



Onco-biome in pharmacotherapy for lung cancer: a narrative review

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Background and Objective: The gut microbiota (GM) was recently recognized to play an important role in modulating systemic immune responses and is known to influence the effects or adverse events of immune checkpoint blockade (ICB) or carcinogenesis by crosstalk with regulators of cancer-related immunity, and this relationship is complex and multifactorial. Diversity in the gut microbiome and the abundance of specific bacterial species have been identified to be associated with better response and prognosis. Therefore, the purpose of the current interest in the gut microbiome is to enable modulation of the immune system in donor cancer patients by the administration of specific bacterial species and enabling their dominance. To understand this “terra incognita” is to uncover the role of the mechanisms underlying unknown organ functions, and this knowledge will lead to enhanced immunotherapy for lung cancer patients.

Methods: In this article, we summarized the literature on the relationship between the microbiome and lung cancer and the potential of the microbiome as a therapeutic target.

Key Content and Findings: This article is organized into the following sections: introduction, methods, microbiota and cancer development, microbiota and lung cancer treatment, future directions, and conclusion.

Conclusions: The gut microbiome is currently becoming the hallmark of cancer research and has an established and critical role in regulating antitumor immunity and the response to ICB in patients with lung cancers.

Keywords: Microbiome; lung cancer; immunotherapy; fecal microbiota transplantation (FMT); consortia

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Introduction

More than 100 trillion bacteria coexist in the gastrointestinal tract, and the flora contains 100 times as many genes as human genes (1,2). With the recent development of technology for metagenomic analysis and the refinement of sterile experimental animals, the composition of the flora and its role in immunity by cross-talk have been

elucidated. Recently, accumulating scientific evidence has shown that dysbiosis in the intestinal flora causes a variety of diseases, including inflammatory bowel disease, diabetes, liver cirrhosis, and reflux esophagitis (3). In addition, the microbiome, comprising the bacteria, archaea, fungi, and viruses that cohabit throughout the body, has been increasingly recognized for its important roles in multiple steps of cancer development or treatment in patients with

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Table 1 The search strategy summary

Items	Specification
Date of search	1, March 2022
Databases and other sources searched	PubMed
Search terms used	Cancer, microbiome
Timeframe	1979–2022
Inclusion and exclusion criteria	Inclusion criteria: English
Selection process	Akiko T. Tateishi and Yusuke Okuma independently conducted the selection; a consensus was obtained

cancer over the last five years. The “Hallmarks of Cancer” developed in 2022 details the crucial factors that should be the focus of future cancer research. In this review article, the discussion of the polymorphic microbiome that modulates the immune system in cancer patients expands the information detailed in “Hallmarks of Cancer” (4,5).

Lung cancer is the leading cause of cancer death worldwide, with 75,000 deaths annually, and half of the patients die within 1 year of diagnosis. Additionally, the 5-year survival rate in metastatic patients with lung cancer is less than 6% (6,7). In metastatic lung cancer patients, including small cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) patients, the crucial treatment axis is pharmacotherapy, such as cytotoxic chemotherapy, molecular targeted agents (MTAs), and immunotherapy. These therapies can be used as single agents or in combinations of each drug modality (8).

The most important agents of pharmacotherapy currently used are MTA and immune checkpoint inhibitors (ICIs). MTA has specific (or limited) efficacy for kinase inhibitors in patients with specific genetic alterations, including those in *EGFR*, *ALK* rearrangement, *ROS1* rearrangement, *BRAF*, *RET* rearrangement, *KRAS* G12C, and *MET* exon Δ14 skipping mutations (9-16), however, they are less effective with ICIs for those who do not have specific genetic alterations (17).

For SCLC, chemotherapy has long been the only treatment strategy, and for NSCLC, targeted therapies such as MTAs with gene mutations have been used. Therefore, the advent of ICIs has had a profound impact on lung cancer treatment. ICIs are characterized by the so-called “tail effect”, which is the long-term nature of the response observed once the patients do respond. The definitive biomarkers are programmed death-ligand 1 (PD-L1) expression on tumor cells or tumor mutation burden (18),

but they are not sufficient. In addition, the toxicities are characterized as immune-related adverse events (irAEs), which are autoimmune-like reactions that are not characteristics of cytotoxic chemotherapy or MTA (19,20). Currently, studies of the gut microbiome are gradually revealing its role in cancer immunity by the collective effects of all the microorganisms that reside in all surfaces of the human body. Optimizing the microbiota composition leads to the modulation of immunity to improve the treatment efficacy of immunotherapy as a therapeutic intervention.

In this narrative review, we focus on the current status of knowledge of the onco-microbiome by the exploration of research from bench to bedside, studies of microbiome-based lung cancer treatment, and future perspectives for clinical applications.

Methods

To obtain relevant literature, we searched Medline (via PubMed) using the keywords “cancer”, and “microbiome” from the inception of the database to March 2022. The search summary is provided in *Table 1*. Our search was restricted to publications in the English language. We retrieved other eligible studies by manual searching of the reference list of included studies. For a search of current trials, a search was performed at Clinical Trials.gov (<https://clinicaltrials.gov/>). We present the following article in accordance with the Narrative Review reporting checklist (available at <https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-299/rc>).

Microbiota and cancer development

Gut microbiota (GM)

The GM is known to have a close relationship with human

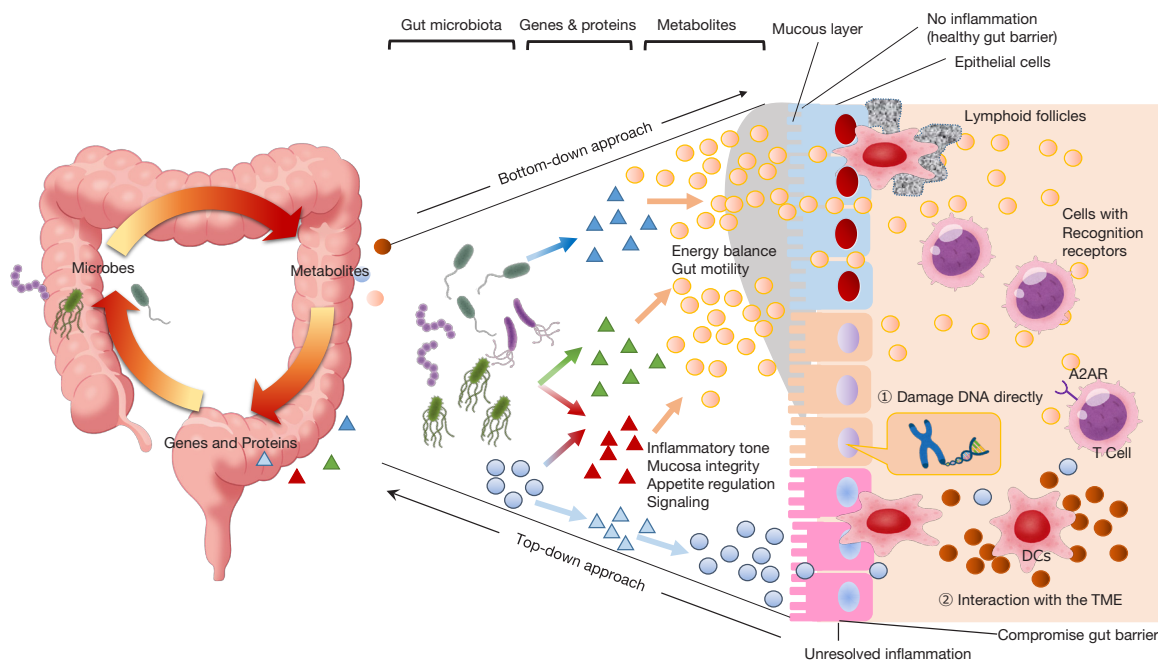


Figure 1 The gut microbiota and the gut are in perfect balance via genes, proteins, and metabolites. Some bacteria improve energy balance and intestinal motility, maintain healthy intestinal mucosa, and improve the immune environment through metabolites, while others directly induce DNA destruction, intestinal mucosal disruption, and inflammation by triggering the immune system through metabolites. The mechanism of gut microbiota carcinogenesis is thought to be due to two effects of the microbiome: ① damage to DNA directly and ② interaction with the TME. TME, tumor microenvironment; DC, dendritic cells; A2AR, adenosine 2A receptor.

health and disease (1). The GM and the intestine are in an exquisite balance via genes, proteins, and metabolites of both bacteria and humans. Some bacteria improve energy balance and intestinal motility, maintain healthy intestinal mucosa, and improve the immune environment through metabolites, while others directly induce DNA destruction, intestinal mucosal disruption, and inflammatory triggering by the immune system through metabolites (Figure 1). In the last decade, with the development of next-generation sequencing and bioinformatic technologies, the relationship between microorganisms and cancer has been studied in more detail, and it has become clear that microorganisms are involved in protective or deleterious effects on cancer development and malignant progression (21). Certain microorganisms have been found to be involved in the carcinogenesis of colorectal cancer (22), liver cancer, biliary tract cancer (23), breast cancer (24,25), and stomach cancer (26). The mechanism of carcinogenesis is thought to be due to two effects of the microbiome. The first effect is mutagenesis of the colonic epithelium. This occurs with the production of bacterial toxins and other molecules

that either damage DNA directly, disrupt the systems that maintain genomic integrity, or stress cells in other ways that indirectly impair the fidelity of DNA replication and repair (27). For example, *E. coli* carries the *pks* locus, which demonstrably mutagenizes the human genome and is implicated in conveying hallmark-enabling mutations (28). Secretion of butyrate acid, a metabolite of *Porphyromonas* sp., has been shown to contribute to tumorigenesis by inducing senescence of fibroblasts and epithelial cells (29). In addition to the aforementioned cancer types, the GM is thought to have an effect on other cancers, such as hematologic tumors or lung cancer, through interactions with the tumor microenvironment (TME) (30). Microbial mechanisms can modulate the activity of primary and secondary lymphoid organs of the gut epithelial barrier and regulate the immune tone of the TME (Figure 1).

A recent multicenter, multinational clinical trial demonstrated that higher diversity of intestinal microbiota is significantly associated with lower patient mortality after allogeneic hematopoietic stem cell transplantation (31). Moreover, analysis of more than 10,000 longitudinal

fecal samples in an allogeneic hematopoietic stem cell transplantation trial revealed that immune reconstitution dynamics were closely related to GM composition (32). Links between GM, nutrition, posttransplant bone marrow (BM) and thymic cellularity, and lympho- and myelopoiesis have also been demonstrated in mouse models (33).

Various facts about the impact of the GM on adaptive immunity have also been elucidated. The intestinal ecosystem can influence both local and distant neoplasia by affecting the immune context, the influx of myeloid and lymphoid cells, and the patterns of inflammatory and metabolic processes. Secretory components of the GM can be important; for example, outer membrane vesicles (OMVs) can reprogram the TME toward a pro-TH1 phenotype (CXCL10, IFN- γ) (34). Similarly, commensal microbiota was shown to prime tumor-associated innate myeloid cells for tumor necrosis factor- α (TNF- α) (IL-1 β , IL-12, and Cxcl10) production in response to anti-IL-10R/CpG-ODN treatment, and antibiotics, germ-free, or TLR4^{-/-} status attenuated this response and TNF-dependent early tumor necrosis. Anti-CTLA-4-induced gut barrier dysfunction was also found to be critical for the systemic translocation of Bifidobacterium-derived inosine, in turn promoting TH1 activation and antitumor immunity by agonizing T-cell-specific adenosine 2A receptor (A2AR) signaling in the context of dendritic cell (DC) costimulation (35). These examples demonstrate that barrier injury is accompanied by a deviation in the local microbiome or translocation of microbial metabolites, resulting in mobilization of DCs to gut-associated lymphoid tissues and contributing to infiltration of the tumor bed by activated helper or cytotoxic T cells. The TME is composed of a dense network of adrenergic nerve fibers that influence oncogenesis of brain and non-brain tumors as well as immune components derived from stromal, tumor, endothelial cells, and hematopoietic progenitor-derived immune component (36,37). Enteric nervous system neurons are both affected by the GM and functionally tuned according to their location in the gut. A subset of microbiota-responsive neurons was found to influence metabolic control independent of the central nervous system (38). These findings suggest close relationships between mucosal or tumoral commensals and tumor innervation, which warrants further study.

Lung microbiota

Lung cancer is closely associated with chronic inflammation, but the causes of inflammation and specific immune

mediators have not been fully elucidated.

Healthy lungs have traditionally been believed to be sterile due to the inability to culture bacteria from lower airway samples using routine microbiological approaches. However, recent culture-independent sequencing study have identified that the lower respiratory tract contains a complex diversity of bacteria (39). Changes in this local microbial community have been associated with the exacerbation of several pulmonary disorders, such as chronic obstructive pulmonary disease, asthma, and cystic fibrosis (39). There are multiple lines of evidence that the lung microbiota is associated with lung cancer. Jin *et al.* published important findings of the relationship between the lung microbiota and lung cancer in a study using a genetically engineered mouse (GEM) model (40). They found that the local microbiota, such as *Herbaspirillum* and *Sphingomonadaceae*, which are associated with tumor growth, could promote inflammation and cancer progression via lung-resident $\gamma\delta$ T cells. Depleting the microbiota or inhibiting T cell activity or their downstream effector molecules effectively suppressed lung cancer development. In addition, Tsay *et al.* presented that the signature of lower airway dysbiosis, an imbalance between the types of organisms present in a person's natural microflora, was most prevalent in the group of lung cancer patients with stage IIIB–IV tumor node metastasis and was associated with poor prognosis, as shown by decreased survival among subjects with early-stage disease. They also described that a lower airway microbiota signature was associated with upregulation of the IL-17, PI3K, MAPK, and ERK pathways in the airway transcriptome, and *Veillonella parvula* was the most abundant taxon driving this association (41).

Human lung microbiome and mouse studies revealed that the most common bacterial genera present in the lung microbiome included *Staphylococcus* (~15%), *Streptococcus* (~15%), and *Lactobacillus* (~15%), along with the family *Pasteurellaceae* (~10%) (40,42,43). However, in the lungs of cancer patients, the total bacterial burden is significantly increased, and several bacterial taxa, including *Herbaspirillum* and *Sphingomonadaceae*, were significantly more abundant in the lungs of cancer patients than in healthy lungs (40).

Intratumoral microbiota

It is recognized that bacteria can be detected within solid tumors. To prove this concept, Nejman *et al.* evaluated 1,526 tumors of seven human cancer types (bone, brain, lung,

melanoma, ovary, and pancreas) and extracted bacterial DNA, RNA, and lipopolysaccharide (44). They revealed that the intratumor bacteria are mostly intracellular and are present in both cancer and immune cells.

The microbiota has been similarly detected in genetically engineered *de novo* mouse models of lung and pancreatic cancer, suggesting that the tumor microbiome is functionally involved as a driver of tumor-promoting inflammation and malignant tumor progression (40). The live microflora appears to have a suppressive effect on local antitumor immunity to the TME. Cancer-specific mechanisms of action of microorganisms in tumors have been reported. Beyond lung cancer, there have been reports of secreted genotoxin-mediated mutagenesis in the gastrointestinal tract and urinary tract, chemotherapy resistance due to microbial metabolism and fungal activation of the host C3 complement cascade leading to greater tumor growth in pancreatic cancer, indirect amplification of cancer cell autophagy in intestinal cancer, and tumor upregulation of the levels of matrix metalloproteinases in breast cancer. In lung cancer, inflammation mediated by CagA and IL-17-producing $\gamma\delta$ T cells and metastasis due to decreased tumor immunosurveillance have been reported (40,45).

Microbiota and lung cancer treatment

The role of the GM in immune checkpoint blockade (ICB)

The activity of the GM has many effects on host physiology, including on the development and regulation of immune responses. Recently, multiple publications have indicated that the microbiota can specifically influence the outcome of cancer immunotherapy (46-49). They demonstrate that differential GM signatures exist in patients who respond to treatment and that these favorable signatures are associated with enhanced systemic immunity and increased levels of intratumoral immune infiltrates. The mechanisms through which the GM influences the response to immunotherapy have been investigated in preclinical and clinical studies. The data suggest that gut microbes may impact antitumor immunity via several mechanisms, including the 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) such as Toll-like receptors (TLRs)], which can help prime the adaptive immune response; induction of cytokine production by APCs or lymphocytes; and even through inducing local or distant

effects with microbial metabolites (50).

Vétizou *et al.* presented a study of a mouse model in which CTLA-4 inhibitory therapy resulted in a marked change in the abundance of microbes in the intestinal flora in mice, with a relative increase in *Bacteroidales* and *Burkholderiales* abundances and a decrease in *Clostridiales* abundance (47). In addition, they showed that the efficacy of anti-CTLA-4 therapy was markedly reduced in germ-free mice treated with broad spectrum antibiotics. Furthermore, oral feeding with *Bacteroides fragilis* in combination with either *Bacteroides thetaiotaomicron* or *Burkholderia cepacia* augmented the action of anti-CTLA-4 therapy by eliciting Th1 responses in the lymph nodes and facilitating the maturation of intratumoral DCs. In addition, Griffin *et al.* presented the molecular mechanisms that influence the host response to immunotherapy (51). They showed that members of the bacterial genus *Enterococcus* improve ICI in mouse models. Active enterococci express and secrete orthologs of the Nlpc/p60 peptidoglycan hydrolase SagA that generate immune-active muropeptides. The expression of SagA in nonprotective *E. faecalis* was sufficient to promote the immunotherapy response, and its activity required the peptidoglycan sensor NOD2. Their data suggested that microbiota species with specialized peptidoglycan remodeling activity and muropeptide-based therapeutics may enhance cancer immunotherapy.

Hakozaki *et al.* presented an association between GM and ICI outcomes in NSCLC patients (52). They collected baseline pre-ICI samples and the clinical data of 70 Japanese NSCLC patients who received anti-PD-1/PD-L1 antibodies as a first-line or treatment-refractory therapy. In this research, patients who were treated with antibiotics before ICI had lower alpha diversity at baseline and underrepresentation of *Ruminococcaceae* UCG 13 and *Agathobacter* than those of patients not treated with antibiotics. For antibiotic-free patients at baseline, alpha diversity correlated with OS. *Ruminococcaceae* UCG 13 and *Agathobacter* levels were also higher in patients with better objective response rate (ORR) and progression-free survival (PFS). *Ruminococcaceae* UCG 13 abundance was higher in patients with better overall survival (OS) for more than 12 months. GM differences were observed between patients who experienced low- versus high-grade irAEs. The negative influence of antibiotics on the composition of the GM and identification of the bacterial repertoire in patients experiencing favorable responses to ICI was shown. Oncologists might recall that antibiotics lead to dysbiosis in the gut microbiome for cancer patients who are going

to be treated with ICI. Therefore, the administration of unnecessary antibiotics should be avoided in principle. In other studies, Derosa *et al.* presented the correlation of fecal *Akkermansia muciniphila* abundance and ICI response (53). They performed shotgun-metagenomics-based microbiome profiling in a large cohort of patients with advanced NSCLC (n=338) treated with first- or second-line ICIs to prospectively validate the predictive value of fecal *Akkermansia*. Baseline stool *Akkermansia* abundance was associated with increased ORRs and OS in multivariate analyses, independent of PD-L1 expression, antibiotic treatment, and performance status. Moreover, intestinal *Akkermansia* was accompanied by richer commensalism, including with *Eubacterium hallii* and *Bifidobacterium adolescentis*, and a more inflamed TME in a subset of patients. However, antibiotic use coincided with a relative dominance of *Akkermansia* above 4.8% accompanied by the genus *Clostridium*, both associated with resistance to ICI. Significant differences in the relative abundance of *Akkermansia* may represent a potential biomarker to refine patient stratification.

The role of the GM in chemotherapy

The presence of intratumoral Gammaproteobacteria was found to be associated with resistance to gemcitabine chemotherapy in pancreatic Ductal adenocarcinoma patients (54).

Conventional cytotoxic chemotherapy is also dependent on intact immune responses, thus substantiating the notion that the GM could shape responses to these forms of therapy. For example, platinum-based chemotherapies and cyclophosphamide therapy cause the translocation of commensal bacteria (especially Gram-positive organisms such as *Lactobacillus johnsonii* and *Enterococcus hirae*) into mesenteric lymph nodes and can potentially facilitate robust stimulation of Th17 responses in the spleen and the induction of memory Th1 responses. Immune responses to cyclophosphamide have also been shown to be dependent on MyD88 and TLR signaling (55,56).

The GM and therapeutic toxicities

The GM has also been implicated in modulating the toxicity associated with cancer therapy. Much research has been done on the microbiome and chemotherapy toxicity and the potential interaction of the microbiome with ICIs, but only a few studies on the microbiome and cytotoxic drug toxicity

exist.

Shen *et al.* found that the diversity of GM promotes the development of chemotherapy-induced mechanical hyperalgesia (57). Oxaliplatin-induced mechanical hyperalgesia was reduced in germ-free mice and mice pretreated with antibiotics. Germ-free mice did not suppress mechanical hyperalgesia. These effects appear to be mediated, in part, by TLR4 expressed on hematopoietic cells, including macrophages.

Some intestinal bacteria may be protective against the toxicity of cancer immunotherapy. For example, *Bacteroidetes* has been reported to be more common in patients resistant to colitis caused by ipilimumab, and a higher abundance of these taxa within the gut is also generally associated with a lower incidence of toxicity (58). *Bifidobacterium* can abrogate pathology in a mouse model of immunotherapy-induced colitis (59). Conversely, some bacterial taxa may also be associated with favorable responses as well as toxicity. Bacterial taxa within the Ruminococcaceae family have been reported to be associated with immunotherapy-induced colitis (59).

Modulating the composition of the microbiome

Therapeutic strategies that modulate the microbiome are currently being evaluated to enhance the ICI response or to avoid primary resistance to ICIs (60). GM modulation studies, such as those investigating fecal transplantation, composition, diet, and probiotics, showed that favorable microbiota modulation is related to increased levels of tumor infiltrating lymphocytes and specifically effector CD8⁺ T cells. This CD8⁺ T-cell infiltration is known to be associated with the enhanced intratumoral activity of Th1⁺ cells and DCs and a lower density of immune suppressive cells. Several studies as described below have begun investigating the manipulation of the intestinal microbiota to elicit the effects of immunotherapy. Several factors are known to manipulate the intestinal microbiota, including diet, bacterial administration, and fecal transplantation.

Diet

Dietary intake can also promote differences in microbiome composition, and deep and intensive changes in dietary regimens can significantly alter the GM in a relatively short time (61). In another study, Desai *et al.* found that when dietary fiber is chronically or intermittently deficient, the GM turns to mucus glycoproteins secreted by the host

as a source of nutrition, and the colonic mucus barrier is eroded (62). Some groups have already begun to explore the impact of diet on the GM in cancer patients. The “BE GONE” trial (NCT02843425) is a study conducted by the M.D. Anderson Cancer Center to measure changes in the bacterial population in colorectal cancer patients after adding 1/2 cup of beans per day to their regular diet and supplementing with fiber.

The favorable safety profile, cost, and accessibility of dietary interventions could provide a simple and safe opportunity for assessing the implications of the microbiota and downstream immune manipulation in cancer patient populations.

Bacterial administration

Prebiotics will be an important factor in contemplating strategies to modulate gut microbes (63). Prebiotics comprise dietary compounds, including fibers and inulin, and they may support certain GM or modulate their functionality. The administration of bacterial consortia and designer probiotics might be a more feasible way to manipulate microorganisms in the clinical setting than manipulating the diet.

Recent study in preclinical models suggest that administration of inulin is associated with enhanced anti-humoral immune responses in melanoma (64). Several trials using probiotics in cancer patients have been initiated, with some completed. Clinical trials evaluating the potential effect of dietary modifications and prebiotics in metastatic cancer patients treated with immunotherapy are underway (NCT04552418, NCT04316520). In addition to lung cancer, several trials have been conducted in colorectal cancer, and many of these trials observe clinical course, changes in inflammatory response markers, and adverse events. For example, NCT03782428 is a completed study in which researchers investigated the effects of oral probiotics that contained six viable microorganisms of *Lactobacillus* and *Bifidobacteria* on the clinical course and inflammatory response markers in patients with colorectal cancer (65). They revealed that probiotics reduced levels of proinflammatory cytokines in colorectal cancer patients. In other cancer types, trials are underway to test whether probiotics affect the efficacy of ICIs and cytotoxic chemotherapy.

In lung cancer, several trials are manipulating the gut microbiome (Table 2). Seven clinical trials of oral probiotics are now recruiting patients (NCT03637803, NCT04601402, NCT04699721, NCT04857697,

NCT04909034, NCT04924374, and NCT05094167).

Another possible probiotic treatment is Akkp2611. In a study of the correlation between *Akkermansia* and ICI response (53), Derosa *et al.* suggested that therapeutic supplementation with lyophilized encapsulated Akkp2611 would benefit subgroups of patients not exposed to antibiotics and devoid of endogenous *Akkermansia*, and complex polymicrobial consortia or fecal microbial transplantation may be best suited for patients with prior antibiotic exposure.

Fecal microbiome transplantation and consortia

Fecal microbiota transplantation (FMT) is the most direct means of manipulating the microbiota, and fecal microbiota transplant formulations can be administered to recipient patients via oral administration of lyophilized tablets or packaged capsules or direct administration via colonoscopy or gastroscopy from an identified donor patient (66). This treatment is a recently developed therapy for recurrent *Clostridium difficile* infections and refractory inflammatory bowel diseases such as Crohn’s disease and ulcerative colitis. Clinical trials of FMT in cancer patients have just begun, but the results are highly anticipated. Autologous FMT in acute myeloid leukemia is being trialed in patients undergoing intensive care to increase the diversity of the GM and prevent dysbiosis during the treatment period. Furthermore, the application of FMT is being explored in patients receiving immunotherapy for solid tumor malignancies, particularly those treated with ICIs. A phase 1 single-center trial for metastatic melanoma patients who failed prior immunotherapy is underway wherein FMT from patients with a good response to immunotherapy is administered to refractory patients. The design of an additional trial is currently underway, and the aim is to test the hypothesis that modulation of the GM will improve the response to treatment with ICB. In the area of lung cancer research, there are several studies of the microbiota of lung cancer patients (52,53), and two trials are in preparation that will combine fecal transplantation or intestinal bacteria reconstitution therapy with ICIs (NCT04105270, NCT05008861). NCT04105270 is the randomized, active-controlled, parallel-group, double-blind, phase II trial of intravenous durvalumab (MEDI4736) and chemotherapy in combination with oral recovery microbiota therapy (RMT) or placebo in patients with untreated advanced or metastatic adenocarcinoma NSCLC; NCT05008861 is a study to evaluate the safety of FMT in the treatment of advanced NSCLC and to analyze the effect of FMT on patients’ GM

Table 2 Clinical trials of manipulation of the gut microbiome on lung cancer (recruiting or completed)

Trial number	Patient population	Intervention	Outcomes	Status (country/region)
NCT02771470	Lung cancer	Drug: probiotics	Composition of microorganisms in stool after probiotic intervention; frequency and severity of adverse effects during chemotherapy; the change of immunity and nutrition index	Completed (China)
NCT04056026	Mesothelioma	Fecal microbiota transplant	Primary: PFS	Completed (United States)
NCT03637803	Solid tumor, NSCLC, RCC, melanoma, bladder cancer	MRx0518 with pembrolizumab	Primary: safety and tolerability of MRx0518 in combination with pembrolizumab; clinical benefit of MRx0518 in combination with pembrolizumab; secondary: antitumor effect	Recruiting (United States)
NCT04601402	Solid tumor, NSCLC, head and neck, urothelial carcinoma	GEN-001 with avelumab	Primary: safety; secondary: efficacy	Recruiting (United States)
NCT04699721	NSCLC Stage III	Nivolumab + paclitaxel + carboplatin + BiFico (<i>Bifidobacterium trifidum</i> live powder)	Primary: safety, surgical complications; secondary: efficacy (ORR, recurrence rate, DFS, OS)	Recruiting (China)
NCT04857697	Breast cancer, lung cancer	Oral probiotics	Primary: length of probiotics, adherence of probiotics, percentage of CD8 ⁺ , CD4 ⁺ , and T-reg cells, cytokine counts	Recruiting (China)
NCT04909034	Lung cancer	MS20	Primary: the incidence of treatment-emergent adverse event; secondary: ORR, PFS, DCR, DOR	Recruiting (Taiwan)
NCT04924374	Lung cancer	Dietary supplement: microbiota transplant plus anti-PD-1 therapy	Primary: safety; secondary: efficacy (iRECIST)	Recruiting (Spain)
NCT05094167	NSCLC	<i>Lactobacillus Bifidobacterium</i> V9 (Kex02) with combined carilizumab with platinum chemotherapy	Primary: ORR; secondary: PFS, OS	Recruiting (China)

NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; PD-1, programmed death 1; PFS, progression-free survival; ORR, overall response rate; DFS, disease-free survival; OS, overall survival; DCR, disease control rate; DOR, duration of response; iRECIST, immune response evaluation criteria in solid tumors.

and immune phenotype conducted by treating patients with locally advanced or metastatic NSCLC after primary treatment with PD-1/PDL-1 monoclonal antibodies in combination with PD-1/PDL-1 monoclonal antibody and GM reconstruction therapy (FMT and other therapies).

Consortia, a lab-produced or designed consortium of microbes, monoclonal microbial candidates, and bacterial peptides, might theoretically incorporate a probiotic-like agent with a high safety profile and may induce an FMT-like functional improvement, as these small microbial

communities can work together. Clinical trials are underway to evaluate whether consortia enhance the function of the GM alone and in combination with immunotherapy (NCT03817125, NCT04208958). The consortia are either derived from donors having favorable GM signatures (NCT03817125) or engineered from preclinical models (67). In addition, investigations are underway to reconstruct gut microbial consortia via culturomics (68,69). Culturomics is an application of high-throughput culture conditions from human microbiota and uses matrix-assisted laser

desorption/ionization-time of flight (MALDI-TOF) or 16S ribosomal RNA (rRNA) amplification and sequencing for the identification of growing colonies (70). However, we need to understand the potential complexities of the current scalability and the consistency in manufacturing such products.

Discussion: future directions

A new approach for cancer research in “Hallmarks of Cancer”

Every decade, Hallmarks of Cancer by Hanahan and Weinberg has been guided by cancer research to clarify the mechanism of cancer and lead to treatments (and improve cancer treatment) (71). In 2022, Hanahan published new dimensions of hallmarks of cancer (4). In this statement, eight hallmark capabilities and embodied characteristics that have been sufficiently validated over a decade are discussed. The current review incorporated four additional proposed emerging hallmarks and enabling characteristics involving “unlocking phenotypic plasticity”, “nonmutational epigenetic reprogramming”, “polymorphic microbiomes”, and “senescent cells”. In addition, microbiomes are introduced as ecosystems involving existing bacteria or fungi, and they have a profound impact on human health and diseases, including cancer phenotypes (5,72). However, Hanahan also considers that the onco-biome is the new frontier associated with multiple tissues and organs and that the microbiome is too complex regarding population dynamics and the diversity of macrobacterium to be able to clarify the role of this entity soon. Polymorphic microbiomes are considered to intersect with tumor-promoting inflammation and genomic instability and mutations. This polymorphic influence and interaction will be a quasi-independent variable in cancer development, progression, and response to cancer treatment. The reproducibility has not been guaranteed in each study over the cancer subtypes and the analytic method has not been established. In the 16S rRNA method, the single arm with a moderate sample size has been established, and in the next step, randomized control study of JCOG 2007 (jRCTs031210013) comparing platinum doublets with pembrolizumab and platinum doublets with nivolumab/ipilimumab is underway. In this sub-study (not yet registered), fecal samples will be analyzed to predict the efficacy and safety of each chemotherapy.

In addition, multiple tissue microbiomes are involved

in modulating tumor phenotypes and the tumor response to immunotherapy positively or negatively. In particular, the use of antibiotics will cause dysbiosis in the gut flora and therefore have a negative effect on ICIs (52). Since maintaining the diversity of the GM is important, it has long been recognized that GM is fundamental for the function of the colon to degrade and import nutrients into the body to maintain metabolic homeostasis, and sometimes distortions in microbiota and dysbiosis can result in physiological maladies (73). The mechanisms by which microbiota impart these modulations are still being elucidated; however, two general effects are increasingly well understood for tumor promotion generally and the promotion of specific tumors. Through the interaction of the colonic epithelium with bacteria that promote mutagenesis, consequently, bacteria producing toxins and other harmful molecules influence either DNA damage or disruptions of the systems needed for maintaining genomic stability, or they stress the cells indirectly. In addition, metabolites such as butyrate from the microbiome, induce complex physiologic effects in nascent epithelial and fibroblastic cells (29,74). This process will lead to the expression of a diverse repertoire of cytokines or chemokines that can sculpt the abundance and characteristics of immune cell populations in the colon, comprising colonic epithelia and its underlying stroma and draining lymph nodes.

Additionally, some bacteria can form a protective biofilm and breach the mucus lining the colon, leading to destruction of the epithelial tight junctions between cells, which maintain the physical barrier that normally compartmentalizes the intestinal microbiome. Once bacteria invade the stroma of the gut, they induce innate and adaptive immune responses and elicit the secretion of a repertoire of cytokines and chemokines. Distinctive microbiomes in individual patients can be associated not only with prognosis but also with efficacy or resistance to ICIs by eliciting innate tumor-promoting inflammation and promoting tumor escape of adaptive immune destruction (3,5,75). In patients with melanoma who progressed during prior ICB, fecal transplantation from the responder restored the efficacy of ICB (60,76). Based on these results, the molecular mechanisms by which polymorphic microbiomes indirectly and systemically modulate tumor immunobiology have been demonstrated (77,78). Homeostasis, aging, and cancer, which have both overlapping and distinct species and abundances, are clearly associated with differences in the composition and diversity of the microbiome. In this context, research on the gut microbiome is sometimes

considered to be data-driven research using data mining; nevertheless, the hypothesis of these studies is based on immunological insights that clarify the mechanism and enrich the related molecules to enhance ICB or overcome resistance to ICB with multiomics analysis suggested in the hallmarks of cancer. In this review, we have described a number of new clinical trials of diet, bacterial administration and FMT that intervene in the gut microbiome. It is hoped that some of these trials may lead to new therapies that improve the efficacy of ICIs or influence the attenuation of resistance to these drugs.

Microbiome analytics

Conventionally, the bacterial culture has been unrealistic since some bacterial species are often difficult to culture and identify. However, recent progress in microbiological approaches improved through the extraction of DNA from the GM, and the identification of specific bacterial species has prevented the need for bacterial culture and interactions with the environment and has elucidated changes in genetic pools. This metagenomic approach is based on next-generation sequencing and bioinformatics and has been a breakthrough in improving the comprehensive analysis of the whole genome of the microbiome.

Metagenomic analysis innovatively changes our understanding of microbial communities using a next-generation sequencer by enrichment with bioinformatics analysis. This approach is roughly divided into two methods: meta 16S analysis and full metagenome analysis. Meta 16S analysis specifically amplifies and sequences the *16S rRNA* gene (18S in the case of eukaryotes) unique to prokaryotes from a group of genes extracted from fecal samples (79). The 16S rRNA is one of the components of the 1542-base-long prokaryotic rRNA. The *16S rRNA* gene has a region where systematic mutations are highly preserved across bacterial species, which can be identified by comparison with existing databases. The limitation of 16S rRNA-based sequencing is that it will be biased depending on the specified region analyzed, and there is a potential bias for specific bacterial species as the sequence fragment that can be analyzed by the sequencer is from 100 to 500 base pairs. In addition, DNA extraction of feces is required for analysis, but reproducibility does not occur across each method, including cell crushing and vibration crushing. On the other hand, whole metagenome analysis sequences all bacterial fragmented gene sequences without selecting specific genetic regions. The disadvantage of

whole metagenome analysis compared to 16S rRNA is the enormous amount of genomic data, which leads to a high burden for computational analysis with large amounts of time required as well as higher reagent costs. Therefore, current analysis of patient data is realistically available by 16S rRNA analysis targeting a specific region efficiently.

A recent study clarified the enriched species of the GM in the clinical setting, including the presence of *Akkermansia*, and demonstrated increased ORR and survival, although microbiota-relevant confounding factors should be considered (53). However, this first step of progress is important to show the role of GM modulation in immunotherapy. The translational approach, which is a bench to bedside approach and vice versa, should be further enhanced. In addition, data on clinical implementation of these approaches on the basis of real-world data, such as the use of antibiotics, which may have a negative impact on the clinical outcome, will be available by accumulating evidence.

Additionally, we have a question about whether cross-talk between the human immune system and the gut microbiome realistically contributes to modulating or interferes beyond immunomodulation and genomic alteration, thereby influencing tumorigenesis and progression. At present, there is evidence that specific bacterial species will directly stimulate proliferating signaling via colonic epithelium and modulate immune cell growth suppression by altering tumor suppressor activity in a different compartment of the intestine (72,80). Recent investigations have accumulated evidence for the polymorphic variations in gut microbiomes or other organs that complement modulating tumors, such as tumor growth, inflammation in the TME, immune evasion, genomic instability, and treatment resistance (4).

Currently, the onco-biome is at the clinical trial phase for clinical implementation and has moved from the preclinical phase. In particular, preclinical landmark studies have demonstrated an association between specific GM and the effectiveness of immunotherapy (46-48,50,53). However, the exact mechanism by which the GM supports immunotherapy remains unclear, and how it works on immune cells or specific molecules produced by the microbiota remains unclear. A GM enriched for specific microbes or broad alpha diversity is associated with a favorable prognosis in patients with NSCLC treated with immunotherapy. To improve the efficacy of immunotherapy, it is important to conduct interventional studies with randomized controlled trials that modulate the GM.

Conclusions

The gut microbiome is currently becoming the hallmark of cancer research and has an established and critical role in regulating antitumor immunity and the response to ICB in patients with lung cancers. The microbiome is regarded as an organ that modulates cancer immunity, and it has been clarified that the specific bacterial species that are enriched can both augment and impede. It is important to study the onco-biome to enhance the efficacy of pharmacotherapy and adjuvant treatment.

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