduce or prevent posttherapeutic toxic effects. Some are even specifically combining different methods for targeting the gut microbiota in conjunction with immunotherapy, including microbial ecosystem therapeutics (a defined mixture of live intestinal bacterial cultures derived from the stool of a healthy donor),[70] FMT,[76] and drug-based metabolic modulators consisting of metformin, rosiglitazone, nivolumab, or pembrolizumab.[72] The multitude of clinical trials analyzing the role of the gut microbiota in cancer prognosis and treatment may revolutionize the future paradigm of cancer therapies.

Perspectives

There remains, however, numerous limitations and gaps that need to be addressed through further research. For instance, when identifying microbiome composition, there is considerable variation in analysis methodologies. Some studies may use 16S rRNA sequencing on stool, while others examine bile or saliva; consequently, it is difficult to identify consensus among studies because bacterial compositions likely differ across different sample types. Moreover, especially when considering 16S rRNA sequencing as the de facto

Table. Current Microbiome Cancer Clinical Trials

Trial ID	Study title	Cancer types	Interventions	Status
NCT03812705[66]	Fecal Microbiota Transplantation for Steroid Resistant/Dependent Acute GI GVHD	Hematopoietic and lymphoid cell neoplasm	Procedure: fecal microbial transplantation	Recruiting
NCT04264975[67]	Utilization of Microbiome as Biomarkers and Therapeutics in Immuno-oncology	Solid carcinoma	Procedure: fecal microbial transplantation	Recruiting
NCT04362826[68]	Study to Investigate Efficacy of a Novel Probiotic on the Bacteriome and Mycobiome of Breast Cancer	Breast cancer	Biological: novel probiotic	Not yet recruiting
NCT04552418[69]	Intestinal Microbiome Modification With Resistant Starch in Patients Treated With Dual Immune Checkpoint Inhibitors	Solid tumor	Dietary supplement: potato starch	Recruiting
NCT03686202[70]	Feasibility Study of Microbial Ecosystem Therapeutics (MET-4) to Evaluate Effects of Fecal Microbiome in Patients on Immunotherapy	All solid tumors	Biological: MET-4	Recruiting
NCT03870607[71]	Prebiotics and Probiotics During Definitive Treatment With Chemotherapy-Radiotherapy SCC of the Anal Canal (BISQUIT)	Anal cancer, squamous cell	Dietary supplement: prebiotics in combination with probiotics	Recruiting
NCT04114136[72]	Anti-PD-1 mAb Plus Metabolic Modulator in Solid Tumor Malignancies	Melanoma	Drug: nivolumab or pembrolizumab (dependent on approved indication)	Recruiting
		Renal cell carcinoma	Drug: metformin	
		NSCLC	Drug: rosiglitazone	
NCT04163289[73]	Preventing Toxicity in Renal Cancer Patients Treated With Immunotherapy Using Fecal Microbiota Transplantation	Renal cell carcinoma	Procedure: fecal microbiota transplantation	Recruiting
NCT04193904[74]	A Study of Live Biotherapeutic Product MRx0518 With Hypofractionated Radiation Therapy in Resectable Pancreatic Cancer	Pancreatic cancer	Drug: MRx0518 Radiation: hypofractionated preoperative radiation	Recruiting
NCT03817125[75]	Melanoma Checkpoint and Gut Microbiome Alteration With Microbiome Intervention	Metastatic melanoma	Drug: placebo for antibiotic	Active, not recruiting
			Drug: vancomycin pretreatment	
			Drug: nivolumab	
NCT03772899[76]	Fecal Microbial Transplantation in Combination With Immunotherapy in Melanoma Patients (MIMic)	Melanoma	Procedure: fecal microbial transplantation	Active, not recruiting

Abbreviations: GI, gastrointestinal; GVHD, graft-vs-host disease; mAb, monoclonal antibody; MET, microbial ecosystem therapeutics; NSCLC, non–small cell lung cancer; PD-1, programmed cell death protein 1; SCC, squamous cell carcinoma.

method for taxonomic identification of bacteria strains, there are resolution differences depending on which of the variable regions (V1-9) were measured.[77] A detailed comparative study examining resolution differences between the V2 to V3 and V3 to V4 16S rRNA regions noted, for example, that V2 toV3 analysis had significantly greater resolution of lower-rank taxa (species and genera), thus exemplifying the fact that the region selected for analysis has a considerable role in the precision and accuracy of analysis interpretation.[77] In addition, given that there is a wide variability of bacterial strains among different healthy individuals and a paucity in the functional understanding of many gut microbes—with much less known about the composition of an "ideal" bacteria consortia—caution must be exercised when using any probiotics in patients with cancer.[78] Moreover, even if efficacy is shown in preclinical models, the findings may not necessarily translate for efficacy in humans. For example, probiotic administration of *L rhamnosus* GG following HSCT did not noticeably alter the gut microbiome in clinical trials; moreover, significantly more patients in the probiotic group developed graft-vs-host disease compared with the control group.[79] As knowledge of each bacterial strain improves, so too will the impact and relevance of using probiotics as vectors to administer drugs.

It is further crucial to note that despite all the advancements in cancer therapy in the last few decades in immunotherapy, chemotherapy, radiotherapy, and surgery, most are still not effective in some of the more challenging primary and metastatic tumors. As such, alternative therapies need to be considered that can be effective for such cancers. For instance, dietary modulations, which considerably modify gut composition, hold notable potential in reshaping the therapeutic paradigm. Tumors, including those from the brain, are often highly dependent on fuel sources such as glucose and glutamine.[80] Taking this into consideration, a recent study combining the administration of a calorically restricted ketogenic diet with the glutamine antagonist 6-diazo-5-oxo-L-norleucine demonstrated an effective management of late-stage glioblastoma (GBM) by arresting tumor cell growth and promoting overall survival without toxicity through the therapeutic management of the 2 fuel sources via ketogenic diet for reducing glucose levels and 6-diazo-5-oxo-L-norleucine for targeting glutaminolysis.[81,82] Moreover, the study noted that beyond therapeutic killing of tumor cells, disease symptoms were also reversed, and the prevalence of edema, hemorrhage, and inflammation was reduced.[81] The application of ketogenic diets for patients with cancer has overall demonstrated great potential in facilitating nontoxic drug delivery to tumor sites with lower dosages necessary to achieve therapeutic effects, targeting multiple pathways linked to cancer development, and reshaping gut composition.[81,83]

With the understanding that the microbiome may play critical roles in the tumor microenvironment, holistic approaches that integrate both biological immune and cell-based cancer therapies with tumor resection surgeries should be further explored because the cure to cancer is likely not within a monotherapy but rather a cocktail of therapies. We have previously shown that cytoreduc-

tivesurgery of intracranial GBM tumors increased the prevalence of effector T cells in the tumor site, significantly boosting therapeutic efficacy of targeted on-site delivery of encapsulated gene-engineered stem cells.[84] More recently, we demonstrated how arginine deprivation alters the polarity of glioma-associated microglia into a proinflammatory phenotype that synergizes with radiotherapy to eradicate non–arginine auxotrophic GBM tumors.[85] Pertinently, we speculate the involvement of the gut microbiota in the arginine-deprivation framework, a conjecture that is supported by emerging evidence of bidirectional influence between the gut microbiota and arginine metabolism.[86] Thus, there remains many exciting possibilities to explore. For example, can the combination of cytoreductive surgery and immunotherapy with a healthy (eg, ketogenic) diet, probiotics, and metabolite/amino acid–altering therapy (ie, arginine deprivation) convey even better and more comprehensive antitumorigenic effects? While the answers to these synergistic approaches remain unclear for now, research is already under way to develop and characterize new treatment paradigms that simultaneously promote potent antitumorigenic effects on tumors, including those of the brain, while reducing systemic toxicity—one that effectively manages these universally fatal malignant neoplasms.

Conclusions

Overall, the importance of gut bacteria in cancer therapy cannot be overstated in its potential for ushering in a new era of cancer treatments. With the understanding that the microbiome may play critical roles in the tumor microenvironment, holistic approaches that integrate microbiome-modulating treatments with biological, immune, cell-based, and surgical cancer therapies should be explored.

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Critical revision of the manuscript for important intellectual content: All authors.
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